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Short Communication

*Spe*I Restriction Enzyme Displays Greater Discriminatory Power than *Xba*I Enzyme Does in a Pulsed-Field Gel Electrophoresis Study on 146 Clinical *Burkholderia pseudomallei* Isolates

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SUMMARY: Restriction enzymes *Spe*I and *Xba*I were used in a pulsed-field gel electrophoresis (PFGE) study for molecular characterization of 146 clinical *Burkholderia pseudomallei* isolates. The PFGE parameters were optimized to enable comparable, reproducible, and robust results. The optimized parameters for both *Spe*I and *Xba*I restriction enzymes used in this study were 200 V and a pulse time of 5 to 65 s for a 28-h runtime. Using *Spe*I, 9 different clusters were identified, whereas 6 clusters were identified by *Xba*I digestion, which exhibited 85% similarity to *Spe*I. *Spe*I (discrimination index [*D*] = 0.854) showed higher discriminatory power than *Xba*I did (*D* = 0.464).

Melioidosis is a human and animal disease caused by *Burkholderia pseudomallei*, a Gram-negative soil saprophyte endemic to Southeast Asia and Northern Australia (1), and had been reported in Mauritius, South America, India, China, the Middle East, and Africa (2). Although biochemical methods remain the gold standard for diagnosing the causative agent of melioidosis, these methods are time consuming and lack discriminatory ability. Hence, current research trends involve the use of molecular tools, such as pulsed-field gel electrophoresis (PFGE) to discriminate bacterial isolates from different sources. PFGE is a highly reproducible and discriminating tool for molecular typing of bacteria; it has been successfully applied to a broad range of Gram-negative bacteria, Gram-positive bacteria, and mycobacteria species in epidemiological studies on endemic populations as well as in outbreak situations (3).

The two most commonly used restriction enzymes for PFGE of *B. pseudomallei* are *Xba*I (4,5) and *Spe*I (6,7). However, the discriminatory abilities of *Xba*I and *Spe*I have not been previously compared and reported. Hence, in this study, we compared the discriminatory abilities of *Spe*I and *Xba*I enzymes in the subtyping of *B. pseudomallei* isolates in Malaysia.

A total of 146 *B. pseudomallei* isolates that had previously been cultured from blood, pus, tissues, and body fluids (including urine, sputum, and peritoneal dialysates) from sporadic cases of melioidosis were analyzed. PFGE was conducted according to previously published protocols (8,9). Briefly, *B. pseudomallei* isolates were cultured overnight in nutrient broth at 37°C and then used to prepare 1.0% (w/v) agarose plugs (10). The plugs were lysed overnight at 50°C in 2 ml of cell lysis buffer (50 mM Tris [pH 8.0], 50 mM EDTA [pH 8.0], 1% [w/v] sodium lauroylsarcosine, and 20 mg/ml of proteinase K). The plugs were washed thoroughly with TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA [pH 8.0]). Two DNA plugs (2 mm each) were digested separately overnight with *Spe*I and *Xba*I (New England BioLabs, Beverly, Mass., USA). The DNA fragments were separated by electrophoresis (CHEF DR II; Bio-Rad Laboratories, Hercules, Calif., USA) in a 1% (w/v) agarose gel (Sigma, Poole, UK) in 0.5× TBE buffer for 28 h at 200 V with pulse times ramped linearly from 5 to 65 s at 14°C. The gels were stained with 0.5 μg/ml ethidium bromide, destained with distilled water, and photographed under UV illumination.

Recorded DNA fingerprints were exported to BioNumerics software version 5.1 (Applied Maths, Kortrijk, Belgium), which created a dendrogram using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA). Genetic diversity and *B. pseudomallei* isolates relatedness were compared at 85% similarity at 1% optimization and 1.8% tolerance. Simpson's index of diversity was applied to measure and compare the diversity of the PFGE banding patterns (11).

