

RESEARCH NOTE

DNA FINGERPRINTING OF SEPTICEMIC AND LOCALIZED *BURKHOLDERIA PSEUDOMALLEI* ISOLATES FROM MALAYSIAN PATIENTS

MN Azura¹, A Norazah¹, AGM Kamel² and S Ahmad Zorin²

¹Bacteriology Unit, Institute for Medical Research, Kuala Lumpur; ²Faculty of Biomedical Sciences, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Abstract. We have analysed DNA fingerprinting patterns by pulsed-field gel electrophoresis (PFGE) of 52 unrelated *Burkholderia pseudomallei* strains isolated from septicemic and localized infections from Malaysian subjects. A total of 38 PFGE types were observed among 36 septicemic and 16 localized strains with no predominant pattern. Type 25 was seen in 2 epidemiologically related strains, suggesting human to human transmission. Twelve PFGE types were shared among 26 strains (21 septicemic and 5 localized) showing close genetic relatedness with coefficient of similarity of 0.81 to 1.0. The other 26 strains (15 septicemic and 11 localized) were unrelated as shown by the similarity coefficient of <0.8. This study showed that our *B. pseudomallei* strains in Malaysia were mainly heterogenous with no predominant type both in septicemic or localized strains.

Keywords: *Burkholderia pseudomallei*, melioidosis, DNA fingerprinting, PFGE, Malaysia

INTRODUCTION

Melioidosis is a disease caused by *Burkholderia pseudomallei* and can be fatal if untreated. *B. pseudomallei* is also listed as a bio-weapon agent (Puthuchery and Vadivelu, 2002; Gilad *et al*, 2007). The disease may manifest as septicemia with pneumonia, septicemia without pneumonia and localized infection (Puthuchery and Vadivelu, 2002). The patient may present with various symptoms but fever

is the most common presentation (Mandell *et al*, 1995). Most of the melioidosis cases in Malaysia are from septicemic infection and only 5-20% was from localized infection (Puthuchery and Vadivelu, 2002). Melioidosis with septicemia is commonly seen among patients with underlying clinical conditions, such as diabetes mellitus and chronic lung diseases while localized infections are associated with minor injuries, such as abrasions and wounds on the skin (Dharakul *et al*, 1996; Suputtamongkol *et al*, 1999; Puthuchery and Vadivelu, 2002).

Limited epidemiological data are available on the distribution of virulent strains and the source of melioidosis infection in Malaysia. Many studies have employed pulsed-field gel electrophoresis (PFGE)

Correspondence: Azura Mohd Noor, Bacteriology Unit, Infectious Diseases Research Center, Institute for Medical Research, Kuala Lumpur 50588, Wilayah Persekutuan, Malaysia.
Tel: 603 2616 2462; Fax: 603 2692 4949
E-mail: azura@imr.gov.my

technique to study clonal relatedness because of its high discriminative power (Mulligan and Arbeit, 1991; Ternover, 1997). In this study, we carried out DNA fingerprinting of clinical isolates of *B. pseudomallei* by PFGE to determine clonal relatedness among these strains.

MATERIALS AND METHODS

Strains

B. pseudomallei were isolated from various clinical specimens of melioidosis patients collected for the duration of 1995 to 2007 from several hospitals in Malaysia. The strains were sent to Bacteriology Unit, Institute for Medical Research (IMR) for confirmation of *B. pseudomallei*. They were identified by their characteristic colony morphology on modified Ashdown's medium, positive oxidase reaction, biochemical profiles based on API 20NE and arabinose assimilation (Ashdown, 1979; Dance *et al*, 1989; Wuthiekanun *et al*, 1990, 1996).

PFGE

Preparation of bacterial DNA was carried out following a modification of the method by Currie *et al* (2001). *B. pseudomallei* colonies were cultured on blood agar medium at 37°C for 24 to 48 hours. Bacterial suspension was prepared in cell suspension buffer (50 mM Tris, 100 mM EDTA, pH 8.0) and the bacterial concentration adjusted to 4 McFarland standards. Proteinase K (20 mg/ml) was added into 100 µl of the bacterial suspension and vortexed for 10 minutes. An equal volume of suspension was added to freshly prepared 1% low melting point agarose (Seakem Gold®) and dispensed into plug moulds. The agarose plugs were allowed to solidify and added to 3 ml of fresh cell lysis buffer (25 mM Tris, 50 mM EDTA, 1% sarcosyl, pH 8.0) containing 100 µl proteinase K (20 mg/ml)

and incubated at 54°C with 100 rpm agitation for 24 hours. Plugs were washed 4 times with 0.5x TE buffer (1M Tris, 0.5 mM EDTA) and stored at 4°C until use. Bacterial chromosomes immobilized in the agarose plugs were digested with 60 units of *SpeI* at 37°C for 24 hours and then washed with 0.5x TE buffer. PFGE of the digested chromosomes was carried out with CHEF-Mapper system (BioRad, Hercules, CA) with 10 to 60 seconds pulse ramping time for 23 hours. Gels were then stained with ethidium bromide and visualized under UV light.

