SPECTRUM OF MELIOIDOSIS IN THE SUBURBS OF MANGALORE, S WEST COAST OF INDIA

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Abstract. Melioidosis is an emerging infection in India. Seventeen cases of culture proven melioidosis are reported in this study. The isolation rate was high during the rainy season. Except one case, all the patients had diabetes mellitus as an underlying disease. Eleven patients improved with ceftazidime or combination therapy and maintenance therapy with doxycycline and Cotrimoxazole. The high prevalence of *B. pseudomallei* in this region is a matter for serious concern.

Key words: *Burkholderia pseudomallei,* melioidosis

INTRODUCTION

Burkholderia pseudomallei, the causative agent of melioidosis was first reported by Captain Alfred Whitmore and his assistant Krishnaswami at Rangoon General Hospital, Burma in 1911 (White, 2003). The name melioidosis is derived from the Greek word 'melis', meaning 'distemper of asses' and 'edios', meaning 'resemble' (Raja *et al*, 2005). Melioidosis is widespread in Southeast Asian countries and northern Australia. The clinical presentation varies from a short febrile illness to prolonged fever with or without lymphadenitis, mimicking tuberculosis. It causes localized acute or chronic suppurative infections in internal organs leading to fatal septicaemia (White, 2003) *B. pseudomallei* is a saprophyte in the soil, stagnant water, rice farming areas, rubber plantations, irrigated agricultural sites, drains and ditches (Raja *et al*, 2005). It has also emerged as a major veterinary pathogen. *B. pseudomallei* has been described as a 'Vietnamese time bomb' and is considered as a potential biological weapon (White, 2003). The name of this organism has undergone many systematic revisions to obtain the present nomenclature. The previous nomenclatures includes *Bacillus pseudomallei, Bacillus whitmori, Pfefferella pseudomallei* and *Pseudomonas pseudomallei*. Based on rRNA homology, the organism has now been assigned to the genus *Burkholderia* (Gilardi, 1991).

The first case was reported in india in 1991, in Dapoli Taluk of Maharashtra (Raghavan *et al*, 1991). There have been many reports from various parts of India, including coastal Karnataka (Sengupta *et al*, 1998; Rao and Shivananda 1999; Kanungo *et al*, 2002; Dias *et al*, 2004; Vidyalakshmi *et al*, 2007; Mukhopadhyay *et al*, 2008). Despite disputes regarding the etiology of outbreaks of plague in

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Maharashtra and Gujarat in 1994, it is likely the etiology was *B. pseudomallei* (Bharadwaj *et al*, 1994).

The present study reports a case series at a tertiary care hospital in Mangalore, a west coastal region of southern India. We report 17 cases of *B. pseudomallei* isolated from patients with various clinical manifestations.

MATERIALS AND METHODS

Seventeen patients with culture proven melioidosis were included in the study. Motile gram-negative bacilli with typical bipolar staining showing safety-pin appearance, a positive cytochrome oxidase test, pink dry colonies on MacConkey's agar, characteristic colony morphology on Ashdown media (Ashdown, 1979) resistant to gentamicin, polymyxin B and Colistin were presumptively identified as *B. pseudomallei*, according to the screening profile devised by Dance *et al* (1989). Detailed characterization was done according to the standard procedures which includes: growth at 42ºC, production of catalase, oxidative utilization of glucose, lactose, mannitol and maltose, liquefaction of gelatin, reduction of nitrate, hydrolysis of arginine and inability to decarboxylate lysine (Jesudason *et al*, 1997; Koneman *et al*, 1997). The identification of initial 4 isolates was confirmed at the Christian Medical College, Vellore, Tamilnadu State, India, a reference center for *B. pseudomallei* and non-fermenters. Antimicrobial susceptibility testing was performed by Kirby-Bauer method according to Clinical Laboratories Standard Institute (CLSI) guidelines (2005).

RESULTS

In the present study, the 17 cases described here exhibited a variety of clinical features including abscesses, septicemia , pneumonia, glomerulonephritis and pyrexia (Table 1). In two cases, there were slowly progressive granulomatous lesions with central necrosis mimicking tuberculous osteomyelitis.

