DOI: 10.7860/JCDR/2026/85005.23729

Case Report

Microbiology Section

Pulmonary Melioidosis: Microbiological Insights for Early Diagnosis and Management: A Case Report

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ABSTRACT

Melioidosis, a life-threatening clinical condition and a multisystem disease with varied presentations associated with high mortality. This disease can affect any organ, but pulmonary involvement is the commonest, followed by skin and soft-tissue infections, bones and joint infections, visceral abscesses, and septicaemia. This is a case report of a 45-year-old male, diabetic with poor glycaemic control, an occasional drinker, with a history of travel to an endemic area, prior to his illness, presented with a history of fever for the past one month, evening rise of temperature and cough with dyspnoea for 10 days associated with a history of weight loss. Initial evaluation led to the clinical suspicion of community-acquired pneumonia, pulmonary tuberculosis and other clinical conditions such as interstitial lung disease, post-COVID sequelae with secondary infections, malignancy, Human Immunodeficiency Virus (HIV) related lung infections. Baseline blood workup showed anaemia, leucocytosis, elevated Erythrocyte Sedimentation Rate (ESR) and C-reactive protein. Chest X-ray and High-Resolution Computed Tomography (HRCT) chest were suggestive of lung consolidation that involved the right upper lobe predominantly. Sputum culture and sensitivity yielded the growth of *Burkholderia pseudomallei*, the causative agent of Melioidosis, which resolved the clinical dilemma in diagnosis. He responded well to parenteral Meropenem treatment based on the antimicrobial susceptibility report and showed a good clinical improvement. His glycaemic control was achieved with insulin therapy. Hence, this case report depicts how the microbiological analysis helped in prompt diagnosis and timely management that prevented morbidity and mortality.

Keywords: *Burkholderia pseudomallei*, Diabetes mellitus, Endemic, Septicaemia

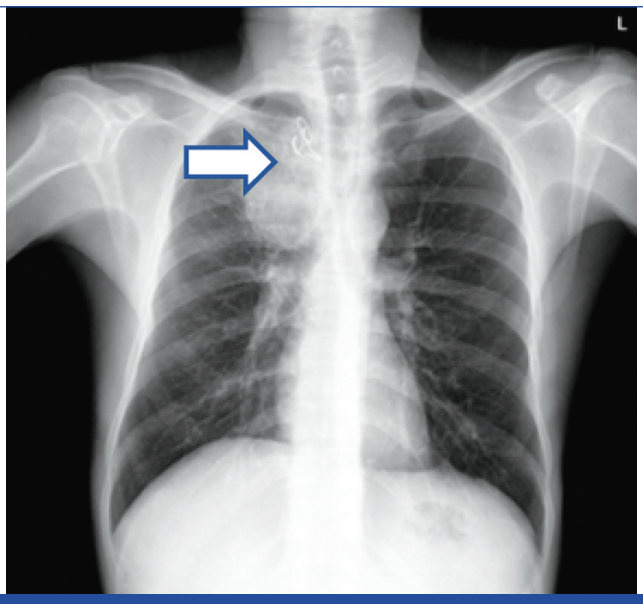
CASE REPORT

A 45-year-old male patient, diabetic on oral hypoglycaemic agents for the past four years with poor glycaemic control, occasional drinker, presented to Medicine department, with chief complaints of fever, continuous type, with evening rise of temperature associated with weight loss for the past one month, cough with expectoration, minimal whitish sputum, not blood stained, associated with mild dyspnoea for 10 days, He was a lorry driver by occupation mainly transporting rice in gunny bags. He gave a history of travel to Puducherry last month, where he had to take care of his hospitalised relative undergoing haemodialysis. At the time of admission, the patient was conscious, oriented to time, place, person and situation. He was febrile with a body temperature of 38.3°C, tachycardia was present, heart rate was 102 beats/min, blood pressure-100/80 mmHg, Respiratory rate-22/min, the respiratory system revealed bronchial breath sounds and fine crepitations in the right supraclavicular region representing the involvement of the upper lobe of the lungs. Other systemic examinations were within normal limits. The above case scenario suggested that the infective foci involved the lungs predominantly, associated with features of sepsis. Community-acquired pneumonia, pulmonary tuberculosis, interstitial lung disease, post-COVID sequelae, HIV related lung infections and malignancy were the differential diagnoses based on presentation and clinical examination. Baseline investigations were performed, and the results are shown in [Table/Fig-1].