All bacterial isolate pulsotypes were reproducible upon one repeated PFGE experiment. *Spe*I endonuclease produced 13 to 23 bands that ranged in size from 20 kb to 1,100 kb, while *Xba*I generated 13 to 18 bands that ranged from 20 kb to 800 kb in size. *Xba*I restriction on all 146 *B. pseudomallei* isolates generated 117 pulsotypes, whereas *Spe*I restriction generated 126 pulsotypes. Two dendrograms were produced on the basis of the pulsotypes generated using *Spe*I (Fig. 1) and *Xba*I (Fig. 2). Among the 146 isolates studied, 9 *Spe*I clusters, designated BS1 to BS9, and 6 *Xba*I clusters, designated BX1 to BX6, were identified using a cutoff value of 85% similarity at 1.0% optimum and 1.8% tolerance (Table 1). The largest cluster generated by *Spe*I was BS9, followed by BS3, BS7, BS5, and BS4. Less than 5% were represented by other smaller clusters (BS1, BS2, BS6, and BS8). A total of 27 (18.5%) of *B.*

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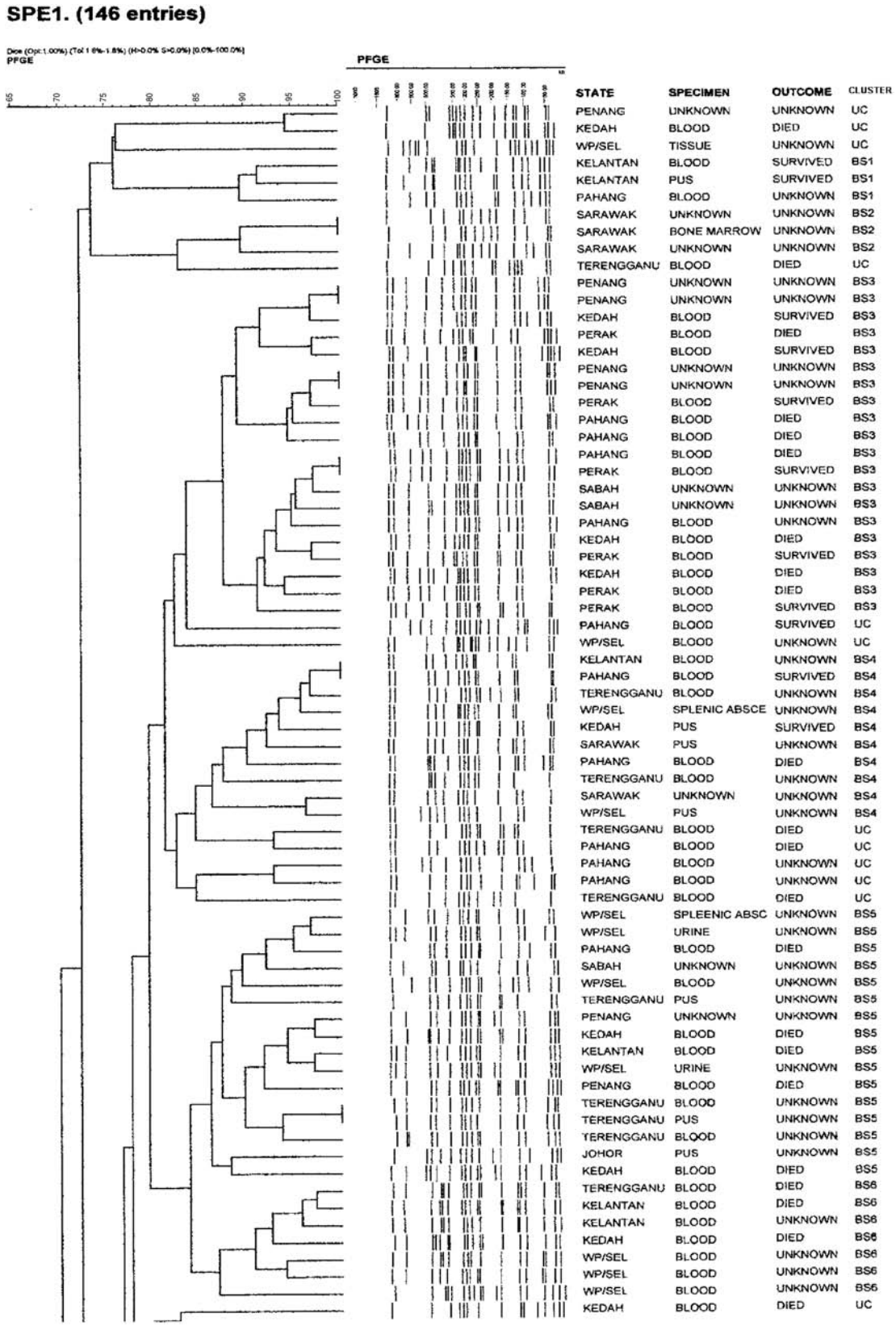

