ISOLATION RATES OF *BURKHOLDERIA PSEUDOMALLEI* AMONG THE FOUR REGIONS IN THAILAND

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Abstract. This study aimed to compare the isolation rates of *Burkholderia pseudomallei* among community-based hospitals located in the central, north, northeast, and south of Thailand. A questionnaire inquiring about the number of isolation of *B. pseudomallei* from various clinical specimens during 1994-95 were mailed to 141 community-based hospitals. Of these, 125 hospitals (88.6%) responded to the questionnaire. Microbiological laboratory was not available in thirty hospitals. Data from 95 remaining hospitals with capability to do bacterial culture showed that *B. pseudomallei* was never isolated in 49 hospitals. Eleven, 9, 19 and 7 hospitals where *B. pseudomallei* has been isolated, are located in the central, north, northeast and south of Thailand respectively. From these 46 hospitals, a total of 1,131 strains of *B. pseudomallei* were isolated from 407,263 specimens in 1994 and 1,165 strains from 440,541 specimens in 1995. However, the isolation was most frequent in northeastern hospitals, which accounted for 890 and 964 strains in 1994 and 1995 respectively while only 94, 76, 71 and 83, 75, 43 strains were simultaneously isolated during the 2-year period in those located in central, north and south respectively. The isolation rates of *B. speudomallei* in 1994 and 1995 were 4.2 and 4.1 per 1,000 clinical specimens in northeastern hospitals as compared to 1.1, 1.8, 1.1 and 1.1, 1.2, 0.7 in those located in central, north and south respectively. Ubon Ratchathani, Nakhon Ratchasima, Buri Ram, Khon Kaen and Udon Thani were the five provinces which exhibited the highest isolation rates as follows; 244, 150, 147, 127, 100 and 218, 128, 114, 119, 58, in 1994 and 1995, respectively. It was concluded that *B.pseudomallei* was most commonly isolated in the northeast of Thailand. Under-recognition of *B. pseudomallei* may prevail not only in other parts of Thailand but in some areas of the northeast as well.

INTRODUCTION

Melioidosis is largely restricted to certain geographical areas. The main endemic areas are located betweeen latitudes 20°N and 20°S; in Southeast Asia, particularly Thailand, Malaysia, Singapore and Vietnam, and northern Australia. It had long been considered to be a rare disease in Thailand until twenty years ago when ten cases with culture-proven melioidosis were reported by Sompone Punyagupta and his associates (1976) at a meeting of the Infectious Disease Group of Thailand. Since then workshop on melioidosis and interhospital case conference organized by Infectious Disease Group of Thailand in collaboration with university hospitals in Bangkok has increased the awareness of young physicians and laboratory personnel to look for melioidosis. By 1985, 795 cases had been identified at the national workshop on melioidosis and extensive studies of melioidosis in various aspects has initiated since then (Punyagupta, 1989). By observation, the isolation of *B.pseudomallei* in Thailand is surprisingly restricted to some provinces in the northeast, namely Khon Kaen and Ubon Ratchathani provinces. There are 100-150 melioidosis cases diagnosed at Sapprasitprasong Hospital in Ubon Ratchathani province and at Khon Kaen provincial hospital in Khon Kaen each year. The incidence of clinical melioidosis in these areas were calculated to be 22.5 to 26.0 cases per 10,000 hospital admissions during 1983 to 1985 (Sookpranee *et al*, 1989) and

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3.6-5.5 cases per 100,000 population in Ubon Ratchathani during 1987-1991 (Suputtamongkol *et al*, 1994) However, only 1-10 cases were reported from a nearby province and none from many hospitals located elsewhere in the country and even from some hospitals in the northeast. It is not known whether this reflects the unequal laboratory facilities and expertise of laboratory personnel among various hospitals or solely due to variation in geographical distribution of the micro-organism.

At this stage, we aimed to obtain basic information about the isolation rates of *B. pseudomallei* from various clinical specimens at all community-based hospitals in Thailand. Data were analyzed by comparing the isolation rates among the four regions of the country. In addition, its frequency of isolation from blood was compared to those of *S.aureus* and *E.coli* both of which were easily isolated from blood in microbiological laboratories of any hospitals in Thailand.

MATERIAL AND METHODS

Questionnaires were mailed to directors of all 141 community-based hospitals in Thailand. Hospitals located in Bangkok, private hospitals and university hospitals were not included in the mailing list. The first mailing was done in February 1996 and follow-up letters were set twice at the most to those who did not respond in one month after delivery. The questionnaires inquired about availability of microbiological laboratory, number of various clinical specimens received for culture each year during 1994-1995, the frequency of isolation of *B.pseudomallei* from various types of clinical specimens during the period and numbers of isolation of *E.coli* and *S.aureus* from blood only. Data were collected and analyzed using SPSS software version 6.0 on Windows version 3.11.