Test parameter	Result	Reference range
Haemoglobin (g/dL)	10.2	13.0-17.0
Total leucocyte count (cells/µL)	15,000	4000-10,000
Erythrocyte sedimentation rate (mm/hr)	88	0-10
C-reactive protein (mg/L)	200	Below 10- normal, 10 and above- reactive, 200 and above- Critical value
HbA1C (%)	11.0	Normal- 4.8-5.7, diabetic- >=6.5
Fasting Blood sugar (FBS) (mg/dL)	314	70-100
Postprandial Blood Sugar (PPBS) (mg/dL)	425	80-140
Urine sugar {Glucose oxidase Peroxidase Reaction (Reagent strip) / Benedict's copper reduction test (Manual method)}	2+	nil
Blood Urea Nitrogen (BUN) (mg/dL)	6	7-18
Serum creatinine (mg/dL)	0.73	0.7-1.3

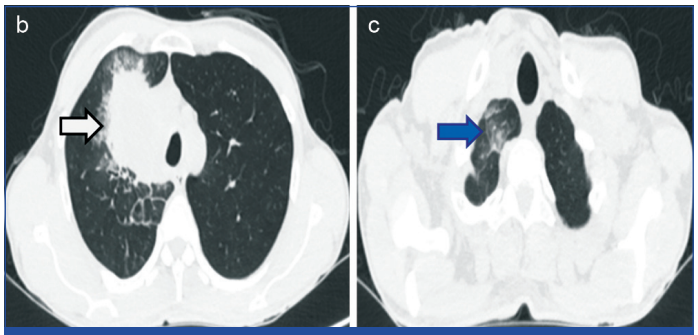
[Table/Fig-1]: Baseline investigations.

Chest X-ray showed homogenous opacity involving the right upper lobe s/o consolidation [Table/Fig-1a].



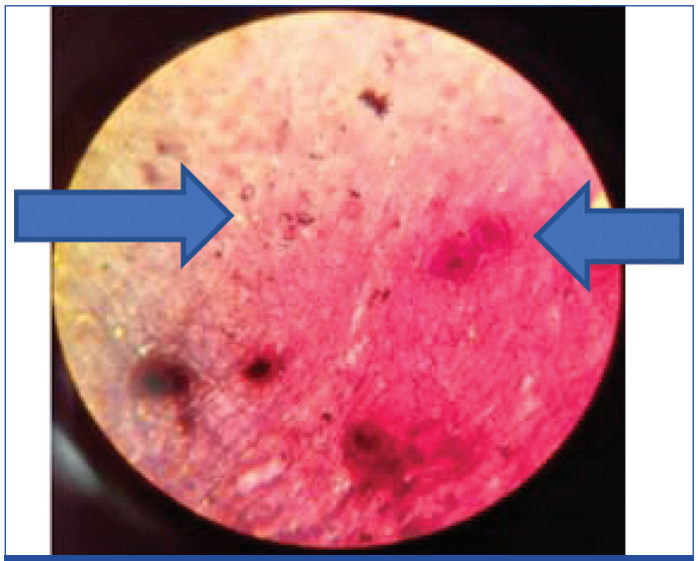
[Table/Fig-1a]: Chest X-Ray- Homogenous opacity right upper lobe.

HRCT chest-showed consolidatory changes involving the anterior and apical segment of right upper lobe, centrilobular nodules scattered in the right upper and middle lobe [Table/Fig-1b,c] and a heterodense lesion with central fluid density invading the mediastinum, measuring 4.7×5.5×5.7 cm was noted in the right paratracheal region.



[Table/Fig-1bc]: b) HRCT Chest-showing consolidatory changes involving anterior segment of right upper lobe; c) HRCT Chest-showing consolidatory changes involving anterior segment of right upper lobe.