STATE	SPECIMEN	OUTCOME	CLUSTER
PENANG	UNKNOWN	UNKNOWN	UC
KEDAH	BLOOD	DIED	UC
WP/SEL	TISSUE	UNKNOWN	UC
KELANTAN	BLOOD	SURVIVED	BS1
KELANTAN	PUS	SURVIVED	BS1
PAHANG	BLOOD	UNKNOWN	BS1
SARAWAK	UNKNOWN	UNKNOWN	BS2
SARAWAK	BONE MARROW	UNKNOWN	BS2
SARAWAK	UNKNOWN	UNKNOWN	BS2
TERENGGANU	BLOOD	DIED	UC
PENANG	UNKNOWN	UNKNOWN	BS3
PENANG	UNKNOWN	UNKNOWN	BS3
KEDAH	BLOOD	SURVIVED	BS3
PERAK	BLOOD	DIED	BS3
KEDAH	BLOOD	SURVIVED	BS3
PENANG	UNKNOWN	UNKNOWN	BS3
PENANG	UNKNOWN	UNKNOWN	BS3
PERAK	BLOOD	SURVIVED	BS3
PAHANG	BLOOD	DIED	BS3
PAHANG	BLOOD	DIED	BS3
PAHANG	BLOOD	DIED	BS3
PERAK	BLOOD	SURVIVED	BS3
SABAH	UNKNOWN	UNKNOWN	BS3
SABAH	UNKNOWN	UNKNOWN	BS3
PAHANG	BLOOD	UNKNOWN	BS3
KEDAH	BLOOD	DIED	BS3
PERAK	BLOOD	SURVIVED	BS3
KEDAH	BLOOD	DIED	BS3
PERAK	BLOOD	DIED	BS3
PERAK	BLOOD	SURVIVED	BS3
PAHANG	BLOOD	SURVIVED	UC
WP/SEL	BLOOD	UNKNOWN	UC
KELANTAN	BLOOD	UNKNOWN	BS4
PAHANG	BLOOD	SURVIVED	BS4
TERENGGANU	BLOOD	UNKNOWN	BS4
WP/SEL	SPLENIC ABSCE	UNKNOWN	BS4
KEDAH	PUS	SURVIVED	BS4
SARAWAK	PUS	UNKNOWN	BS4
PAHANG	BLOOD	DIED	BS4
TERENGGANU	BLOOD	UNKNOWN	BS4
SARAWAK	UNKNOWN	UNKNOWN	BS4
WP/SEL	PUS	UNKNOWN	BS4
TERENGGANU	BLOOD	DIED	UC
PAHANG	BLOOD	DIED	UC
PAHANG	BLOOD	UNKNOWN	UC
PAHANG	BLOOD	UNKNOWN	UC
TERENGGANU	BLOOD	DIED	UC
WP/SEL	SPLEENIC ABSC	UNKNOWN	BS5
WP/SEL	URINE	UNKNOWN	BS5
PAHANG	BLOOD	DIED	BS5
SABAH	UNKNOWN	UNKNOWN	BS5
WP/SEL	BLOOD	UNKNOWN	BS5
TERENGGANU	PUS	UNKNOWN	BS5
PENANG	UNKNOWN	UNKNOWN	BS5
KEDAH	BLOOD	DIED	BS5
KELANTAN	BLOOD	DIED	BS5
WP/SEL	URINE	UNKNOWN	BS5
PENANG	BLOOD	DIED	BS5
TERENGGANU	BLOOD	UNKNOWN	BS5
TERENGGANU	PUS	UNKNOWN	BS5
TERENGGANU	BLOOD	UNKNOWN	BS5
JOHOR	PUS	UNKNOWN	BS5
KEDAH	BLOOD	DIED	BS5
TERENGGANU	BLOOD	DIED	BS6
KELANTAN	BLOOD	DIED	BS6
KELANTAN	BLOOD	UNKNOWN	BS6
KEDAH	BLOOD	DIED	BS6
WP/SEL	BLOOD	UNKNOWN	BS6
WP/SEL	BLOOD	UNKNOWN	BS6
WP/SEL	BLOOD	UNKNOWN	BS6
KEDAH	BLOOD	DIED	UC