RESULTS

Of 141 hospitals in the mailing list, 125 hospitals (88.6%) participated in the study. Microbiological laboratory service was not available in 30 hospitals. Data from the remaining 95 hospitals with capability to do bacterial culture further revealed that *B. pseudomallei* was never reported in 49 of these hospitals. Thus data from only 46 hospitals remained in the analysis. Of 46 hospitals where *B. pseudomallei* had been isolated, nineteen hospitals (41%) were located in the northeast, while other 11 (24%), 9 (20%), and 7 (15%) hospitals were located in the central, north and south of Thailand, respectively. Only 4 hospitals (8%) in the northeast region were unable to isolate the micro-organism during the study period. Details of types of hospitals classified by availability of microbiological laboratory facilities and ability to isolate *B. pseudomallei* are shown in Table 1. From these

Table 1

Types of hospital classified by availability of microbiological laboratory and isolation of *B. pseudomallei*.

Region	No. of hospitals with microbiological laboratory		
	Available		Not available
	B. pseudomallei isolated	*B. pseudomallei* never isolated	
Central (n=41)	11 (24%)	22	8
North (n=29)	9 (20%)	15	5
Northeast (n=38)	19 (41%)	4 (8%)	15
South (n=17)	7 (15%)	8	2
Total (n=125)	46 (100%)	49 (100%)	30

hospitals, a total of 1,131 strains of *B. pseudomallei* were isolated from 407,263 specimens in 1994 and 1,165 strains from 440,541 specimens in 1995. However, the isolation was most frequent again in the northeastern region and accounted for 890 and 964 strains in 1994 and 1995 respectively while only 94, 76, 71 and 83, 75, 43 strains were simultaneously isolated during the 2-year period in those located in central, north and south respectively. The isolation rates of *B. pseudomallei* in 1994 and 1995 were 4.2 and 4.1 per 1,000 clinical specimens in northeastern hospitals as compared to 1.1, 1.8, 1.1 and 1.1, 1.2, 0.7 in those located in central, north and south respectively. Ubon Ratchathani, Nakhon Ratchasima, Buri Ram, Khon Kaen and Udon Thani were the five provinces where highest numbers of *B. pseudomallei* isolates were discovered as follows: 244, 150, 147, 127, 100 and 218, 128, 114, 119, 58, in 1994 and 1995 respectively. When numbers and rates of isolation of *B. pseudomallei* were calculated by region and year, 4.2 and 4.1 isolates per 1,000 specimens were discovered from all specimens submitted to the laboratory for bacterial culture in the northeast and 0.7-1.8 per 1,000 specimens in other regions. Numbers of *B. pseudomallei* isolates from blood were overall slightly higher than those from non-blood specimens. Details of the overall isolation rates in each region are shown in Table 2. Isolation rates of *B. pseudomallei* per 1,000 specimens of blood, sputum, pus and urine by region and year are shown in Table 3. The hospitals in the northeast were seen to contribute to the highest yields especially when blood, sputum and pus specimens were taken into consideration in both years. Table 4 revealed the frequency of isolation of *B. pseudomallei* per 1,000 blood samples compared to *S. aureus* and *E. coli* by region and year. For blood culture in the northeast, the isolation of *S. aureus* and *E. coli* yielded approximately two times more frequently than those of *B. pseudomallei* while in other regions, three to five times more frequently. Although the overall isolation rates of *S. aureus* and *E. coli* from blood were higher than those of *B. Pseudomallei* in every regions, the latter was higher than the two former in some hospitals located in the northeast as showed in Tables 5 and 6. From these Tables, it could be seen that blood culture contributed to more than half of the isolations of *B. pseudomallei*, especially in the northeast region.

Table 2

Numbers and rates of isolation of *B. pseudomallei* from blood and non-blood specimens by region* and year.

Region	Year 1994			Year 1995		
	No. of isolation Blood: non-blood (total)	Total specimen	No. of isolation per 1,000 specimen	No. of isolation Blood: non-blood: (total)	Total specimen	No.of isolation per 1,000 specimen
Central	55:39 (94)	86,152	1.1	50:33 (83)	76,673	1.1
North	31:45 (76)	43,027	1.8	30:45 (75)	65,053	1.2
Northeast	433:457 (890)	212,656	4.2	498:466 (964)	236,626	4.1
South	55:16 (71)	65,428	1.1	19:24 (43)	62,189	0.7
Total	574:557 (1,131)	407,263		597:568 (1,165)	440,541	

*Only data from hospitals where *B. pseudomallei* was ever isolated, were analyzed.

Table 3

Isolation rates of *B.pseudomallei* per 1,000 clinical specimens of blood, sputm, pus and urine by region* and year.

