

# Epidemiological Surveillance of Melioidosis in Singapore

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## Abstract

During the period 1989 to 1996, a total of 372 cases of melioidosis, with 147 deaths, were reported, giving a mean annual incidence rate of 1.7 per 100 000 population and a case-fatality rate of 39.5%. Majority (89%) of the clinical cases were confirmed by culture of *Burkholderia pseudomallei*, while the others were presumptive cases based on a single blood specimen with an indirect haemagglutination (IHA) antibody titre of  $\geq 1:16$ . The highest incidence rate was reported in those aged 45 years and above (5.7 per 100 000 population), males (2.8 per 100 000 population), and Indian ethnic group (3.0 per 100 000 population). Cases were distributed throughout the island all year round. There was no correlation with rainfall. Most of the cases (77.4%) had other concurrent medical conditions, the most common being diabetes mellitus (57.5%). Factors significantly associated with a higher case-fatality rate were age (55 years and above), septicaemia, smoking history and heart or renal failure. The overall case-fatality rate has been declining from 60% in 1989 to 27% in 1996 due to a greater awareness among medical practitioners to diagnose and treat the disease early. The overall seroprevalence of IHA antibody (titre of  $\geq 1:16$ ) among asymptomatic population groups was 0.2%. *B. pseudomallei* isolated from clinical specimens were sensitive to imipenem (100%), ceftazidime (99.1%), piperacillin (99.7%), ampicillin-clavulanate (98.5%), minocycline (97.4%), chloramphenicol (94.3%), doxycycline (94.3%) and tetracycline (93.9%). Of 395 samples of soil collected during epidemiological investigation of reported cases, 1.8% were positive for *B. pseudomallei*.

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**Key words:** Antibiotic sensitivity, *Burkholderia pseudomallei*, Cutaneous abscess, Septicaemia, Seroprevalence

## Introduction

Melioidosis was first described in 1911 among va-grants and morphine addicts brought into the mortuary in Rangoon, Burma.<sup>1</sup> However, the disease received little attention until the Vietnam War when French<sup>2</sup> and US<sup>3,4</sup> military personnel were affected by it. The disease is found predominantly in Southeast Asia and northern Australia.<sup>5-8</sup> It is highly endemic in Thailand, especially the north-eastern part,<sup>9,10</sup> and has been reported in Malaysia,<sup>11</sup> Hong Kong,<sup>12,13</sup> Taiwan,<sup>14</sup> Indian subcontinent,<sup>15,16</sup> Africa,<sup>17,18</sup> Central and South America,<sup>19</sup> Papua New Guinea,<sup>20,21</sup> Guam<sup>22</sup> and New Zealand.<sup>23</sup> The disease is rare in Europe and North America where virtually all the cases reported were soldiers<sup>3,4,24</sup> or travellers<sup>25-28</sup> who have resided in Southeast Asian countries or immigrants from endemic countries.<sup>29</sup>

The disease may manifest as an acute, subacute or chronic form. The acute form presents as pneumonia or fulminant septicaemia, both associated with a high case-fatality rate, or as localised abscess. The subacute or chronic form may last for months or years affecting many organs.

Although the first case of melioidosis in Singapore was reported in 1920,<sup>30</sup> it was not recognised as an important public health problem until three fatal septicaemic cases among apparently healthy young adults were reported in 1989. The disease was made administratively notifiable in October 1989, and guidelines on the clinical diagnosis, laboratory investigation and clinical management of the disease were circulated to all medical practitioners. The findings of our epidemiological investigations of melio-

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idosis for the period 1989 to 1996 are described in this paper.

## Materials and Methods

### *Epidemiological Investigation*

For every case of melioidosis notified to the Quarantine & Epidemiology Department, Ministry of the Environment, a thorough epidemiological investigation was carried out by a trained environmental health officer using a standard questionnaire form. The investigation entailed an interview with the patient, or immediate family members (if the patient had died), and a review of the hospital records. The information collected included demographic profile of the patient (age, sex, ethnic group and occupation), residential address, place of work, date of onset of illness, signs and symptoms, presence of other concurrent medical conditions, and results of laboratory and radiographic investigations. To determine the possible sources of infection and mode of transmission, enquiries were also made regarding exposure to soil and surface water (as in physical training, playing games, fishing, trekking, gardening and camping), contact with pets, and visits to construction sites, zoo, park, jungle, and other places.