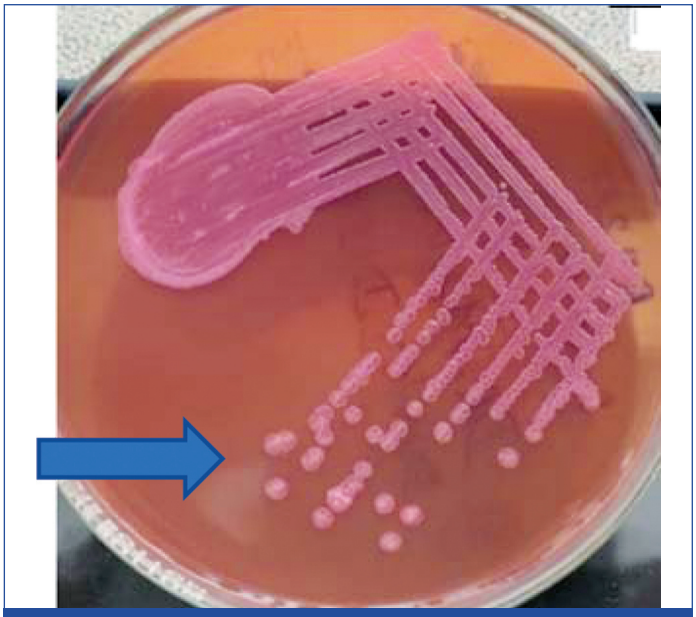
Hence, chest X-ray and HRCT chest were suggestive of lung consolidation that involved the right upper lobe predominantly and centrilobular nodules, particularly in the right upper and middle lobes in HRCT chest. Clinical microbiology lab received a sputum sample for bacterial culture and sensitivity, acid-fast stain and GeneXpert Rif/Ultra. Gram stained sputum smear was examined initially under low power objective of microscope (10x) to assess the quality of sputum by Bartlett's criteria [1] and the sample was found to be acceptable (>25 puscells/LPF and <5 epithelial cells/LPF) for culture, then same smear examined under 100x oil immersion objective of microscope which showed many pus cells, moderate Gram-negative bacilli and few Gram-positive cocci in pairs and chains [Table/Fig-2].



[Table/Fig-2]: Direct Gram stain - showing pus cells and Gram negative bacilli.

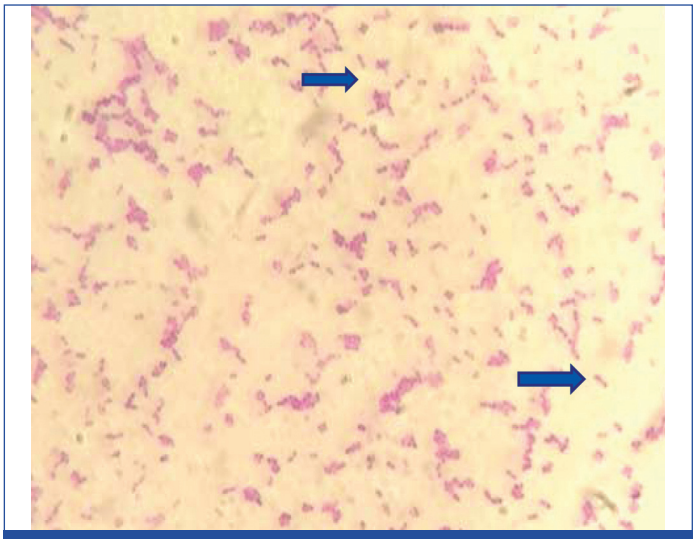
After inoculation and overnight incubation, MacConkey agar plate showed pale, colourless, small, non lactose fermenting, smooth creamy colonies with a metallic sheen initially, turning slowly into pink to purple-coloured colonies on further incubation, after 2-3 days of incubation at 37°C in bacteriological incubator, rough and corrugated slightly wrinkled pink to purple-coloured colonies with a central umbo [Table/Fig-2a] was isolated. Blood agar plate [Table/Fig-2b] showed beta-haemolytic, initially small, smooth greyish white colonies with a metallic sheen, which turned into dry, wrinkled colonies after 2-3 days of incubation at 37°C under aerobic conditions. Gram stain from the colonies showed typical bipolar stained Gram-negative bacilli with safety pin appearance [Table/Fig-2c]. Organism was actively motile.

