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Characterization of *Burkholderia pseudomallei* isolated in Thailand and Malaysia

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Abstract

A total of 35 *Burkholderia pseudomallei* isolates from Thailand (16 clinical and eight soil isolates) and Malaysia (seven animal, two isolate each from clinical and soil) were investigated by their antimicrobial resistance, plasmid profiles and were typed by randomly amplified polymorphic DNA analysis. All isolates were found to be resistant to six or more of the 12 antimicrobial agents tested. Only two small plasmids of 1.8 and 2.4 megadalton were detected in two clinical isolates from Thailand. RAPD analysis with primer GEN2-60-09 resulted in the identification of 35 RAPD-types among the 35 isolates. The constructed dendrogram differentiated the 35 isolates into two main clusters and a single isolate. The wide genetic biodiversity among the 35 isolates indicate that RAPD-PCR can be a useful method to differentiate unrelated *B. pseudomallei* in epidemiological investigation.

1. Introduction

Burkholderia pseudomallei (previously known as *Pseudomonas pseudomallei*), are major pathogens for humans and animals in Southeast Asia and Northern Australia (Dance 1991; Idris et al. 1998; Supputtamongkol et al. 1994; Yabuuchi & Arakawa 1993), found in water and wet tropical soils. Clinical manifestations of melioidosis include acute septicaemia, chronic pulmonary infection, and visceral and soft-tissue abscesses. The infection carries a high mortality in people with underlying risk factors such as diabetes and renal disease (Chaowagul et al. 1989; Currie et al. 1993). Previous studies suggests that clinical and environmental isolates of *B. pseudomallei* from Thailand could be readily distinguished from each other by their ability to utilise the sugar L-arabinose as a sole energy source for growth (Sirisinha et al. 1998; Wuthiekanun et al. 1996). This raises the question of their genomic relatedness, and hence the reason for alternative techniques to be used in epidemiologic studies for strain comparisons. The present study was conducted to evaluate the genomic diversity of *Burkholderia pseudomallei* isolates from clinical, soil and animal specimens. In addition, the *B. pseudomallei* isolates were characterized for their antimicrobial resistance and plasmid profiles.

2. Materials and methods

2.1. Bacterial isolates

The bacterial isolates were obtained from clinical specimens of hospitalized patients and soil samples from the district of Khon Kaen, Thailand. Isolates from clinical (two), animals (seven) and soil (two) sources isolated in Malaysia were also included. The 35 isolates of *B. pseudomallei* had been confirmed with API tests (bioMereiux). Arabinose utilization was determined by growth on minimal salts agar containing 0.2% L-arabinose.

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2.2. Antimicrobial susceptibility tests

Disk diffusion tests were performed with antibiotic-containing disks obtained from BBL Microbiology System, Cockeysville, MD, by the method recommended by the National Committee for Laboratory Standards (1997). The antimicrobial agents tested included ampicillin (10 μg), bacitracin (10 μg), carbenicillin (100 μg), ceftriaxone (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg) and tetracycline (30 μg).

2.3. DNA isolation

Plasmid DNA was extracted, followed by electrophoresis, essentially as described by Sambrook et al. (1989). The approximate molecular mass of each plasmid was determined by comparison with plasmids of known molecular mass of *Escherichia coli* V517 (Macrina et al. 1978). Prior to amplification, chromosomal DNA was isolated by phenol-chloroform extraction as described by Wilson (1989).

2.4. RAPD-PCR amplification

In preliminary experiments with a subset of 5 isolates, ten 10-mer arbitrary primers obtained from Genosys Biotechnologies, Inc. were screened. Based on the fingerprint clarity and discrimination obtained, primer GEN2-60-09 (5′-CCTCATGACC-3′) was used for RAPD-PCR analysis of all 35 *B. pseudomallei* isolates. PCR mixtures (final volume 25 μl) contained 20–30 ng of purified DNA, 2 μM primer, 1 mM (final conc.) each of dNTP, 2.5 mM $MgCl_2$, one unit *Taq* DNA polymerase (Promega). PCR conditions were as follows: 30 cycles of 1 min at 94°C, 1 min at 52°C and 2 min at 72°C. A final elongation step at 72°C for 5 min was included. The PCR amplification products were visualized by running 10 μl of the reaction on a 1.2% agarose gel and detected by staining with ethidium bromide. DNA ladder was used as DNA size markers. RAPD-PCR gel photographs were scanned with Gel Compar Ver. 4.1 image analysis software (Applied Maths, Kortrijk, Belgium). The images were calibrated, and the presence or absence of bands was recorded, generating a matrix of similarity coefficients. Dendrogram based on the similarity coefficients was then generated using mathematic averages (UPGMA) cluster analysis.

