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CASE SERIES OPEN ACCESS

Case Series of Melioidosis in a Tertiary Health Care Centre in Puducherry, India

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Abstract

Melioidosis, a potentially fatal disease caused by the bacterium *Burkholderia pseudomallei* continues to be neglected in the Indian Subcontinent despite bearing about 44% of the global burden. Diagnosis poses a significant challenge since the disease presents a wide range of symptoms and closely mimics tuberculosis and pneumonia both of which are endemic in India. Sophisticated diagnosis and treatment often become unaffordable for patients from rural or low-income backgrounds. We present five cases of melioidosis from a tertiary care hospital (Mahatma Gandhi Medical College and Research Institute) in Pondicherry that exhibited predominantly high-grade fever, abdominal pain, and vomiting. Radiological imaging revealed abnormalities in the brain (1/5, 20%), lung (3/5, 60%), liver (2/5, 40%), spleen (2/5, 40%), kidney (2/5, 40%), and prostate gland (1/5, 20%). *Burkholderia pseudomallei* infection was confirmed through blood culture. Treatment with meropenem or ceftazidime was initiated immediately. Neuromelioidosis was confirmed in one patient. The clinical diagnoses for the remaining cases were as follows: septic shock, melioidosis with urosepsis, and refractory shock. Three patients required intensive care and of the five, one patient was discharged, one died, and three discontinued treatments against medical advice. In the case of the deceased patient, the clinical diagnosis encompassed refractory shock accompanied by lactic acidosis, melioidosis, and community-acquired pneumonia, which subsequently progressed to acute respiratory distress syndrome (ARDS). Notably, this patient presented with co-morbidities, notably type 2 diabetes mellitus. This exemplifies the difficulty faced by patients from low-income backgrounds which forces them to discontinue expensive treatment. The true burden of melioidosis in the Indian Subcontinent is uncertain as many cases remain undiagnosed. Unawareness of the disease, low index of suspicion among medical professionals, incorrect treatment, and discontinuation contribute to the disease burden. It is therefore imperative that melioidosis is brought to the attention of healthcare policymakers to determine the true burden of the disease by prioritizing nationwide surveillance and diagnosis.

Keywords: Melioidosis, *Burkholderia pseudomallei,* Meropenem, Neuromelioidosis

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INTRODUCTION

Melioidosis, also known as Whitmore's disease, is a potentially fatal infectious disease caused by the saprophytic environmental Gram-negative bacterium *Burkholderia pseudomallei*, resulting in about 89,000 deaths per year. Melioidosis is predominantly a tropical disease, with the Indian subcontinent bearing 44% of the global disease burden.[1] It is, however, classified as a rare disease because of its similarity to tuberculosis, diverse clinical symptoms, lack of awareness among clinicians, and dearth of proper testing facilities.[2]

The pathogen infects both humans and animals through direct contact with contaminated soil and water and can be naturally acquired through skin abrasion, ingestion, or inhalation.[3] Environmental factors like tropical storms and rain, and specific occupations like rice farming, increases the chance of exposure.[4,5] Diabetes mellitus is the major risk factor along with chronic kidney, pulmonary, and coronary diseases.[2] The mean incubation period of *B. pseudomallei* in humans is nine days but can vary from days (acute) to years (chronic) depending on risk factors, inoculum size, route of entry, and the presence or absence of virulence factors.[6] Acute melioidosis manifests as pneumonia, multiorgan abscesses, and fulminant septicemia, whereas the chronic infection presents as symptoms mimicking tuberculosis (TB) with or without bloody sputum and multiorgan abscesses with occasional neurological involvement.[2,7] However, unlike TB, scarring and calcification of the lungs are rarely observed in chronic melioidosis. The latent form of the disease may lie dormant for years, sometimes decades, and may reactivate in an immunocompromised state or with comorbidities including diabetes, renal failure, and alcoholism.[8]