Catalase and oxidase tests were positive [Table/Fig-2d,e]. Indole was not produced, Citrate was utilised, Triple Sugar Iron agar (TSI)



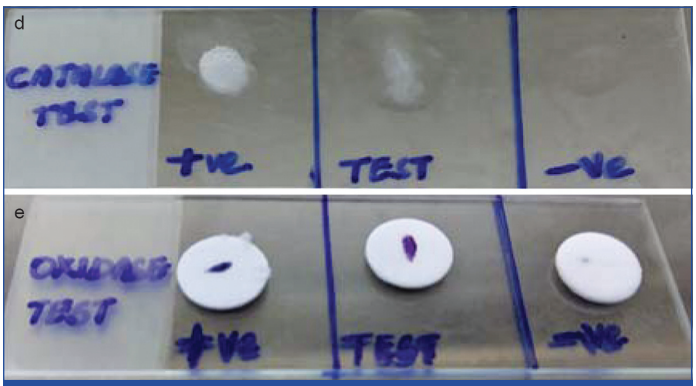
[Table/Fig-2a]: MacConkey agar plate- showing pink coloured, corrugated, wrinkled colonies with a central umbo.

[Table/Fig-2b]: Blood agar plate-showing beta-haemolytic white colonies with a metallic sheen.

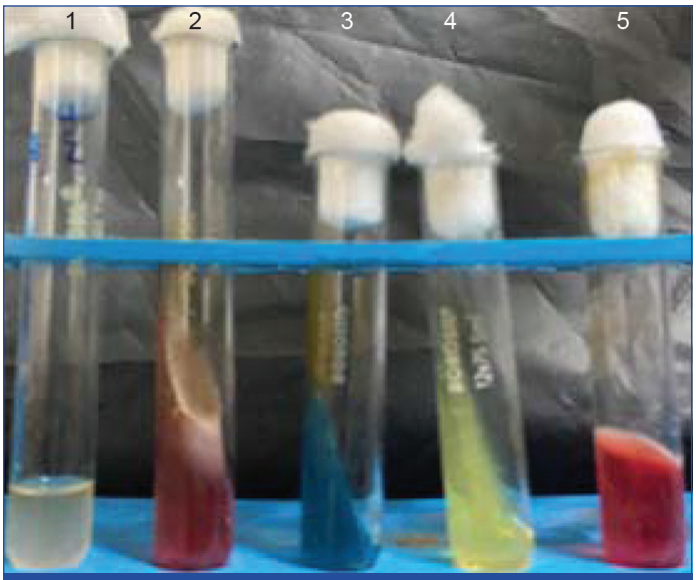


[Table/Fig-2c]: Gram stain- Gram negative bacilli, bipolar staining, safety pin appearance.

test showed alkaline slant/alkaline butt without gas/H_2S, sugars were not fermented but oxidatively utilised [Table/Fig-2f].



[Table/Fig-2]: d) Catalase test-positive; e) Oxidase test-positive.



[Table/Fig-2f]: 1) Indole test-Negative; 2) TSI- alkaline slant/alkaline butt without gas/H2S; 3) Citrate-utilised; 4) Urea- not hydrolysed; 5) Mannitol motility medium-non-fermented and motile.

Growth was present at 42°C. Trypticase soy agar was used which showed growth at 42°C, where initially the colonies were moist, creamy with a metallic sheen, which after 2-3 days of incubation, showed wrinkled colonies with a characteristic central umbo formation [Table/Fig-2g].

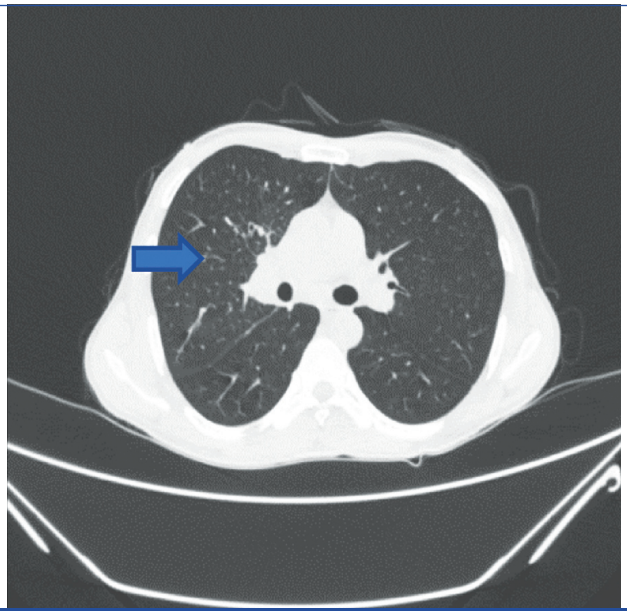
[Table/Fig-2g]: Trypticase soy agar showing growth at 42°C- wrinkled colonies with a characteristic central umbo.