Region	Blood		Sputum		Pus		Urine	
	1994	1995	1994	1995	1994	1995	1994	1995
Central	4.0	4.7	1.9	1.0	2.5	1.6	0.3	0.4
North	3.4	1.7	0.5	0.4	1.9	0.9	1.0	0.2
Northeast	10.6	8.8	6.2	6.6	11.7	8.0	0.6	1.2
South	1.9	1.0	0.6	0.7	0.7	1.8	0.1	0.1
Total	5.0	4.1	2.3	2.2	4.2	3.1	0.5	0.5

*Only data from hospitals where *B. pseudomallei* was ever isolated, were analyzed.

Table 4

Isolation rates of *B. pseudomallei,S. aureus* and *E. coli* per 1,000 specimens of blood by region* and year.

Region	*B.pseudomallei*		*S.aureus*		*E.coli*	
	1994	1995	1994	1995	1994	1995
Central	4.0	4.7	13.6	11.5	16.1	14.7
North	3.4	1.7	17.9	15.8	22.2	20.8
Northeast	10.6	8.8	26.1	15.6	21.8	23.0
South	1.9	1.0	14.7	14.8	9.5	10.8
Total	5.0	4.1	18.1	17.4	17.4	17.4

*Only data from hospitals where *B. pseudomallei* was ever isolated, were analyzed.

Table 5

Average numbers of isolation of *B. pseudomallei* per 100 strains of *E. coli* from blood culture by region*

Region	Year 1994			Year 1995		
	mean±SD	Range	95%CI	mean±SD	Range	95%CI
Central	10.5±9.8	0-27.3	3.0,18.0	22.3±18.3	3.7-66.7	8.2,36.4
North	18.0±12.6	0-33.3	4.9,31.2	18.0±33.5	0-100	-9.6,46.5
Northeast	67.5±64.6	0-250.0	31.7,103.3	88.3±138.8	3.7-600	16.9,159.7
South	20±15.8	0-47.6	6.3,35.6	8.9±11.4	0-33.3	-1.7,19.5

*Only data from hospitals where *B. pseudomallei* was ever isolated, were analyzed.

Table 6

Average numbers of isolation of *B. pseudomallei* per 100 strains of *S. aureus* from blood culture by region*.

Region	Year 1994			Year 1995		
	Mean±SD	Range	95%CI	Mean±SD	Range	95%CI
Central	21.5±25.4	0-81.8	5.3,42.0	20.9±12.8	4.8-46.7	11.1,30.8
North	30.1±34.5	0-100.0	-1.8,61.9	25.5±34.5	0-100	-3.3,54.3
Northeast	68.6±67.7	0-250.0	29.5,107.7	62.6±45.3	71-150.0	39.3,85.9
South	17.1±13.6	0-36.8	4.5,29.7	8.0±8.8	0-22.2	-0.1,16.1

DISCUSSION

The total numbers of isolates of *B. pseudomallei* from various specimens were 1,131 in 1994 and 1,165 in 1995. Under current circumstances, the annual report of culture-proven cases may be less than these figures because *B. pseudomallei* could be isolated from more than one source in case with disseminated septicemic melioidosis. However, positive isolation was reported from only 46 out of 141 community-based hospitals. It is difficult at this moment to estimate a number which is close to the true number of clinical cases each year. Among the hospitals with an available microbiological laboratory, 19 (83%) out of 23 hospitals in the northeast reported the isolation of *B. pseudomallei* while in other regions, approximately one-half of the hospitals did so. If another 15 hospitals in the northeast which lack facility for bacterial culture are able to set up their own microbiological laboratories, the number of cases reported may increase significantly each year in this region. Again, the highest average yields of isolation of *B. pseudomallei* from clinical specimens were seen in the northeastern hospitals which were about three times more often than in other regions. Our findings confirmed that the northeast region is currently endemic for clinical melioidosis and *B. pseudomallei* could be isolated from sputum and pus in the northeast in higher rates than other regions (Table 3). Since positive culture is usually obtained from cases with severe infection, data can be interpreted that clinical melioidosis is more common or more severe or both in the northeast. The former was confirmed by a recent study on the etiology of acute undifferentiated febrile illness in 1,218 patients from the four regions of Thailand. The result of serological study revealed a higher seropositivity rate for melioidosis in the patients from the northeast, *ie* 2.1% in the northeast versus 1.2, 1.1 and 1.3 % in the north, central and south respectively (unpublished data). The reason why melioidosis is common in the northeast needs explanation, though we can only hypothesize that differences in environment, virulence factors of the micro-organism, host immunity as well as the chance of exposure to *B. pseudomallei* may play roles in the endemicity and pathogenesis of this disease. Since a subclinical form of melioidosis also exists and affects antibody production against the micro-organism, the cut-off titer for any test that employs antibody level should be carefully selected if the test will be used in endemic area, to distinguish clinical melioidosis from subclinical or other infections. The cut-off titer in an endemic area may be different and higher than in other regions or countries where melioidosis is rare (Leelarasamee, 1985).