B. pseudomallei can be detected in blood, throat and rectal swabs, sputum, and pus from abscesses but their presence in clinical samples is limited. Melioidosis can be confirmed by culturing biological specimens in selective media and antimicrobial activity assays. However, care must be taken not to misinterpret results as *Pseudomonas, Burkholderia cepacia*, or *Bacillus* species, infections.[7] Under light microscopy, *B. pseudomallei* appear as safety-pin-shaped bacilli which can be confirmed by immunofluorescence microscopy. Although very specific, these methods are limited in sensitivity.[9] Serological tests like hemagglutination and latex agglutination coupled with ELISA can be used to screen for suspected infection but one should be cautious to avoid false positives from *B. thailandensis* infection and take into account differences in antibody titers from geographic areas.[10] Molecular diagnosis with PCR using 16S rRNA or *B. pseudomallei*-specific probes can be confirmatory. However, logistics and the availability of sophisticated instruments in rural areas limit the use of advanced molecular methods. Radiological imaging reveals multifocal nodular lesions in the lungs under X-ray and chronic infection may present lesions that resemble tuberculosis. Abscesses in organs, especially the liver and spleen, appear as a honeycomb pattern in computerized tomography (CT) scan. In neuronal involvement, magnetic resonance imaging (MRI) scan of the brain often shows ring-enhancing lesions and hyperintense changes but must be distinguished from cystic tumors by using diffusion-weighted imaging.[11]

The treatment of melioidosis follows a two-step regimen - an intravenous intensive phase and an eradication phase. The intensive phase of therapy (10-14 days) employs antibiotics like ceftazidime and/or carbapenems. The eradication phase commences after clinical improvement from the intensive treatment and the choice of drugs is a combination of trimethoprim-sulfamethoxazole (TMP-SMX) or amoxicillin-clavulanic acid for 3-6 months.[12]

Individuals with open skin wounds, diabetes mellitus, and chronic kidney disease should avoid contact with soil and water, especially in endemic areas. Wearing boots can prevent infection of the lower limbs and feet. Patients presenting symptoms should be diagnosed with a high level of suspicion. Awareness among clinicians and healthcare workers can aid in the early diagnosis of melioidosis.

This case study underlines the recent increase in melioidosis in India and highlights the broad spectrum of clinical symptoms presented to better equip healthcare workers in diagnosing the

Table 1. Underlying conditions, clinical manifestations, and outcomes of patients with melioidosis

Patie No.	Age/ Sex diagnosis	Clinical features	Clinical Diagnostic test	Abnormal findings in	Comorbidities	ICU	Treatment nt	Outcome
1	57/ M	Septic shock	Fever for one week and breathlessness for two days, burning sensation all over the body, decreased appetite, giddiness, orthopnoea, and pedal edema	CT thorax - multiple soft tissue density nodules in the right posterior basal segment of the lungs with peripheral ground glass opacities. USG of abdomen -hepatosplenomegaly and a simple cortical cyst in the kidney without calculus Routine test – ESR 140, CRP positive, deranged RFT, blood in urine, *Klebsiella pneumoniae* in urine	CKD for 2 yr, T2DM for 5 yr hypertension for 5 yr, anemia of chronic disease, coronary artery Disease, and inflammatory bowel disease	No	MER and TMP/SMX	DAMA
2	20/ M	Melioidosis	Complaints of high-grade fever, vomiting, dry cough, breathlessness, and abdominal pain for 15 days Trauma while playing kabaddi 15 days back	CT abdomen- hepatosplenomegaly with multiple discrete and conglomerated hypodense, hypoenhancing lesions in the liver and spleen. Routine test – thrombocytopenia, hyponatremia, and transaminitis	No known comorbidities	No	MER and TMP/SMX	Discharged
3	34/F	Neuromelioid -osis, demyelinating disorder with left eye optic neuritis	Blurring of vision in the left eye, pain in the left ear, with complaints of giddiness and headache for one week	MRI brain – optic neuritis, inflammatory granuloma with increased perilesional white matter edema CT brain - subtle ill-defined hypodense lesions in the right frontoparietal lobe, capsuloganglionic region, and left thymus. CT thorax - focal ground glass opacity in the left upper lobe. Dependent opacities were noted in the right lower lobe posterior basal segments. LP – 430 cells/mm^3 Routine tests - leukocytosis	No known comorbidities History of typhoid fever two months back	Yes	MER and TMP/SMX	DAMA

Table 1. Cont...