PFGE results were analyzed using FPQuest software (BioRad, Hercules, CA). The Dice metric was used to calculate similarity coefficient of pairs of samples and a dendrogram of cluster analysis was constructed using Unweighted Pair Group Method with Arithmetic (UPGMA) method. Strains showing Dice coefficient of ≥ 0.8 are grouped into the same PFGE type and subtypes.

RESULTS

A total of 52 clinical isolates were identified as *B. pseudomallei* and all the strains were arabinose negative. Sixteen isolates were obtained from pus, swab, tissue, peritoneal fluid, sputum and chest tube drainage specimens (Table 1). The patients' age ranged from 8-77 years (mean 43.8). The ratio of male to female was 4:1. Thirty-six isolates were obtained from blood and urine specimens and were categorized as septicemic strains. Septicemic patients' age ranged from 21-75 years old (mean age 51.4) with male to female ratio being 30:1 and only one patient had diabetes mellitus. PFGE analysis of *SpeI*-digested *B. pseudomallei* resulted in the characterization of 38 types of PFGE fingerprints and all the strains were typable (Fig 1). A total

Dice (Opt:0.50%) (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
PFGE(a)

PFGE(a)

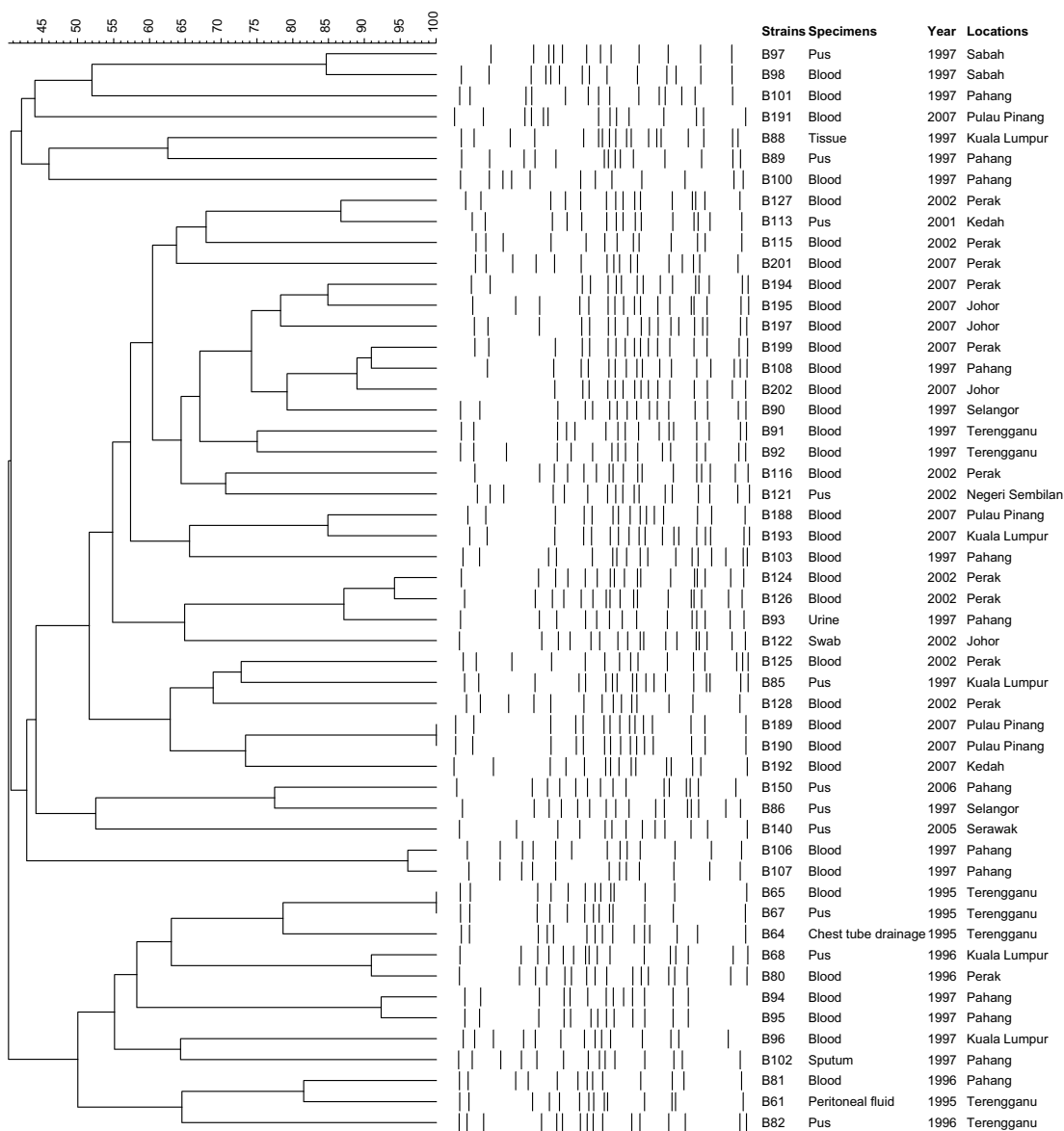


Fig 1–Dendrogram of PFGE fingerprints of 52 clinical *B. pseudomallei* strains.