Clinical specimens such as blood, pus, sputum and respiratory aspirate were submitted by the attending physicians to various clinical laboratories in the government, restructured, private and university hospitals for isolation by culture of *Burkholderia pseudomallei*,<sup>31</sup> and if isolated, they were tested for antibiotic sensitivity.<sup>32</sup> In some cases, serological test was also carried out. It was based on the indirect haemagglutination (IHA) test using melioidin, an extract of *B. pseudomallei* developed by the Department of Microbiology, National University of Singapore (NUS). A clinical case in which *B. pseudomallei* was not isolated, but the IHA antibody showed a titre of  $\geq 1:16$  was considered a presumptive case. The cut-off titre of 1:16 was used as Yap and colleagues<sup>33</sup> have shown that culture-positive cases in Singapore had titres ranging from 16 to 32 768, while the majority of healthy population had titres of  $< 16$ .

### *Seroprevalence Surveys*

To determine the actual extent of infection with *B. pseudomallei*, seroepidemiological surveys were carried out in selected population groups e.g. construction workers, dumping ground workers and zoo keepers. About 2 ml of blood was taken from each volunteer after consent had been obtained. At the end of each day's collection, the sera were separated, stored in polypropylene tubes at  $-20^{\circ}\text{C}$  and tested in batches at the Department of Microbiology, NUS, by the IHA test. An IHA antibody titre of  $\geq 1:16$  was considered seropositive.

### *Surveillance of B. pseudomallei in the Environment*

Soil and water samples were routinely taken from various localities where cases had sustained injuries or had direct contact prior to their onset of illness. The samples were sent to the Central Veterinary Laboratory, Primary Production Department for analysis using the enriched trypticase soy broth incorporating 5 mg of crystal violet per litre and 20 mg of colistin per litre and subcultured to Ashdown medium.<sup>34</sup> The genotype of *B. pseudomallei* isolated from the environment and clinical specimens were further investigated using restriction endonuclease analysis by pulsed-field gel electrophoresis (REA-PFGE)<sup>35</sup> at the Department of Microbiology, NUS.

### *Data Analysis*

All clinical, epidemiological and laboratory data collected were analysed using the microcomputer-based Microsoft Excel 7.0, Epistat Statistical Package and Statistical Package for Social Science (SPSS). Differences in proportions observed were initially compared by  $\chi^2$  test using Yates' continuity correction, and probability (*P*) values less than 0.05 were considered significant. To determine factors significantly associated with case-fatality, after adjusting for possible confounders, a stepwise logistic regression analysis was used.

## Results

### *Epidemiology*

Between 1989 and 1996, a total of 372 cases of melioidosis were reported, giving a mean annual rate of 1.7 per 100 000 population (range 1.0 to 3.0 per 100 000 population). Of these, 331 (89%) cases were confirmed by culture of *B. pseudomallei* from clinical specimens, while 41 (11%) cases were presumptive (Table I).

The ages of the reported cases ranged from 2 to 91 years (mean 53.1 years; median 55 years), with the highest incidence rate among persons aged 45 years and above (5.7 per 100 000 population). The male to female ratio was 4.5:1. Among the three major ethnic groups, Indians had the highest mean annual incidence rate (3.0 per 100 000 population) compared with Malays (2.7 per 100 000 population) and Chinese (1.3 per 100 000 population) (Table II). Based on the analysis of 160 cases reported in the period 1995 to 1996 by occupational group, over a quarter (26.9%) were cleaners, labourers and related workers, and drivers.

Cases occurred singly and sporadically in all parts of the island all the year round. A discernible peak was noted in July 1995. There was no correlation between disease incidence and rainfall. There was also no difference in incidence between flat dwellers and residents of landed property.