Patie No.	Age/ Sex diagnosis	Clinical features	Clinical Diagnostic test	Abnormal findinds in	Comorbidities	ICU	Treatment nt	Outcome
4	49/ M	Melioidosis with urosepsis	Complaints of vomiting and constipation for TMP/SM one week with decreased urine output since 11.00 pm the previous day	USG - Prostatomegaly, bladder wall thickening with dependent debris, and right renal minimally complex cyst with internal septation Routine – Microcytic hemolytic anemia, dyselectrolytemia, Urine culture indicated UTI	T2DM – 15 yr, Coronary artery disease – 5 yr dyslipidemia, I&D of left foot abscess (2010), I&D of right-hand abscess	Yes	MER and X	DAMA
5	31/ M	Refractory shock with lactic acidosis, Melioidosis, Community-acquired pneumonia-ARDS	Complaints of abdominal pain and vomiting for the past three days, fever for one week, and burning micturition for the last two days	Chest X-ray and HRCTthorax - consolidation in the right upper and middle lung lobe Routine test – anemia, thrombocytopenia, Urine - ketones positive, metabolic acidosis,	T2DM, appendicectomy - 2 yr back	Yes	MER and TMP/SMX	Dead

Abbreviations: ARDS – acute respiratory distress syndrome, CKD – chronic kidney disease, CT- computed tomography, DAMA – discharged against medical advice, HRCT – high-resolution computed tomography, I&D – incision and drainage, LP – lumbar puncture, MER – meropenem, MRI – magnetic resonance imaging, RFT – renal function test, T2DM – type II diabetes mellitus, TMP-SMX – trimethoprim-sulfamethoxazole

Table 2. Antibiotic susceptibility testing

Patient number	Antibiotic susceptibility				
	Amoxicillin/ clavulanic acid	Imipenem	Meropenem	Trimethoprim-sulfamethoxazole	Ceftazidime
1	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
2	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
3	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
4	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
5	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

disease in its earlier stages. The need for sustained treatment for better recovery and eradication of the pathogen is emphasized.

Case Presentation

Within a five-month period from October 2021 to February 2022, a tertiary healthcare center in Puducherry, South India, treated five cases of melioidosis, predominantly affecting men (Table 1). Three patients had a history of type II diabetes mellitus (T2DM), and one suffered from chronic kidney disease. Chief complaints included high-grade fever, abdominal pain, and vomiting. Case 1 presented with blurring of vision and ear pain, alongside complaints of giddiness and headache. Case 2 experienced recent trauma while playing kabaddi, and Case 4 underwent incision and drainage of an arm abscess. All patients tested negative for COVID-19. Routine blood analysis revealed elevated white blood cell counts in three patients (Case 1, 2 and 3), thrombocytopenia (Case 2 and 3) in two, and urinary tract infections in two (Case 1 and 4). Radiological tests indicated lung ground glass opacities and hepatosplenomegaly in three patients, with hypodense lesions in the liver and spleen in one patient. One patient exhibited a cyst in the urinary bladder with prostatomegaly (Case 4). Case 3's brain MRI revealed an inflammatory granuloma with increased perilesional white matter edema, and a CT scan showed ill-defined hypodense lesions in various brain regions. A lumbar puncture of Case 3 indicated 430 cells/cu.mm (lymphocytes 80%, neutrophils 20%), suggesting CNS inflammation. Blood culture and antibiotic sensitivity tests (Table 2) confirmed *B. pseudomallei* infection in all five patients.