of 26 out of 38 types (Type 2, 3, 4, 5, 6, 8, 9, 11, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 26, 27, 28, 29, 32, 35, 36 and 38) were single PFGE types with similarity coefficient

ranged from 0.44 to 0.79. Another 12 PFGE types (Type 1, 7, 10, 12, 18, 20, 25, 30, 31, 33, 34, and 37) were shared by 26 unrelated clinical strains of *B. pseudomallei* with simi-

Table 1

Localized and septicemic strains of *Burkholderia pseudomallei* examined in this study.

Localized		Arabinose status	Septicemic		Arabinose status
Specimen	Number of strains		Specimen	Number of strains	
Pus	11	Ara -	Blood	35	Ara -
Swab	1	Ara -	Urine	1	Ara -
Tissue	1	Ara -			
Peritoneal fluid	1	Ara -			
Chest tube drainage	1	Ara -			
Sputum	1	Ara -			
Total	16		Total	36	
Male:female	4:1		Male:female	30:1	
Age (yr)(mean)	43.8		Age (yr)(mean)	51.4	

larity coefficient ranging from 0.81 to 1.0. Type 12 and 20 were identified as the commonest PFGE types where each of the type had 3 subtypes. Types 1, 7, 10, 18, 30, 33, 34 and 37 were represented by 2 subtypes each. The remaining Type 25 and Type 31, were shared by 2 identical strains having the same PFGE profiles.

Interestingly we noted that strain B189 and B190, which were isolated from different patients from the same ward within the same week of isolation, shared the same PFGE pattern (Type 25). Septicemic strain B65 and localized strain B67 shared the same Type 31 profile. These strains were isolated from different patients admitted in different wards.

Eight strains (4 septicemic and 4 localized) shared 4 PFGE patterns, namely, Type 1, Type 7, Type 33 and Type 37 with coefficient similarity ranging from 0.84 to 0.9 (Table 2). Strain B97 (localized) was related to strain B98 (septicemic) at the level of coefficient similarity 0.84 designated (Type 1a and 1b, respectively). Strain B68 (localized) and B80 (septicemic) were related at similarity coefficient of 0.90

(Type 33a and 33b). Type 7a and 7b was present in B113 (localized) and B127 (septicemic), respectively, and related at 0.86 coefficient similarity. Type 37a and 37b was present in strain B81 (septicemic) and B61 (localized), respectively, and related at 0.84 coefficient of similarity. All these strains were isolated from different areas and years except for strain B97 and B98 that were isolated from same geographic location in the same year.

Six PFGE types were observed among 10 septicemic strains with coefficient of similarities of 0.84 to 0.96. The rest of the septicemic and localized strains were each represented by a single unique type of PFGE pattern (coefficient of similarities 0.44 to 0.79).

DISCUSSION

Twenty-six isolates from 52 septicemic and localized cases were shown to share the same PFGE fingerprint types, suggesting that there is a close genetic relatedness among the strains even though they were isolated from different patients who were

Table 2
PFGE fingerprint patterns of septicemic and localized strains of *B. pseudomallei*.

PFGE fingerprint type	Strain		PFGE fingerprints type	Strain	
	Septicemia(%)	Localized(%)		Septicemia(%)	Localized(%)
1a		B97(84.6)	20a	B124(94.1)	
1b	B98(84.6)		20b	B126(94.1)	
			20c	B93(87.1)	
2	B101(51.9)		21		B122(64.8)
3	B191(44.0)		22	B125(72.7)	
4		B88(62.5)	23		B85(72.7)
5		B89(62.5)	24	B128(68.9)	
6	B100(45.9)		25	B189(100.0)	
			25	B190(100.0)	
7a	B127(86.7)		26	B192(73.3)	
7b		B113(86.7)			
8	B115(67.9)		27		B150(77.4)
9	B201(63.7)		28		B86(77.4)
10a	B194(84.9)		29		B140(52.5)
10b	B195(84.9)				
11	B197(78.2)		30a	B106(96.0)	
			30b	B107(96.0)	
12a	B199(90.9)		31	B65(100.0)	
12b	B108(90.9)		31		B67(100.0)
12c	B202(88.9)				
13	B90(79.2)		32		B64(78.6)
14	B91(75.0)		33a		B68(90.9)
			33b	B80(90.9)	
15	B92(75.0)		34a	B94(92.3)	
			34b	B95(92.3)	
16	B116(70.6)		35	B96(64.3)	
17		B121(70.6)	36		B102(64.3)
18a	B188(84.9)		37a	B81(81.5)	
18b	B193(84.9)		37b		B62(81.5)
19	B103(65.5)		38		B82(64.5)