More than three-quarters (77.4%) of the cases had

TABLE I: METHOD OF DIAGNOSIS OF 372 REPORTED CASES OF MELIOIDOSIS IN SINGAPORE, 1989 TO 1996

Diagnostic method	No. of cases	%
<b>Culture only</b>	<b>259</b>	<b>69.6</b>
blood	145	39.0
pus	46	12.4
sputum/respiratory aspirate	22	5.9
blood and sputum/respiratory aspirate	18	4.8
blood and pus	5	1.3
blood, respiratory aspirate and pus	1	0.3
urine and others*	10	2.7
cerebrospinal fluid and others	2	0.5
joint aspirate and others	2	0.5
gastric fluid and others	1	0.3
pericardial fluid	1	0.3
peritoneal fluid	1	0.3
tissue (lung, liver, joint, throat )	5	1.3
<b>Serology# only</b>	<b>41</b>	<b>11.0</b>
<b>Both serology# and culture</b>	<b>72</b>	<b>19.4</b>
Total	372	100

\* Blood, pus or sputum/respiratory aspirate

# IHA antibody titre  $\geq 1:16$ 

TABLE III: FREQUENCY AND CASE-FATALITY RATE OF SPECIFIC CONCURRENT MEDICAL CONDITIONS\* OF 372 REPORTED CASES OF MELIOIDOSIS, 1989 TO 1996

Medical condition	Case n = 372		Death n = 147	
	No.	(%)	No.	Case-fatality rate (%)
Any concurrent medical condition	288	(77.4)	119	(41.3)
Diabetes mellitus	214	(57.5)	78	(36.4)
Diseases of the circulatory system				
- hypertensive disease	58	(15.6)	25	(43.1)
- ischaemic heart disease	39	(10.5)	24	(61.5)
- heart failure	9	(2.4)	8	(88.9)
Diseases of the respiratory system				
- asthma	23	(6.2)	9	(39.1)
- chronic obstructive pulmonary disease	14	(3.8)	7	(50.0)
Pulmonary tuberculosis	27	(7.3)	10	(37.0)
Chronic renal failure	21	(5.6)	17	(81.0)
Neoplasms	17	(4.6)	9	(52.9)

\* Cases may have more than one concurrent medical conditions

other concurrent medical conditions, the most common being diabetes mellitus (57.5%) (Table III).

The main clinical presentations were fever and respiratory symptoms (cough, dyspnoea and chest pain/discomfort). Other clinical presentations include gastrointestinal and urinary symptoms (Table IV). It is noted that, generally, there has been a decrease in the proportion of cases presenting with septicaemia from 90.9% in 1990 to 42.9% in 1996, while there has been a

TABLE II: MEAN ANNUAL INCIDENCE AND CASE-FATALITY RATES OF 372 REPORTED CASES OF MELIOIDOSIS BY GENDER, AGE AND ETHNICITY IN SINGAPORE, 1989 TO 1996

Characteristic	No. of cases	Mean annual rate per 100 000*	No. of deaths	Case-fatality rate (%)
All	372	1.73	147	39.5
Gender				
Male	305	2.80	117	38.4
Female	67	0.63	30	44.8
Age group (y)				
0-4	2	0.11	0	0
5-14	7	0.22	0	0
15-24	27	0.75	2	7.4
25-34	27	0.59	7	25.9
35-44	43	1.18	13	30.2
45-54	80	4.09	30	37.5
55-64	86	5.91	38	44.2
65+	100	7.71	57	57.0
Ethnic group				
Chinese	214	1.28	90	42.1
Malay	83	2.73	33	39.8
Indian	45	2.95	13	28.9
Others	7	2.99	5	71.4
Foreigner	23	-	6	26.1