Initially, broad-spectrum antibiotics were administered based on the initial diagnosis and symptoms. However, once *B. pseudomallei* infection was confirmed, all five patients were switched to intravenous meropenem (1-2 g) or ceftazidime (1 g) (i.v.) or a combination thereof within a week of their hospital admission. During the treatment course, Case 2, exhibiting asymptomatic and hemodynamically stable conditions, was discharged. Conversely, Cases 1 and 4, advised to continue medical treatment, opted for discharge against medical advice, citing logistical reasons. For Cases 3 and 5, the severity warranted intensive care, leading to admission to the intensive care unit (ICU). Unfortunately, Case 5 faced a fatal outcome during the intensive treatment phase.

Although, *B. pseudomallei* acute pulmonary infection was detected within 3 days of hospital admission and promptly started on i.v. meropenem, the patient developed sudden cardiac arrest on the 7th day preceded by bradycardia and could not be revived. This brings the mortality rate to 20% in the study, assuming the three patients who withdrew from treatment survived.

DISCUSSION

The present study reports five cases of melioidosis within a span of five months, describing the symptoms, diagnosis, treatment, and outcome at a teaching hospital in South India. Of the five patients, one patient (Case 2) was discharged after significant improvement. One patient (Case 5) died despite correct treatment[13] within 48 hr of hospital

admission. Three withdrew from treatment against medical advice and their outcome is not known. This highlights a mounting problem in the treatment of melioidosis and probably reflects the high mortality rate due to this disease in India. All the *B. pseudomallei* isolates displayed susceptibility to the antibiotics when assessed using the MIC method (Table 2). Melioidosis is acquired generally from the environment and individuals exposed to contaminated soil and water are the most common victims. These often include people from low-income backgrounds especially daily laborers and farmers who often find it difficult to afford expensive medical treatment and hence discontinue medical advice. Since most primary and secondary healthcare centers including government hospitals are not sufficiently equipped to diagnose the disease, the true incidence of the disease may be an underestimate. Owing to diverse clinical presentations, diagnosis is surprisingly challenging, especially for rural health centers that lack awareness about the disease and proper infrastructure. Symptoms of melioidosis mimic other tropical diseases for which India is endemic and this exacerbates the issue further as the principal suspicion points to diseases like tuberculosis or pneumonia.

There has been a steady increase in melioidosis cases in India with a large number of reports from the southern parts of the country.[14,15] The recent decade has witnessed an alarming rise in the number of cases with the highest of nearly 600 cases being reported last year.[8] Whether this increase reflects a recent surge in incidence or diagnostic improvements, is unknown. However, there remains a significant lack of awareness and suspicion among clinicians and healthcare workers which often leads to misdiagnosis and incorrect treatment. Although a large number of scientific publications report the increase in melioidosis incidence in India, a national surveillance study to evaluate the exact number of cases and encourage the government to include melioidosis in health policies is largely lacking. A large-scale seroepidemiological study conducted in India reported a 29% seropositivity among adults in the Udupi district of Karnataka. Interestingly, however, seropositivity was similar in both farmers and non-farmers. Other factors like gender, socio-demographic status, occupation, and environmental factors were not correlated with seropositivity. However, it was also noted that the discrepancy in seropositivity rates and disease development may be dependent on the level of inoculum exposure. Also, high seropositivity does not necessarily mean a high incidence rate of melioidosis. A high antibody titer in some could also result from exposure to the closely related but non-virulent pathogen, *B. thailandensis*, that naturally inhabits soil.[16]