Identification was made by conventional methods (based on colony morphology, culture characteristics, Microscopic examination (Gram staining), biochemical identification) and confirmed by VITEK 2 compact automated ID system. Antimicrobial susceptibility testing was carried out by broth dilution method for the following antibiotics- ceftazidime, cotrimoxazole, minocycline, amoxicillin-clavulanate tested at the concentration range between 0.25 µg/mL to 32 µg/mL and polymyxin B tested at the concentration range between 0.25 µg/mL to 512 µg/mL for MIC (Minimal inhibitory concentration) determination and the antibiotic Meropenem MIC [Table/Fig-2h] was determined by using gradient E-strip and the concentration range used for susceptibility testing was between 0.002 µg/mL to 32 µg/mL and interpreted according to CLSI guidelines [2,3], where the isolated organism was susceptible to ceftazidime (MIC-1 µg/mL), cotrimoxazole (MIC- (0.05/0.95 µg/mL), minocycline (MIC- 1 µg/mL) and amoxicillin-clavulanate (MIC- 1/0.5 µg/mL) by broth dilution method as well as by VITEK 2 compact system and the MIC values are as follows- ceftazidime (MIC-2 µg/mL), cotrimoxazole (MIC- <=20 µg/mL), minocycline (MIC- 2 µg/mL), meropenem (MIC- 1 µg/mL).

[Table/Fig-2h]: Antibiotic Meropenem MIC determined by using gradient E-strip.

The sputum sample tested by acid-fast stain was negative for acid fast bacilli and GeneXpert test was also negative for *Mycobacterium tuberculosis*. Blood culture was sterile. He was initially started on parenteral ceftriaxone 2 gm i.v. BD and Tab. azithromycin 500 mg OD, being a standard empirical therapy in case of suspected community-acquired pneumonia, in addition, azithromycin has immunomodulatory and anti-inflammatory effects. In view of fever spikes, poor clinical response and evolving sepsis, on day 2, antibiotics were escalated to parenteral meropenem 1 gm i.v. TDS and linezolid 600 mg i.v. BD empirically. This combination therapy started in view of suspected pneumonia due to other Gram-negative organisms such as *Pseudomonas aeruginosa*, ESBL-producing Enterobacterales, which don't respond to inj. ceftriaxone and inj. linezolid was added to cover suspected MRSA infections. Within 24 to 48 hours of commencing the above treatment, his fever spikes settled. His treatment was focused mainly on community-acquired pneumonia, as clinical diagnosis supported by radiological evidence of pneumonic consolidatory changes in the right upper lobe was present. Isolation and identification of *Burkholderia pseudomallei* from sputum culture made a great turning point in their diagnosis to confirm as pulmonary melioidosis, which often resembles various other clinical conditions like pulmonary tuberculosis, malignancy,

etc. in its presentation. Hence, microbiological diagnosis helped the treating physician in making a correct diagnosis, early and timely intervention. Based on the sputum culture and sensitivity report, parenteral meropenem 1 gm i.v. TDS was continued. At discharge, he was conscious, well oriented to time, place, person, situation, afebrile, vitals were stable, systemic examinations were within normal limits and were showing clinically good improvement without any organ damage or septicaemia. Advised with parenteral meropenem 1 gm i.v. TDS for five days, to continue oral cotrimoxazole {160 mg trimethoprim/800 mg sulphamethoxazole (double strength tablet) -2tablets BD} for a period of three months. Subcutaneous insulin regimen consisted of human regular insulin and human NPH insulin, to be administered as follows: 10 units of regular insulin and 10 units of NPH insulin in the morning, 10 units of regular insulin in the afternoon, and 8 units of regular insulin with 8 units of NPH insulin at bed time, to repeat blood sugar test after two weeks and to review in medicine OPD was advised at the time of discharge. During his recent visit to OPD, he had almost completed his full course of oral eradication therapy and was doing well; his general and systemic examinations were within normal limits, his blood sugar levels were FBS-167 mg/dL and PPBS -228 mg/dL, (normal ref. range- FBS-70-100 mg/dL, PPBS-80-140 mg/dL). Advised to take his regular diabetic medications (currently on Tab. metformin SR 500 mg BD), as he is on irregular treatment on and off and to follow-up at the diabetic clinic for periodic monitoring of his blood sugar levels, also advised with healthy lifestyle modifications. Follow-up at the end of two weeks, he was symptomatically better, fever subsided, dyspnoea reduced, he was on maintenance therapy with oral cotrimoxazole. Total leucocyte count reduced to 11,700 cells/µL (Normal ref. range-4000-10,000 cells/µL). After two months follow-up, he was afebrile, dyspnoea significantly reduced, vitals were stable, leucocyte count reduced to 5700 cells/µL (Normal ref. range- 4000-10,000 cells/µL), HRCT chest showed resolution of consolidatory changes in the lung fields [Table/Fig-3]. Hence, this reveals that the patient was recovering from his illness following treatment.