\* Based on 1990 census population

TABLE IV: CLINICAL PRESENTATION\* OF 372 REPORTED CASES OF MELIOIDOSIS, 1989 TO 1996

Clinical presentation	No.	%
Fever (with/without chills and rigors)	293	78.8
Respiratory symptoms		
- cough	177	47.6
- dyspnoea	94	25.3
- chest pain/discomfort	62	16.7
Gastrointestinal symptoms		
- abdominal pain/discomfort	38	10.2
- vomiting	19	5.1
- diarrhoea	17	4.6
Urinary symptoms		
- dysuria, haematuria	13	3.5
Cutaneous abscess	65	17.5

\* Cases may have more than one clinical presentations

corresponding increase in the proportion of cases presenting with cutaneous infection from 13.6% to 24.3% (Table V). Cases presenting with septicaemia were significantly older (mean 58 years; median 59 years) than those presenting with cutaneous infection (mean 36.4 years; median 33.5 years) ( $P < 0.0001$ ).

#### Case Fatality

A total of 147 cases died, giving an overall case-fatality rate of 39.5%. The annual case-fatality rate declined

from 60% in 1989 to 27.1% in 1996. The overall case-fatality rate of those with septicaemia (55.4%; range 44% to 100%), was significantly higher than that among those with cutaneous infection (4.6%; range 0% to 66.7%) ( $P < 0.0001$ ) (Table V). Factors significantly associated with a higher case-fatality rate, by univariate analysis, were age  $\geq 55$  years (51.1%), septicaemia (55.4%), smoking history (45.3%) and heart or chronic renal failure (84.8%) (Table VI). Using stepwise logistic regression analysis where the four covariates were included into the model, the adjusted case-fatality rate was significantly associated with septicaemia ( $P < 0.0001$ ), heart or renal failure ( $P = 0.0005$ ) and smoking history ( $P = 0.02$ ).

#### Antibiotic Sensitivity of Clinical Isolates of *B. pseudomallei*

*B. pseudomallei* isolated from clinical specimens were sensitive to imipenem (100%), ceftazidime (99.1%), piperacillin (99.7%), ampicillin-clavulanate (98.5%), minocycline (97.4%), chloramphenicol (94.3%), doxycycline (94.3%) and tetracycline (93.9%). However, only 9.5% were sensitive to co-trimoxazole, 5.3% to amikacin, and 2.1% to gentamicin. All were resistant to ampicillin and cephalixin (Table VII).

#### Seroprevalence

A total of 1854 blood specimens were collected from selected population groups and tested for IHA antibody

TABLE V: FREQUENCY AND CASE-FATALITY RATES OF REPORTED MELIOIDOSIS CASES PRESENTING WITH SEPTICAEMIA\* AND CUTANEOUS INFECTION,\*\* 1989 TO 1996

Year	All cases			Cases with septicaemia			Cases with cutaneous infection		
	No. of cases	No. of deaths	Case-fatality rate (%)	No. of cases (%)	No. of deaths	Case-fatality rate (%)	No. of cases (%)	No. of deaths	Case-fatality rate (%)
1989	5	3	60.0	3 (60.0)	3	100	0 (0)	-	-
1990	22	12	54.5	20 (90.9)	10	50.0	3 (13.6)	2	66.7
1991	43	20	46.5	29 (67.4)	17	58.6	7 (16.3)	0	0
1992	46	24	52.2	25 (54.3)	18	72.0	6 (13.0)	0	0
1993	56	29	51.8	40 (71.4)	26	65.0	10 (17.9)	1	10.0
1994	40	12	30.0	25 (62.5)	11	44.0	9 (22.5)	0	0
1995	90	28	31.1	50 (55.6)	23	46.0	13 (14.4)	0	0
1996	70	19	27.1	30 (42.9)	15	50.0	17 (24.3)	0	0
Total	372	147	39.5	222 (59.7)	123	55.4 <sup>a</sup>	65 (17.7)	3	4.6 <sup>b</sup>

\* Confirmed by blood culture

\*\* Including cases with other clinical presentations

a versus b:  $P < 0.0001$ ; odds ratio, 25.7; 95% confidence interval, 7.5 to 62.8