Of 17 cases reported in the study, the frequency of isolation was high during June- September, which constituted the monsoon season ,with heavy rainfall in this region. Fourteen patients belonged to the coastal regions of Karnataka State and 3 from coastal regions of Kerala State. Of these 17 cases, 12 were males and 5 were females aged 34-65 years old. Seventy percent of case were male.

Nine of the cases acquired the infection due to contact with contaminated soil through abrasions by agricultural exposure or gardening. The details of the patients, clinical manifestations, predisposing factors and outcomes are described in Table 1.

Co-morbid conditions such as malignancy, acute respiratory distress syndrome, renal failure, liver abscess were present in few of our patients. Pyrexia and non-disseminated septicemia were the most frequent clinical findings in our study. Chest involvement was very low in this study. Except one patient, all the patients had DM as an underlying disease.

All the strains of *B.pseudomallie* isolated in this study were uniformly resistant to gentamicin, colistin, polymyxin B and showed variable resistance to cephalosporins. All 17 strains were sensitive to amoxicillin-clavulanic acid, ciprofloxacin, Cotrimoxazole, doxycycline, chloramphenicol and ceftazidime. Eleven patients improved with proper treatment (ceftazidime, Co-trimoxazole, doxycycline), whereas 6 expired in spite of treatment. All the recovered patients were put on maintenance

Table 1
Details of melioidosis cases documented in the study.

Sl. No.	Age in years	Sex	Specimen	Predisposing factors	Clinical presentation	Duration of illness	Treatment	Outcome
1	62	F	Pus	DM	Fever, cellulitis in the neck, sepsis	2 weeks	CAZ, CIP	Expired
2	48	M	Pus	DM	Chronic osteomyelitis mimicking TB	1 month	CAZ, CTX	Recovered
3	55	M	Pus	DM, hepatitis	Chronic osteomyelitis mimicking TB	1 month	MEM, CTX	Recovered
4	65	M	Pus	DM, epilepsy	Parieto- occipital scalp abscess	1 year	MEM	Expired
5	45	F	Pus	DM	Lateral chest wall abscess	3 weeks	CTX, AMC	Recovered
6	52	M	Pus	DM	Fever, septic arthritis of hip, gluteal abscess, sepsis	3 weeks	CAZ, CTX	Recovered
7	65	M	Pus	Nil	Submandibular abscess	10 days	LVX, CTX	Recovered
8	50	F	Pus	DM	Cellulitis of the leg	2 months	CAZ, CTX	Recovered
9	41	M	Blood	DM	Fever, sepsis	2 months	CAZ, CTX	Recovered
10	55	M	Blood	DM, alcoholism	Fever, septicemia septic arthritis hip, gluteal abscess	1 month	CFT, AKN	Expired
11	34	M	Blood	DM, Renal impairment, steroid therapy	Fever, acute renal failure, sepsis glomerulonephritis	2 months	CAZ	Improved
12	42	M	Blood	DM	Fever, septicemia bilateral pneumonia, respiratory failure	2 months	PTZ	Expired
13	55	M	Blood urine	DM, alcoholism tuberculosis	Fever, diabetic gangrene of toe, liver abscess, sepsis	1 month	CFT, DOXY	Discharged against medical advice
14	61	M	Blood	DM	Fever, sepsis multiloculated encysted pleural effusion	2 weeks	CAZ, CTX	Recovered
15	63	F	Blood, urine	DM, carcinoma of lung	Fever, septicemia respiratory failure, renal failure	1 month	CEF	Expired
16	40	M	Sputum	DM	Fever, chronic cough mimicking tuberculosis	1 month	CAZ, CTX	Recovered
17	50	F	Pleural fluid	DM, Chronic UTI	Multiple liver abscess, splenic abscess, pleural effusion	6 weeks	CEF, AKN, CIP	Expired

DM, Diabetes mellitus; F, Female; M, Male; CAZ, Ceftazidime ; CFT, Cefotaxime; CEF, Ceftriaxone; CTX, Cotrimoxazole; CIP, Ciprofloxacin; LVX, Levofloxacin; MEM, Meropenem; AKN, Amikacin; AMC, Amoxycillin- Clavulanic acid; DOXY, Doxycycline

therapy with doxycycline and Cotrimoxazole for 2-3 months.