[Table/Fig-3]: HRCT chest- post-treatment- resolving lesions.

DISCUSSION

Melioidosis, also known as Whitmore's disease, caused by *Burkholderia pseudomallei*, a Gram negative, facultative bacteria, mainly dwelling in soil and surface water, can affect both humans and animals through following modes such as inhalation, cutaneous inoculation following minor abrasions, cut injuries, trauma or by ingestion. This disease is common during rainy season and endemic in South East Asia and northern Australia [4]. The diagnosis of Melioidosis is often missed, as it is a "great mimicker" of several clinical conditions in its presentation like Tuberculosis. Case fatality rate [4-7] is around 10-50% and is even higher, around 40-70% in case of septicaemia, if not diagnosed and managed on time. Major risk factors associated with this disease are diabetes mellitus, the most common, similar to this case report and a study by Chowdhury S et al., [8] which found a strong association between diabetes mellitus and the increased risk of melioidosis, chronic kidney disease, lung disorders like chronic obstructive pulmonary disease, which can predispose to pulmonary melioidosis, malignancy, immunocompromised states like patients undergoing haemodialysis, on long standing steroid therapy, following liver or renal transplants, Thalassaemia etc., [4-7]. Various virulence mechanisms involved in pathogenesis are the presence of capsular polysaccharide, intracellular survival within host cells, mediated by actin-based and flagellar motility, type III and VI secretion system, biofilm formation, quorum sensing and the formation of Multinucleated Giant Cells (MNGC), remains the hallmark of melioidosis. Depending on the immune status of the individual, clinical presentation [9] varies from an acute fulminant, localised or disseminated infection with or without septicaemia, chronic infection, reactivations /relapses and categorised into acute (<2 months duration) and chronic illness (>2 months duration) based on duration. The incubation period typically falls within a range of 1-21 days with an average duration of nine days. Melioidosis, a multisystem disease, can affect any organ, pulmonary involvement being the commonest [7]. Other presentations are cutaneous infections, osteomyelitis, septic arthritis, encephalomyelitis, meningoencephalitis, intracerebral abscess, genitourinary infections, other visceral abscess most commonly the liver, spleen, kidney, adrenals, in a few cases, prostatic abscess also seen; it can even involve lymph nodes, pericardium, parotids (in children) [4]. Pulmonary melioidosis in this case report presented with symptoms suggestive of pneumonia such as high grade fever, productive cough, dyspnoea similar to the case report by Chang CY and the case report by Thorve SM et al., and also often mimics various other clinical conditions, leading to a misdiagnosis which was detailed in a case series study conducted by Rajendran A et al., where diverse pulmonary presentations which were initially misidentified as lung abscess, empyema, lung malignancy and later on diagnosed as melioidosis based on microbiological culture [10-12]. It can present as a primary pneumonia as in this present case and similar to a study by Meumann EM et al., where primary pneumonia accounted for 91% of the pulmonary melioidosis cases [9] and in the same study 20% cases were with secondary pneumonia presentations associated with positive blood cultures, hence pulmonary melioidosis can also present with pneumonia secondary to a bacteraemia and also commonly associated with relapses/ reactivation [7]. Radiologically presents as upper lobe infiltrations, consolidation, similar to the case report by Chang CY or can present with cavitation, lung abscess or an isolated pleural effusion etc., [10]. Outcome is often highly fatal especially with pulmonary melioidosis, if not diagnosed and treated on time [5,6]. Diagnosis is usually based on the detailed history, clinical examination findings, epidemiological information, baseline investigations along with radiological imaging and bacterial culture, where culture isolation and identification from the appropriate clinical specimen remains the mainstay in diagnosis. The organism can be isolated in routine culture media like MacConkey agar, Blood agar and selective media, Ashdown's agar shows dry, wrinkled colonies with a characteristic central umbo after 2-3 days of incubation at 37°C, bipolar staining (safety pin appearance), catalase and oxidase test positive, motile, sugars not fermented but oxidatively utilised, growth at 42°C, intrinsically resistant to polymyxin B. Automated diagnostic modalities such as VITEK 2 compact system, MALDI-TOF and Molecular assays such as PCR, Genomic sequencing helps in rapid diagnosis. Serological tests such as Indirect haemagglutination assay, Enzyme-Linked Immunosorbent Assay (ELISA), lateral flow antigen detection tests, Immunofluorescence assays can be used as an adjunct to culture - based diagnosis especially in endemic areas, where early diagnosis is essential. According to Australian treatment guidelines, Darwin melioidosis treatment guidelines and