TABLE VI: FACTORS ASSOCIATED\* WITH CASE-FATALITY OF 372 REPORTED CASES OF MELIOIDOSIS, 1989 TO 1996

	Case	Death	Case-fatality rate (%)	Odds ratio	(95% CI)	<i>P</i> value
All	372	147	39.5	-	-	-
Age						
<55 years	186	52	28.0	1		
55+ years	186	95	51.1	2.7	1.7 - 4.2	<0.0001
Septicaemia						
present	222	123	55.4	6.5	3.8 - 11.2	<0.0001
absent	150	24	16.0	1		
Smoking history						
yes <sup>®</sup>	245	111	45.3	2.1	1.3 - 3.4	0.002
no	127	36	28.3	1		
Concurrent medical condition						
present	288	119	41.3	1.4	0.8 - 2.4	ns
absent	84	28	33.3	1		
Heart or chronic renal failure						
yes	30	25	83.3	9.0	3.2 - 27.6	<0.0001
no	342	122	35.7	1		

\*  $\chi^2$  test using Yates' continuity correction

<sup>®</sup> Includes smokers and ex-smokers

CI: confidence interval; ns: not significant

against *B. pseudomallei*. All the subjects were asymptomatic and apparently well at the time of blood collection. The antibody prevalence of local construction workers (1.6%) was not significantly higher than the overall prevalence (0.2%). However, the antibody prevalence of foreign construction workers (28.3%) was found to be significantly higher than that of local construction workers ( $P < 0.0001$ ) (Table VIII). Further breakdown of these workers by various countries of domicile showed that the seroprevalence rates were high among those from Thailand (90/213 or 42.3%), the Indian subcontinent (13/120 or 10.8%) and Malaysia (2/31 or 6.5%).

#### Surveillance of the Environment

During the period 1992 to 1996, a total of 395 samples of soil and 46 samples of surface water were collected for isolation of *B. pseudomallei*. The organism was isolated from 7 (1.8%) of the soil samples obtained from 3 different sites implicated during epidemiological investigation. Using REA-PFGE, the genotype of *B. pseudomallei* isolated from 4 samples in 1 locality was similar to that isolated from the elbow abscess of an adult who had sustained an injury there. None of the water samples tested were positive for *B. pseudomallei*.

TABLE VII: ANTIBIOTIC SENSITIVITY PATTERN OF *BURKHOLDERIA PSEUDOMALLEI* ISOLATED FROM VARIOUS CLINICAL SPECIMENS, 1992 TO 1996

Antibiotic	Number sensitive/ Number tested	%
Cephalosporins		
Ceftazidime	316/319	99.1
Ceftriaxone	23/26	88.5
Cephalexin	0/28	0
Penicillins		
Piperacillin	306/307	99.7
Amoxicillin-clavulanate	133/135	98.5
Ampicillin-sulbactam	22/27	81.5
Ampicillin	0/209	0
Tetracyclines		
Tetracycline	246/262	93.9
Doxycycline	33/35	94.3
Minocycline	75/77	97.4
Quinolones		
Ciprofloxacin	8/40	20.0
Aminoglycosides		
Amikacin	13/245	5.3
Gentamicin	5/243	2.1
Netilmicin		
$\beta$ -lactams		
Imipenem	117/117	100
Aztreonam	5/13	38.5
Others		
Chloramphenicol	283/300	94.3
Co-trimoxazole	29/306	9.5