Fig. 1. Dendrogram of *Spe*I-generated PFGE and cluster analysis.

pseudomallei isolates did not belong to any cluster; they harbored unique profiles.

*Xba*I generated 6 clusters as shown in Table 1. The largest cluster was BX1 with 71.9%, followed by BX4 with 12.3%. Other smaller clusters (BX2, BX3, BX5, and BX6) were less than 7% each. An unnamed cluster of 2 isolates contributed 1.4%. In Table 1, column BX1 clearly shows that the largest cluster, and BX1 from *Xba*I could be further differentiated into 7 clusters using the *Spe*I enzyme. This also indicates that all the

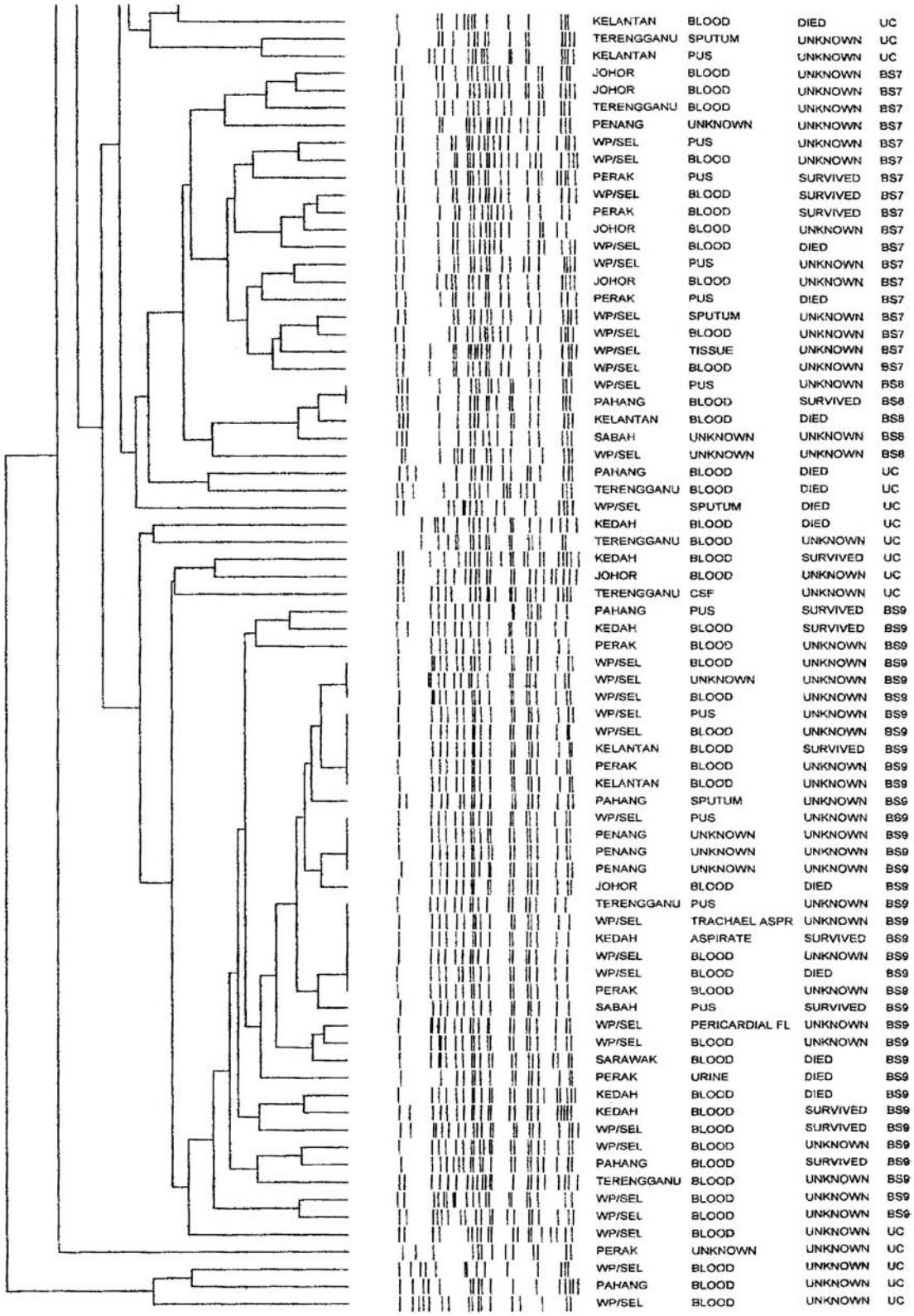

Fig. 1. (Continued)

bacteria grouped under cluster BX1 could be further subdivided on the basis of the different pulsotypes generated using *Spe*I. Therefore, the use of the DNA restriction enzyme *Spe*I was found to be more discriminatory in PFGE typing than *Xba*I was for *B. pseudomallei.* The overall result was also supported by Simpson's index diversity analysis; *Spe*I-digested *B. pseudomallei* isolates resulted in a discriminatory index of 0.854, compared to 0.464 for *Xba*I.

In general, the choice of restriction enzymes depends on the GC content of the particular organism. The GC content of *B. pseudomallei* is 64%; therefore, *Spe*I and *Xba*I are suitable for generating a reasonable number of bands for easy analysis. Based on the findings of previ-

