GENOTYPIC AND PHENOTYPIC RELATIONSHIP IN BURKHOLDERIA PSEUDOMALLEI INDICATES COLONIZATION WITH CLOSELY RELATED ISOLATES

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Abstract. Seven isolates of Burkholderia pseudomallei from cases of melioidosis in human (2 isolates) and animal (2 isolates), cat (one isolate) and from soil samples (2 isolates) were examined for in vitro sensitivity to 14 antimicrobial agents and for presence of plasmid DNA. Randomly amplified polymorphic DNA (RAPD) analysis was used to type the isolates, using two arbitrary primers. All isolates were sensitive to chloramphenicol, kanamycin, carbenicillin, rifampicin, enrofloxacin, tetracycline and sulfamethoxazole-trimethoprim. No plasmid was detected in all the isolates tested. RAPD fingerprinting demonstrated genomic relationship between isolates, which provides an effective method to study the epidemiology of the isolates examined.

INTRODUCTION

Burkholderia pseudomallei (previously known as Pseudomonas pseudomallei), is the causative agent of melioidosis in humans and a large variety of animals (Supputtamongkol et al, 1994). The major areas where melioidosis is endemic are Southeast Asia and Northern Australia (Dance, 1991). In Malaysia the disease has been reported in humans and animals (Embi et al, 1992; Idris et al, 1998; Heng et al, 1998). The spectrum of melioidosis ranges from subclinical disease to chronic pulmonary infection to fulminant septicemia with metastatic abscesses. Improve therapy has decrease the mortality in severe melioidosis from 80% to 40-50% (Supputtamongkol et al, 1994). The epidemiology and pathogenesis of melioidosis remain unclear. Because of the lack of a sensitive enough method that allows two isolates to be defined as identical strains, no correlation between isolates of soil, human and animal sources has been proven (Lew and Desmarchelier, 1993). Randomly amplified polymorphic DNA (RAPD) analysis is a PCR-based method using a single short random primer. This method is now widely used for the study of population genetics in a large variety of species (Kersulyte et al, 1992; Haase et al, 1995). In the work reported here, B. pseudomallei isolated from human, animals and soil were examined for their antibiotic resistance, plasmid profiles and random amplification of polymorphic DNA analysis.

MATERIALS AND METHODS

Bacterial strains

The seven strains of B. pseudomallei had been identified by the forwarding laboratory (Regional Veterinary Laboratory, Kuantan, Pahang) by standard tests. In this case study, two isolates each were isolated from a child admitted to the hospital with melioidosis, a dead goat reared by the child’s family and soil samples from the surrounding compound of the family’s house in a rural village. A single unrelated isolate from a cat was also included.

Sensitivity to antimicrobial agents

Antimicrobial sensitivity tests were performed by the disc diffusion method according to National Committee for Clinical Laboratory Standard (1997). The B. pseudomallei strains were tested against the following antibiotics discs (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) on Mueller Hinton agar: ampicillin (10 µg), carbenicillin (100 µg), cephalxin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), enrofloxacin (5 µg), kanamycin (30 µg), latamoxef (30 µg), furazolidone (100 µg), penicillin (10 µg), rifampicin (30 µg), streptomycin (15 µg), sulfamethoxazole-trimethoprim