In the Indian Subcontinent, about 80% of melioidosis cases are diabetics.[17-19] Immunocompromised patients and those with chronic kidney disease (CKD) are more susceptible to the disease.[20] Our study also indicates that diabetics and patients with CKD have a stronger predisposition to melioidosis. This aids in the differential diagnosis and patients presenting with high-grade fever, abdominal pain, vomiting, pneumonia, skin infections, and abscess with co-morbidities like type 2 diabetes mellitus (T2DM), CKD, liver disease, blood disorders, and cancer should be diagnosed with a high degree of suspicion keeping melioidosis in mind. In addition to the acute and chronic forms of melioidosis, *B. pseudomallei* can remain latent in its dormant state for many years. The major proportion of the bacteria may be neutralized by the host immune response in immunocompetent individuals or be eradicated incompletely by broad-spectrum antibiotics at sub-optimal doses. This paves the way for the recurrence of infection even after several years.[8]

In a resource-limited country like India, especially the rural areas, diagnosing melioidosis which mimics other diseases, is a challenge. Awareness among clinicians and a strong suspicion can act as a primary diagnostic checkpoint enabling early transfer to higher centers of care thereby preventing misdiagnosis and incorrect treatment. Microbial culture analysis remains the gold standard for *B. pseudomallei* identification. However, it is often time-consuming and requires selective media like Ashdown agar that rural laboratories hardly have any access to. Sophisticated techniques like mass spectrometry, PCR, and sequencing may also be used to identify *B. pseudomallei* infection and genetic variants if any. These techniques are also, however, restricted to higher research centers and medical

laboratories. Successful treatment requires prolonged administration of expensive antibiotics that have proven effective even in deep-seated infections. Patients from rural or low-income strata of society can seldom afford such treatments and often discontinue resulting in relapse.[21,22]

CONCLUSION

Melioidosis remains to be a neglected disease in India and is not under the purview of the Integrated Disease Surveillance Program (IDSP) run by the Indian National Center for Disease Control (NCDC) despite a higher predicted number of global deaths than dengue and leptospirosis which are among the top 12 priority diseases of IDSP. Melioidosis, therefore, warrants more critical attention from the health ministry and can only be achieved through a consorted approach by researchers and medical professionals to pool their resources and present the true nature of the disease in India. The recent surge in melioidosis cases in India has prompted active initiatives by institutes like Kasturba Medical College, Manipal, India, leading to the inception of the Indian Melioidosis Research Forum (IMRF). IMRF aims to rope in regional health centers and network with centers in other South as well as South East Asian countries to monitor and enhance communication about the disease to assess the true burden of the disease and bring it under the attention of policymakers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Institutional Humans Ethics Committee, Mahatma Gandhi Medical College & Research Insititute, Puducherry, India, with Faculty Project/04/2021/06.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

REFERENCES

1. Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol* 2015;11;1(1):15008. doi: 10.1038/nmicrobiol.2015.8
2. Koshy M, Jagannati M, Ralph R, et al. Clinical Manifestations, Antimicrobial Drug Susceptibility Patterns, and Outcomes in Melioidosis Cases, India. *Emerg Infect Dis.* 2019;25(2):316-320. doi: 10.3201/eid2502.170745
3. McCormick JB, Sexton DJ, McMurray JG, Carey E, Hayes P, Feldman RA. Human to human transmission of *Pseudomonas pseudomallei. Ann Intern Med.* 1975;83(4):512-513. doi: 10.7326/0003-4819-83-4-512
4. Currie BJ. Melioidosis: Evolving concepts in epidemiology, pathogenesis, and treatment. *Semin Respir Crit Care Med.* 2015;36(1):111-125. doi: 10.1055/s-0034-1398389
5. Limmathurotsakul D, Kanoksil M, Wuthiekanun V, et al. Activities of Daily Living Associated with Acquisition of Melioidosis in Northeast Thailand: A Matched Case-Control Study. *PLoS Negl Trop Dis.* 2013;7(2):e2072. doi: 10.1371/journal.pntd.0002072
6. Limmathurotsakul D, Peacock SJ. Melioidosis: a clinical overview. *Br Med Bull.* 2011;99(1):125-139. doi: 10.1093/bmb/ldr007
7. Wiersinga WJ, Virk HS, Torres AG, et al. Melioidosis. *Nat Rev Dis Prim.* 2018;4:17107. doi: 10.1038/nrdp.2017.107
8. Mohapatra PR, Mishra B. Burden of melioidosis in India and South Asia: Challenges and ways forward. *Lancet Reg Heal - Southeast Asia.* 2022;2:100004. doi: 10.1016/j.lansea.2022.03.004
9. Sheridan EA, Ramsay AR, Short JM, Stepniewska K, Wuthiekanun V, Simpson AJH. Evaluation of the Wayson stain for the rapid diagnosis of melioidosis. *J Clin Microbiol.* 2007;45(5):1669-1670. doi: 10.1128/JCM.00396-07
10. Lowe W, March JK, Bunnell AJ, O'Neill KL, Robison RA. PCR-Based Methodologies Used to Detect and