3. Results and discussion

Table 1 showed the 35 *B. pseudomallei* isolates examined in this study. For the 35 isolates, duplicate culturing and determination of susceptibility gave reproducible inhibition diameters. All the *B. pseudomallei* were resistant to six or more of the 12 antimicrobial agents tested. This investigation suggests that the resistance of the *B. pseudomallei* isolates from the different sources may well reflects the extent of antibiotic usage in the study area. The development of antimicrobial resistance among these *B. pseudomallei* isolates may have consequences for the treatment of infection in humans due to relapse after cessation of therapy (Silbermann et al. 1997). Only two clinical isolates carried plasmids of approximately 1.8 and 2.4 megadalton (MDa) as determined by relative mobility in agarose gels (data not shown).

It is increasingly common to use molecular techniques

Table 1
Burkholderia pseudomallei Isolates Examined in this Study

Strain[a,b]	Source	Antimicrobial resistance[c]	Subtype as determined by RAPD type with primer GEN2-60-09[d]
T1	Human	BErGmKfKmSm	1 (1)
T2	Human	ApBCroErGmKfKmSmTe	2 (1)
T3	Human	ApBErGmKfKmSm	3 (1)
T4	Human	ApBErGmKfKmNaSmTe	4 (2)
T5	Human	ApBCarCroErGmKfKmNaSmTe	5 (1)
T6	Human	ApBErGmKfKmNaSm	6 (1)
T7	Human	ApBCroErGmKfKmSm	7 (1)
T8	Human	ApBCroErGmKfKmNaSmTe	8 (1)
T9	Human	ApBCroErGmKfKmSmTe	9 (1)
T10	Human	ApBCarCroCmErGmKfKmNaSmTe	10 (1)
T11	Human	ApBErGmKfKmSmTe	11 (1)
T12	Human	ApBCroErGmKfKmSmTe	12 (1)
T13	Human	ApBCroErGmKfKmNaSmTe	13 (1)
T14	Human	ApBErGmKfKmSmTe	14 (1)
T15	Human	ApBErGmKfKmSmTe	15 (2)
T16	Human	ApBCroErGmKfKmSmTe	16 (1)
T17	Human	ApBCroErGmKfKmSmTe	17 (1)
T18	Human	ApBErGmKfSm	18 (1)
T19	Soil	ApBErGmKfKmSm	19 (1)
T20	Soil	ApBCroErGmKfKmSmTe	20 (1)
T21	Soil	ApBCarCroErGmKfKmSmTe	21 (1)
T22	Soil	ApBCroErGmKfKmNaSmTe	22 (1)
T23	Soil	ApBCarCroErGmKfKmSm	23 (1)
T24	Soil	ApBCroErGmKfKmNaSm	24 (1)
M25	Sheep	BCroErGmKfSm	25 (1)
M26	Sheep	ApBCroErGmKfSm	26 (1)
M27	Human	ApBCroErGmKfKmSmTe	27 (1)
M28	Human	ApBCroErGmKfSm	28 (1)
M29	Goat	ApBCroErGmKfSm	29 (1)
M30	Soil	ApBCroErGmKfKmSm	30 (s)
M31	Soil	ApBErGmKfKmSm	31 (1)
M32	Cat	ApBErGmKfKmSm	32 (1)
M33	Cat	ApBCroErGmKfKmSm	33 (1)
M34	Cat	ApBCroErGmKfKmSm	34 (1)
M35	Deer	ApBCroErGmKfSm	35 (1)

[a] T designates isolates from Thailand and M designates isolates from Malaysia.

[b] Isolates T10 and T11 contained two plasmids each of 1.8 and 2.4 megadalton. All the other isolates were plasmidless.

[c] Tested for ampicillin (Ap), bacitracin (B), carbenicillin (Car), ceftriaxone (Cro), cephalothin (Kf), chloramphenicol (Cm), erythromycin (Er), gentamicin (Gm), kanamycin (Km), nalidixic acid (Na), streptomycin (Sm) and tetracycline (Te).

[d] Number 1 and 2 in parenthesis indicates cluster groups and (s) denotes the single isolate.