#### Discussion

Melioidosis is endemic in Singapore. As in other countries in the region,<sup>7,36-38</sup> the risk factors associated with the infection include male gender, older age group, concurrent medical illness (especially diabetes mellitus), and exposure to soil (construction workers). However, the seroprevalence *B. pseudomallei* infection (0.2%) is very low compared with Thailand (21% to 47%),<sup>39-42</sup> northern Queensland (7.8% to 29%),<sup>43</sup> Malaysia (1.9% to 15.8%),<sup>44</sup> Vietnam (6.4% to 31.8%)<sup>45</sup> and Hong Kong (14%).<sup>46</sup> *B. pseudomallei* is also extremely uncommon in the environment unlike in the 1960s when the bacterium was isolated in 5.9% of 136 samples of surface water collected from forest, roadside drains and sports fields.<sup>47</sup> In north-eastern Thailand, the bacterium was found in 68% of the 167 samples of soil,<sup>48</sup> while in southern Thailand, it was found in 60.9% of soil and surface water samples from rubber plantations, and 78.1% of bottom sediments of rice fields.<sup>49</sup> In West Malaysia, an isolation rate of 14.5% to 33.3% was reported in wet rice fields and fields of newly planted oil palm compared with 0.8% to 3.2% in surface water of forested lands.<sup>50</sup> Another study in Malaysia found 2.9% of 1120 water samples and 1.9% of 1078 soil samples positive for *B. pseudomallei*.<sup>51</sup> In China, 4.2% of 1366 samples of soil and water were positive for the bacteria.<sup>52</sup>

Despite Singapore's highly urbanised environment with 88% of the population living in high-rise flats, the incidence of melioidosis is relatively high (1.7 per 100 000 population) in comparison with other countries, e.g. Thailand (4.4 per 100 000 population).<sup>38</sup> The apparent increase in incidence of the disease in Singapore in

TABLE VIII: PREVALENCE OF INDIRECT HAEMAGGLUTINATION (IHA) ANTIBODY (TITRE OF  $\geq 1:16$ ) TO *BURKHOLDERIA PSEUDOMALLEI* IN SELECTED POPULATION GROUPS IN SINGAPORE, 1990 TO 1993

Population group	Number positive/ Number tested	%
Construction workers		
- local	2/217	1.6 <sup>a</sup>
- foreigners	105/371	28.3 <sup>b</sup>
Inmates of a home for the mentally disabled	2/500	0.4
Dumping ground workers	0/43	0
Blood donors	0/50	0
Cemetery workers	0/58	0
Sand quarry workers	0/79	0
Zoo keepers	0/149	0
Incineration plant workers	0/247	0
Sewage workers	0/601	0
Total of local population groups	4/1854	0.2 <sup>c</sup>

a versus c:  $P$  = not significant

b versus a:  $P < 0.0001$ ; odds ratio, 42.4;  
95% confidence interval, 10.2 to 104.0

recent years is attributed partly to the greater clinical awareness and improved diagnostic methods. However, it has been suggested that it could be due to excessive soil excavations creating aerosols and disseminating the bacteria into the air.<sup>53</sup>

The increase in the proportion of cutaneous infection was probably attributed to more cases of skin infection being sent for culture. The 3 fatal cases (2 in 1990 and 1 in 1993) presenting with cutaneous infection (mainly septic sores) and who later developed septicaemia, were either elderly (87 years old) or had concurrent debilitating illnesses (1 had diabetes mellitus and the other had both diabetes mellitus and chronic renal failure) that contributed to the progression of infection to fulminant septicaemia and death.

The source of infection could not be determined for most of the cases. In young healthy adults with localised cutaneous infections and abscesses,<sup>54</sup> there is often a history of occupational or recreational exposure to soil. The isolation of *B. pseudomallei* from the cutaneous abscess of one of the cases with the same genotype by REA-PFGE as that detected in soil provided convincing evidence that contact with contaminated soil in some localities could spread the infection. There have also been other incidents involving animals which succumbed to the disease through contact with contaminated soil. The higher seroprevalence among construction workers could be due to occupational exposure to contaminated soil and water in land development areas.

A greater awareness among the medical practitioners to diagnose and treat the disease early, resulted in a decline of the case-fatality rate from 60% in 1989 to 27% in 1996. As the disease often mimics other diseases, doctors should continue to bear a high index of suspicion and consider melioidosis in the differential diagnosis of a high risk patient who presents with a fever, localised abscess or a clinical picture of pulmonary cavitary tuberculosis.<sup>10,55,56</sup> Persons doing outdoor activities should be advised to take simple precautionary measures such as covering all open wounds with waterproof dressings, and wearing boots and gloves when in contact with water-logged soil.

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