DISCUSSION

B.pseudomallei, an emerging pathogen in India, is overlooked or under reported due to lack of awareness, low index of suspicion and morphological resemblance to *Pseudomonas* species. Typical pink dry colonies on MacConkey's agar, due to the production of oxalic acid from amino acids and the alteration of pH (Shankar and Rao, 2005), a characteristic microscopic appearance, a positive cytochrome oxidase test, dry, wrinkled, purple colored colonies with corrugated edges on Ashdown medium and antimicrobial resistance pattern will be helpful in the presumptive identification. Other media available for the selective isolation of *B. pseudomallei* are *B. pseudomallei* selective agar (BPSA) (Howard and Inglis, 2003) and *B. cepacia* medium. According to a recent study, sensitivity of detection of *B. pseudomallei* colonies on these media remain the same, but the selectivity of BPSA was lower than the other two media (Peacock *et al*, 2005).

Heavy rainfall, damp soil, proximity to agricultural land in this region, occupational contact during agriculture have been contributing factors for infection with *B. pseudomallei*. The fact that a large population in India is exposed to the agricultural land and direct contact with wet soils is a matter of concern. Nine of these cases acquired the infection due to contact with contaminated soil. Melioidosis mainly affects people who have underlying diabetes mellitus (DM), renal disease, alcoholism, cirrhosis, chronic lung disease, or immunosuppression by chronic disease or drugs. The present findings are consistent with previous studies. Two patients had multiple liver and splenic abscess (commonly reported in Thailand), while genitourinary infections and prostatic abscesses (commonly reported in Australia) were not common in this study (Vatcharapreechasakul *et al*, 1992; Cheng and Currie, 2005). In contrast to studies of melioidosis from other regions, chest involvement was found to be only 8% in Mukhopadhyay *et al* (2008), 36% in Vidyalakshmi *et al* (2007) and 23.5% in the present study. These findings need to be evaluated further.

Except for one patient, all the patients in our study had DM as an underlying disease (random blood sugar level ranging from 200- 540 mg/dl at the time of admission). A high incidence of DM has been reported in other studies from this geographical region [76% by Vidyalakshmi *et al* (2007) and 56% by Mukhopadhyaya *et al* (2008)]. Isolated case reports from India also showed DM as a predisposing factor (Anuradha *et al*, 2003). India being the country with one of the greatest incidence of diabetics of about 35 million (Ramachandran *et al*, 2004; Mohan *et al*, 2005), high incidence of diabetes seen in this study is not surprising. Hence it would seem appropriate to further evaluate *Pseudomonas*-like organisms cultured from diabetic patients to rule out the presence of *B. pseudomallei*.

The recommended treatment regimen for melioidosis is 10-14 days of ceftazidime or meropenem or imipenem with or without Cotrimoxazole, and eradication therapy is 3 months with Cotrimoxazole with or without doxycycline (Currie, 2005). Eleven patients improved with treatment (ceftazidime, Cotrimoxazole, and doxycycline), whereas 6 expired in spite of treatment. Treatment of *B. pseudomallei* is a challenge since it can survive intracellularly and can produce biofilms and microcolonies sheltered from beta-lactam drugs (Pruksachartvuthi *et al*, 1990). A combina-

tion of ciprofloxacin and a macrolide has been suggested as a good regimen to overcome this problem and achieve intracellular killing as cited by Raja *et al* (2005). Ciprofloxacin and azithromycin were found to achieve high concentration intracellularly by Chetchotisakd *et al* (2001). However, in the eradication phase, Cotrimoxazole and doxycycline were found to be more effective than ciprofloxacin-azithromycin combination (Chetchotisakd *et al*, 2001). The 100% sensitivity of all the isolated strains to Cotrimoxazole is an interesting finding in this study. Hence, using Cotrimoxazole for treatment and maintenance therapy appears appropriate. All patients who survived in our study were put on maintenance therapy with doxycycline and Cotrimoxazole for 2-3 months.

The high incidence of *B. pseudomallei* in Mangalore and its suburbs raises the possibility this coastal region is endemic for this organism, which needs to be proved by analyzing the soil and evaluating its seroprevalence in the community.

The increased emergence of melioidosis from this area in the last three years raises the question as to whether this may be a post-Tsunami effect. This is a matter which warrants further attention. We believe awareness among clinicians and microbiologists in this region is important when attempting to estimate the true magnitude of melioidosis in India, and to prevent outbreaks in the further.

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