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Research Article

Health Risks to Agricultural Communities: Impact of *B. pseudomallei* Contamination in Soil and Water Sources in Goa, India

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Abstract

Burkholderia pseudomallei, the etiological agent responsible for the potentially life-threatening condition known as melioidosis, has been identified in few farmers in Goa, India, marking the documented evidence of its presence in this region. Melioidosis, an infectious disease prevalent in tropical and subtropical regions, is caused by the soil-dwelling bacterium *Burkholderia pseudomallei*. In areas such as South West India, where heavy rainfall is common, most human infections occur during these periods, although the reasons behind this seasonal trend remain incompletely understood. Despite India being among the countries projected to have a high burden of melioidosis, there is limited information available regarding the environmental presence of *B. pseudomallei* and the factors influencing its distribution. This study sought to address this gap by investigating the prevalence of *B. pseudomallei* in infected farmers from South Goa India.

This case series study investigates the epidemiological factors, clinical manifestations, and drug susceptibility patterns of 12 microbiologically confirmed cases of melioidosis at South Goa Tertiary care hospital, over a 3 years period. The diagnosis of Burkholderia infection was established through culture of blood or specific body fluids (such as joint fluid aspirate or pus aspirate) on Ashdown's medium/Sheep Blood agar. The study reveals that Burkholderia infection is most frequently observed in middle-aged and elderly individuals, with a predominance among males and a notable association with diabetes mellitus. Field workers Like paddy field farmers represent the most common occupational group affected by this infection. Monobactams are frequently effective against Burkholderia strains; however, there is an emerging challenge posed by strains resistant to ceftazidime, highlighting the need for vigilant clinical management strategies.

Keywords: *Burkholderia pseudomallei*; Farmers; Ceftazidime; Antibiotic Resistance; Melioidosis

Abbreviations

µg: Microgram; µl: Microlitre; µM: Micromolar; ATCC: American Type Culture Collection; BAM: Bacteriological Analytical Manual; BHI: Brain Heart Infusion (broth); BP: Base Pairs; CLSI: Clinical and Laboratory Standards Institute; CFU: Colony Forming Units; CAI: Community-acquired Infection; UTI: Urinary Tract Infection

Introduction

Melioidosis, caused by the bacterium *Burkholderia pseudomallei*, manifests with a diverse clinical spectrum encompassing asymptomatic infection, chronic pneumonia, leg ulcers, septic arthritis, liver abscess, splenic abscess, brain abscess, and septicemic shock. First described in 1912 by Krishnaswami and Whitmore, the

disease is prevalent in Southeast Asian countries, including India. Notably, a considerable number of cases have been reported from Indian states like Goa, Karnataka and Tamil Nadu. The upsurge in cases of diabetes mellitus, chronic kidney disease, and other immunocompromised states has contributed to the increased incidence of melioidosis [2,3]. Furthermore, its resemblance to tuberculosis has led to cases where patients were initially treated for tuberculosis until a definitive diagnosis of melioidosis was established.

Our estimates suggest that melioidosis is severely underreported in the 45 countries in which it is known to be endemic and that melioidosis is likely endemic in a further 34 countries which have never reported the disease. The large numbers of estimated cases and fatalities emphasise that the disease warrants renewed attention from public health officials and policy makers [4].

Burkholderia pseudomallei is a small, gram-negative, oxidase-positive, motile, aerobic bacillus with occasional polar flagella. Its characteristic bipolar "safety pin" appearance is observed on gram staining. The organism is commonly found in soil and surface water in endemic regions. Human infection can occur through percutaneous inoculation, inhalation, aspiration, or ingestion. Immunocompromised individuals, particularly the elderly suffering from conditions like diabetes mellitus and alcoholism, are at heightened risk of infection.

Upon infection, the activation of toll-like receptor-5 (TLR-5) by lipopolysaccharide (LPS) leads to the rapid recruitment of innate immune cells such as neutrophils, macrophages, and natural killer cells. The incubation period for melioidosis can vary from a few days to several months [6,7].

Pneumonia represents the most common clinical manifestation of melioidosis, which can manifest as acute fulminant or chronic, resembling tuberculosis. Other clinical presentations include multiple abscesses in the lungs and various internal organs, skin abscesses or ulcerations, soft tissue abscesses, joint pain, septic arthritis, and a bacteremic phase characterized by sepsis and septic shock. Additionally, melioidosis can lead to severe complications such as encephalomyelitis and brain abscesses [6].

Definitive diagnosis of melioidosis necessitates a positive culture of *Burkholderia pseudomallei*. Blood, urine, synovial fluid, throat swab, and skin swab specimens can be utilized for culture purposes. Although *B. pseudomallei* readily grows in commercially available blood culture media, it is often misidentified as Pseudomonas or other *Burkholderia* species. Ashdown's medium, containing gentamicin, can be employed for the selective growth of *B. pseudomallei* [17].

Identification of *B. pseudomallei* can be accomplished by combining the use of commercial API 20NE or 20E biochemical kits with a simple screening system involving Gram staining, oxidase reaction, typical growth characteristics, and resistance patterns to antibiotics such as polymyxin B and aminoglycosides [1].