data from MERTH study [13], the therapy involves a long term course - an initial intensive parenteral therapy with ceftazidime or meropenem for a duration of 10-14 days, in case of severe sepsis, this may be further extended, in the case report by Rajeev A and Surendran ST, treatment of melioidosis with parenteral ceftazidime was successful and in the study by Cheng AC et al., treatment with parenteral meropenem is a better alternative to ceftazidime and also outcome was better in patients with severe sepsis associated with melioidosis then a maintenance phase or oral eradication therapy with trimethoprim-sulphamethoxazole (cotrimoxazole) for a period of three months given [14,15]. Doxycycline or amoxicillin-clavulanate is preferred in individual intolerant to oral cotrimoxazole, pregnant women and young children. Treatment is often difficult as this organism is resistant to wide range of antibiotic classes such as penicillin, 1st, and 2nd generation cephalosporin, aminoglycosides, quinolones, macrolides, polymyxin and colistin. Relapse rate and recurrence rate are around 15-30% [7] and 5-28% respectively, in melioidosis. Though the outcome in cases of melioidosis has several scenarios such as post discharge relapse, recurrences, readmissions, death as analysed in a study by Chantratita N et al., [16] the present study based on follow-up, revealed that the patient had recovered from his illness following appropriate and timely management.

CONCLUSION(S)

This study would be a representative case for the treating clinicians to learn to recognise the frequently misdiagnosed, highly fatal disease and to have a high-index of suspicion as in this present case with associated risk factor diabetes mellitus, travel to endemic area prior to illness, primarily lung involvement with clinical picture of pneumonia, the commonest presentation of pulmonary melioidosis. Though a diagnostic challenge was faced initially with presentations similar to varied clinical conditions like community-acquired pneumonia, pulmonary tuberculosis, etc., and was initially managed differently with empirical therapy, early microbiological confirmation based on conventional methods of culture identification, biochemical tests, and later on confirmed with the automated VITEK-2 compact system ended the clinical dilemma. Treatment based on antimicrobial susceptibility report and the importance of prolonged treatment course especially with 2- phase treatment (intensive and eradication phase), helped in resolution of lung lesions. Overall, this case report emphasises and underscores the significance of microbiological insights which helped to overcome the clinical diagnostic dilemma and in early, timely management that prevented the mortality and morbidity.

Authors' contribution: PS, PA designed and conceptualised this case report. JML, MMK supported PA in data collection. PA had done the literature search, prepared manuscript and also did the manuscript editing work. PS thoroughly did the manuscript review and gave overall support in completing this manuscript successfully.

Acknowledgement

I am thankful to my Professor and Head, Department of Microbiology, for the great support provided to this case report.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 06, 2025
- Manual Googling: Mar 21, 2026
- iThenticate Software: Mar 24, 2026 (2%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Oct 23, 2025**
Date of Peer Review: **Dec 22, 2025**
Date of Acceptance: **Mar 27, 2026**
Date of Publishing: **Jul 01, 2026**