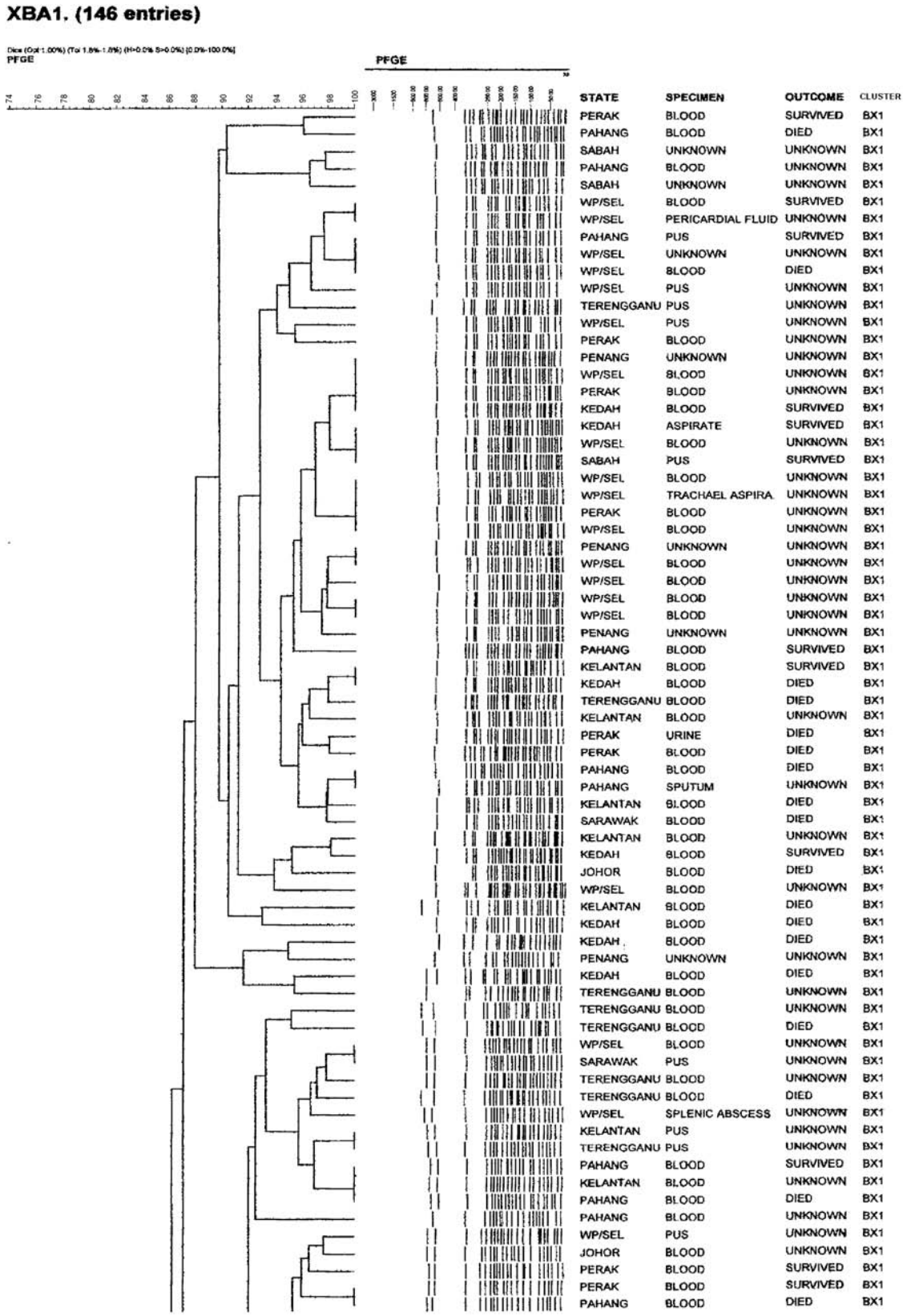

Fig. 2. Dendrogram of *Xba*I-generated PFGE and cluster analysis.

ous studies, optimal PFGE parameters for both *Xba*I and *Spe*I were defined as 5–65 s at 28 h. The pulse time is relatively long since both enzymes gave rise to fragments with large molecular weights of 700 kb and 1,135 kb, respectively. Pulse time and runtime were maintained throughout the *B. pseudomallei* study for band separation. Matsumoto et al. reported that it is very important to standardize the PFGE protocol to obtain reproducible and comparable results (3).