Differentiate the *Burkholderia pseudomallei* Complex: *B. pseudomallei, B. mallei,* and *B. thailandensis. Curr Issues Mol Biol.* 2013;16(1):23-54.

11. Liang CC, Chen SY, Chen TY, Chen ST. Central Nervous System Melioidosis Mimics Malignancy: A Case Report and Literature Review. *World Neurosurg.* 2016;89:732.e19-e23. doi: 10.1016/j.wneu.2016.01.093
12. Koshy M. Melioidosis: An emerging infection in India. *Curr Med Issues.* 2020;18(2):94-97. doi: 10.4103/cmi.cmi_72_19
13. Treatment | Melioidosis | CDC. 2021. Accessed January 18, 2024. https://www.cdc.gov/melioidosis/treatment/index.html
14. Gopalakrishnan R, Sureshkumar D, Thirunarayan M, Ramasubramanian V. Melioidosis :an emerging infection in India. *J Assoc Physicians India.* 2013;61(9):612-614.
15. Mukhopadhyay C, Shaw T, Varghese GM, Dance DAB. Melioidosis in South Asia (India, Nepal, Pakistan, Bhutan and Afghanistan). *Trop Med Infect Dis.* 2018;3(2):51. doi: 10.3390/tropicalmed3020051
16. Vandana KE, Mukhopadhyay C, Tellapragada C, et al. Seroprevalence of *Burkholderia pseudomallei* among Adults in Coastal Areas in Southwestern India. *PLoS Negl Trop Dis.* 2016;10(4):e0004610. doi: 10.1371/journal.pntd.0004610
17. Behera B, Mohanty S, Mahapatra A, et al. Melioidosis in Odisha: A Clinico-Microbiological and Epidemiological Description of Culture-Confirmed Cases over a 2-Year Period. *Indian J Med Microbiol.* 2019;37(3):430-432. doi: 10.4103/ijmm.IJMM_19_367
18. Chowdhury FR, Jilani MSA, Barai L, et al. Melioidosis in Bangladesh: A Clinical and Epidemiological Analysis of Culture-Confirmed Cases. *Trop Med Infect Dis.* 2018;3(2):40. doi: 10.3390/tropicalmed3020040
19. Corea EM, De Silva AD, Thevanesam V. Melioidosis in Sri Lanka. *Trop Med Infect Dis.* 2018;3(1):22. doi: 10.3390/tropicalmed3010022
20. Sridharan S, Princess IB, Ramakrishnan N. Melioidosis in Critical Care: A Review. *Indian J Crit Care Med.* 2021;25(Suppl 2):S161-S165. doi: 10.5005/jp-journals-10071-23837
21. Halim I, Kokkayil P, Kirti R, et al. Melioidosis in Bihar, India: unearthing the first of many? *Access Microbiol.* 2021;3(9):260. doi: 10.1099/acmi.0.000260
22. Halim I, Shaw T, Tellapragada C, Vandana K, Mukhopadhyay C. Melioidosis: Reinfection Going Incognito as Relapse. *Indian J Med Microbiol.* 2017;35(4):593-596. doi: 10.4103/ijmm.IJMM_17_140