B. pseudomallei was commonly isolated from blood of patients with clinical sepsis. In this study, blood was the only source from which more than half of total number of *B. pseudomallei* was isolated during 1994-1995. The finding may signify that bacteremic melioidosis was more commonly encountered than its other forms in the central, northeast and southern regions, though less frequent in the north, or but regional practice variation in obtaining specimens from various organs may exist. Whether the lower ratio of bacteremic to non-bacteremic forms found in the north as compared to other regions suggest that strains of *B. pseudomallei* in the north were comparatively less invasive than the strains in other regions, needs to be elucidated by further study. Further comparative study of virulence factors and DNA or RNA typing of strains isolated from different regions might give a clue concerning the above hypothesis. In Table 3, the isolation yield per 1,000 blood specimens was still highest in the northeast and was calculated to be 8.8-10.6 per 1,000 blood specimens compared to 1.0-4.7 in other regions. When the isolation rates of *S. aureus* and *E. coli* from blood were also taken into account as controls for laboratory usage by physicians and laboratories' capability to perform bacterial culture and isolation, it was found that bacteremic melioidosis was 3-5 times more common in the northeast than in the north while frequency of bacteremia due to *S. aureus* and *E. coli* was comparable in both regions. It was also 6-8 times more common in the northeast than in the south while bacteremia due to *S. aureus* and *E. coli* was about 2 times more common by the same comparison. Thus bacteremic melioidosis was about 3-5 times on average more common in the northeast than in other regions of Thailand. Recent study on the etiology of acute unexplained fever in 1,214 cases indicated that *B. pseudomallei* was isolated from blood in only one case but positive serology for melioidosis was detected by indirect hemagglutination test in 11 cases. By estimation, with 574 strains isolated from blood in 1994 in this study, there would be an additional 6,314 cases left undetected if serological testing was not performed in patients with undifferentiated febrile illness.

Though bacteremia due to *S. aureus* and *E. coli* were on average more common than *B. pseudomallei,* as seen in Table 4, it could be seen from Tables 5 and 6 that bacteremic melioidosis may be found 2.5-6.0 and 1.5-2.5 times more frequently than *E. coli* and *S. aureus* septicemia in some individual hospitals in the northeast. This finding supports the observation that melioidosis is very common in certain areas in the northeast and empirical antimicrobial therapy for patients with septicemia should include antimicrobials which are effective against *B. pseudomallei.* In this survey, Ubon Ratchatani, Nakhon Ratchasima, Buri Ram, Khon Kaen and Udon Thani were the five provinces where the highest numbers of *B. pseudomallei* isolates were reported as follows; 244, 150, 147, 127, 100 and 218, 128, 114, 119, 58 in 1994 and 1995 respectively. In one hospital located in the northeast, melioidosis accounted for 19% of hospital admissions and 40% of deaths from community-acquired septicemia in a year in one hospital in the northeast (Chaowagul *et al*, 1989). There is the possibility that and outbreak was confined to these areas as reported in Australia (Currie *et al*, 1993). However, the difference of isolation rate among the hospitals in the northeast may just reflect the unequal facilities and expertise between these and other provinces. The latter hypothesis was substantiated by geographical distribution of the positive isolation which showed that some hospitals located in a province reported a negative isolation while another hospital located in the same or adjacent province reported abundant isolation of *B. pseudomallei.* In 1979 Ashdown described a selective medium containing gentamicin and crystal violet which is recommended for the isolation of the organism from contaminated specimens (Ashdown, 1979). Recently, a latex agglutination method has been proposed for the identification of suspect colonies (Smith, 1993) since it can be misidentified as *B. mallei. B. cepacia. P. stutzeri* or *Flavobacteria* spp. Isolates may generally be identified by a kit system such as API 20 NE (Dance, 1989). The development of improved methods of isolation and identification of *B. pseudomallei* will result in a better estimation of total cases with culture-proven melioidosis in Thailand, as occurred in Australia (Ashdown, 1991) and Singapore (Tan, 1990).

Finally, we realize that the reported cases of culture-proven melioidosis in this survey may be only the tip of the iceberg due to the uneven distribution of appropriate diagnostic facilities. Though developing an overall picture remains an elusive undertaking, we urgently need basic data about the actual number of isolations from various specimens in various regions in Thailand. Serosurveillance should be simultaneously done with appropriate technique. Data from the two methods should yield the best estimate of true picture and spectrum of the disease. Then ribotyping may be used to see if a particular strain exists in a certain area, which would assist in delineation of endemicity and pathogenicity. We believe that with greater awareness and better diagnostic capabilities, recognition of the number of people afflicted with *B. pseudomallei* will increase dramatically in many areas of Thailand.

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