As shown in Table 1, 34 isolates were from fatal

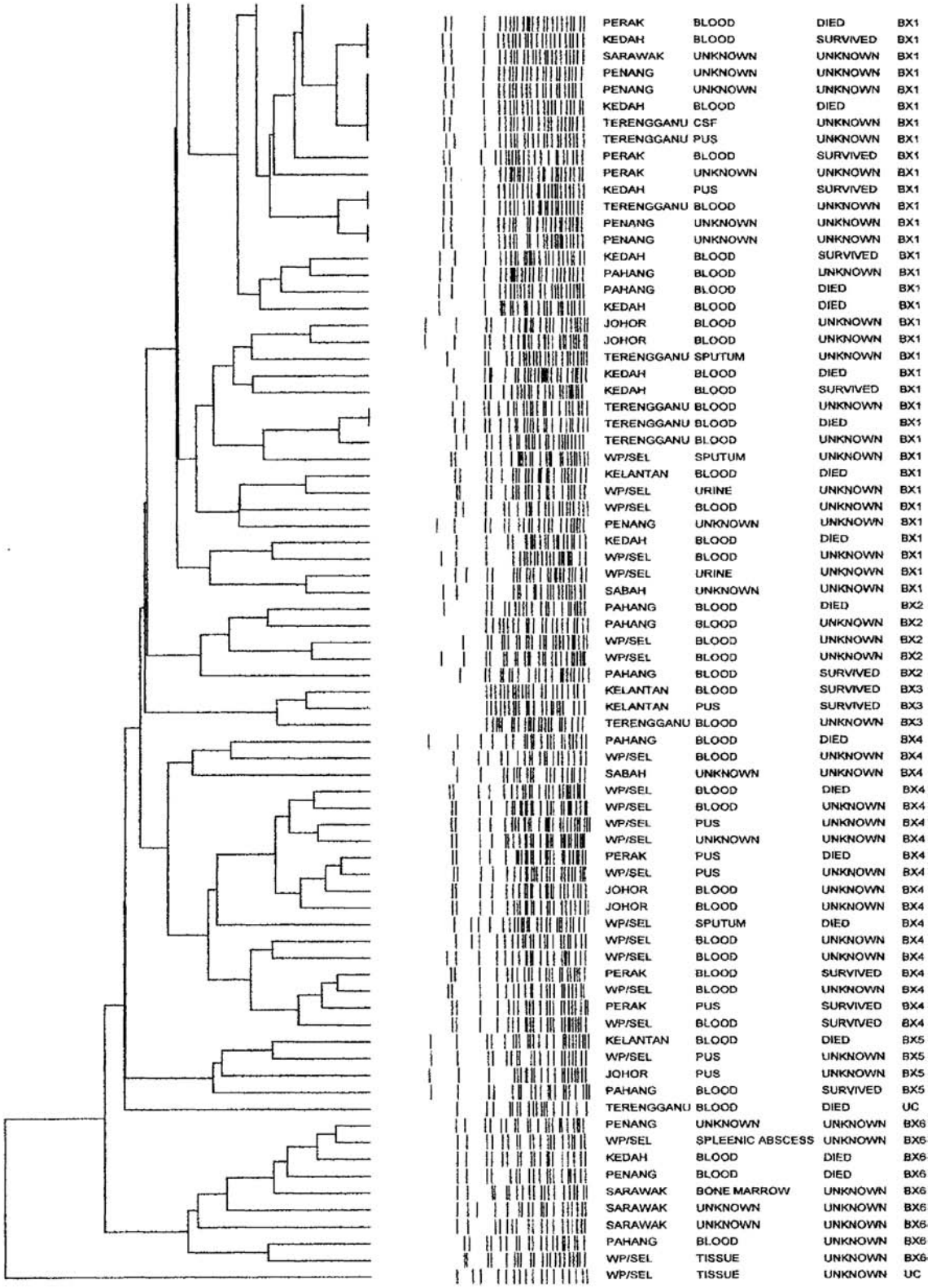

Fig. 2. (Continued)

cases. With use of *Xba*I, 76.5% ($n = 26$) of the isolates were grouped under BX1, and 1 to 3% were scattered throughout BX2, BX4–BX6, and the unnamed cluster. However, with use of *Spe*I, the 34 isolates spread out among the 7 different pulsotypes from BS3 to BS9 as well as in the unnamed cluster. Fatal cases that were grouped under pulsotypes BS3 and BS5 were from the hyperendemic state of Pahang, but those from Kedah were from clusters BS5, BS6, and an unnamed cluster. Therefore, the molecular epidemiological benefits and advantages of using *Spe*I include increased discriminatory power; correlation with patient outcome, source, and origin may be less accurate with the use of the *Xba*I enzyme.

Table 1. Comparison between *Spe*I and *Xba*I restricted PFGE clusters of *B. pseudomallei*

*Spe*I	*Xba*I							
	BX1	BX2	BX3	BX4	BX5	BX6	Unnamed cluster	Fatal case
BS1		1	2					0
BS2						3		0
BS3	20							7
BS4	10							1
BS5	10	1			1	4		5
BS6	6			1				3
BS7	5			12			1	2
BS8				2	3			1
BS9	37							5
Unnamed cluster	17	3	1	3		2	1	10
Fatal case	26	1	0	3	1	2	1	Total 34

Based on our results, the restriction enzyme *Spe*I was determined to be more suitable and accurate for PFGE profiling and typing of *B. pseudomallei* for future molecular and epidemiological studies.

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Conflict of interest None to declare.

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