%, Percentage of similarity coefficient

from different locations. The best example is shown by 3 unrelated septicemic strains B199, B108 and B202 isolated from Perak, Pahang and Johor, respectively. Taking the similarity coefficient above 0.8 as the cut-off point for the determination of close relatedness (Struelens *et al*, 1996; Chong *et al*, 2003), the patterns of these strains showed

high similarity to each other and this may suggest that there is an endemic strain in Malaysian soil. These 3 strains might be descendent of clones of the same progeny, which were disseminated to different areas especially during the wet season (Strauss *et al*, 1969; Haase *et al*, 1995; Struelens *et al*, 1996; Puthuchery and

Vadivelu, 2002; Chong *et al*, 2003). The sharing of same PFGE pattern by a septicemic (B65) and a localized strain (B67) showed that *B. pseudomallei* can infect multiple sites, as observed in other studies (Haase *et al*, 1995; Vadivelu *et al*, 1997; Koonpaew *et al*, 2000). The other 26 strains (15 septicemic and 11 localized) were represented by a single PFGE types with similarity coefficient <0.8, suggesting a heterogeneity of our *B. pseudomallei* strains (Struelens *et al*, 1993; Chong *et al*, 2003).

The identification of the PFGE Type 25 that was identical in strains B189 and B190 raises the possibility of human to human transmission of *B. pseudomallei*. Strain B189 was isolated from a diabetic patient admitted for perforated ulcer and strain B190 was from a patient admitted for intestinal obstruction. These patients were located in the same surgical ward, in the same cubical and their beds were side by side. Strain B190 was isolated 3 days after B189. Human to human transmission of melioidosis has been reported in Thailand, involving a woman who was diabetic and infected with melioidosis through her brother who was treated for melioidosis (Kunakorn *et al*, 1991). However, the patients's wife who was not a diabetic was not infected even when she was in close contact with the patient for a much longer time. Septicemic melioidosis cases reported in Thailand are significantly associated with diabetes mellitus (Chaowagul *et al*, 1989).

Two distinct biotypes of *B. pseudomallei* have been defined recently by their differential ability to assimilate L-arabinose (Liu *et al*, 2002). Arabinose negative (Ara-) type *B. pseudomallei* was identified as a virulent strain and clinically significant in causing melioidosis infection (Wuthiekanun *et al*, 1996; Brett *et al*, 1997; Smith *et al*, 1997; Liu *et al*, 2002). Based on

a study by Wuthiekanun *et al* (1996) on environmental isolates of *B. pseudomallei* in rice paddies in the northeast and central origin of Thailand, only Ara- environmental isolates are intergenically identical to clinical isolates obtained from melioidosis patients, while arabinose positive isolates were strikingly different. Furthermore, all Ara- isolates were isolated from northeastern Thailand where melioidosis was common. Smith *et al* (1997) also found that the inability to assimilate L-arabinose is more strongly associated with virulence than Ara+ and this was observed also in their experimental animal models. In our study we found that all *B. pseudomallei* isolates obtained from melioidosis patients in various hospitals in Malaysia were Ara-type and this may suggest that Malaysian clinical isolates are highly virulent.

Our isolates were mainly from male patients aged above 40 and most cases were from rural areas. Even though we could not get their occupational history, occupational factor could not be ruled out in these cases. Occupational or outdoor activities that have contact with soil is one of the risk factors for contracting melioidosis (Merianos *et al*, 1993; Suputtamongkol *et al*, 1999). This was observed in Thailand where most of the melioidosis cases in northeastern area are males who worked as rice farmers (Chaowagul *et al*, 1989; Wuthiekanun *et al*, 1990). A survey by Strauss *et al* (1969) on antibody detection of *B. pseudomallei* among outdoor workers, found 1.9 to 15.8% of positive sera are from forest dwellers, rubber and oil-palm estate workers, military camp groundskeepers, army engineers residing in rice-growing areas and healthy army recruits.

In summary, our study showed that clinical *B. pseudomallei* isolates were heterogeneous and there were no predominant PFGE fingerprint profiles among septic-

mic and localized strains. Similar PFGE types were shared by localized and septicemic strains. The strains have the characteristic of being highly virulent as shown by their arabinose negative type. Further studies on environmental isolates should shed more light on the epidemiology of *B. pseudomallei* in Malaysia.

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