Burkholderia pseudomallei displays intrinsic resistance to numerous clinically used antibiotics and has a propensity to induce relapse even after successful initial and maintenance therapy. Furthermore, it possesses the capability to be employed as a biological weapon and can lead to infections with severe ramifications, particularly among individuals with immunosuppression, alcoholism, diabetes mellitus, and chronic renal failure [2].

Treatment for melioidosis typically involves intensive therapy followed by eradication therapy. For intensive therapy, a minimum duration of 10-14 days is recommended with one of the following antibiotics:

- **Ceftazidime:** 50 mg/kg every 6 hours (up to 2 g per dose)
- **Meropenem:** 25 mg/kg every 8 hours (up to 1 g per dose)
- **Imipenem:** 25 mg/kg every 6 hours (up to 1 g per dose).

Additionally, any of the above antibiotics can be combined with trimethoprim-sulfamethoxazole (6/30 mg/kg, up to 320/1600 mg) every 12 hours, especially for cases involving neurologic, cutaneous, bone, joint, and prostatic melioidosis. But now new drug Ceftazidime avibactam has introduced and work well in case of multidrug resistance case.

For eradication therapy, a minimum duration of 3 months is recommended with trimethoprim-sulfamethoxazole (6/30 mg/kg, up to 320/1600 mg) every 12 hours [17].

Melioidosis poses a significant threat as a cause of fatal community-acquired pneumonia, with *Burkholderia pseudomallei* being widespread in various geographic areas worldwide, leading to spo-

radic cases and outbreaks. Therefore, periodic assessment of antibiotic susceptibility patterns of *B. pseudomallei* is crucial to guide initial empiric therapy. This study presents the clinical presentation and antibiotic susceptibility data of *B. pseudomallei* isolates from patients with melioidosis treated at South Goa tertiary care hospitals for a period of time [3].

Materials and Methods

This retrospective analysis examined 12 cases of melioidosis admitted to South Goa tertiary care hospitals and in between a time period of 3 years. Patients with culture-proven melioidosis were identified and included in the study.

Ashdown's medium, a selective medium for *Burkholderia pseudomallei*, was utilized for culturing the bacteria. Antibiotic susceptibility testing was performed using either the disk diffusion method or BACTEC, employing the automated BioMérieux Vitek2 compact system and also used Molecular tools to further confirmation of identification of bacteria by using sanger sequencing.

Report of microbial identification

- DNA was isolated from the culture provided by the scientist. Its quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA has been observed.
- Fragment of 16S rDNA gene was amplified by 27F and 1492R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel.
- The PCR amplicon was purified to remove contaminants.
- Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer
- Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software.

Clinical specimens, including blood, cerebrospinal fluid (CSF),urine, pus, bone marrow, and knee joint aspirate, obtained from suspected sites of infection, were sent to the Microbiology laboratory for culture and antibiotic sensitivity testing.

Result

Among the 12 patients included in the study, 8 (60%) were male and 4 (40%) were female. Five (40%) patients were aged less than 40 years, while the remaining 7 (60%) were over 40 years old. The majority of patients were from border areas of Goa, Karnataka and Maharashtra.

Of the 12 patients, 8 (60%) had diabetes, 3 (20%) were alcoholics, and 1 (10%) had both diabetes and alcoholism. All patients (100%) presented with fever lasting more than 2 weeks, indicating a common initial symptom. Field workers Like paddy field farmers represent the most common occupational group affected by this infection.

Pulmonary involvement was observed in 6 (50%) patients, with 4 (30%) exhibiting bilateral pneumonia and 2 (20%) showing pleural effusion. Additionally, 4 (30%) patients had skin ulcers, while 2 (20%) had septic arthritis, splenic micro abscesses, portal peri-splenitis, and portal vein thrombosis. 2 (20%) patients presented with a brain abscess. Furthermore, 4 (30%) patients presented with sepsis and acute kidney injury (AKI).

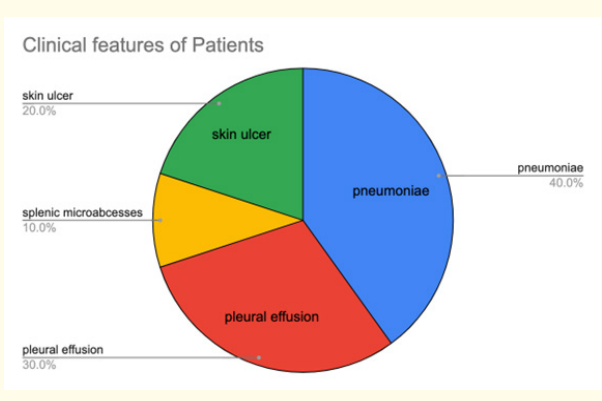

Figure 1: Clinical presentation.

Antibiotic susceptibility of patients

Among the 12 patients, *B. pseudomallei* was isolated from different clinical specimens: synovial aspirate from 2 patient with septic arthritis, bronchoalveolar lavage from 1 patient with pneumonia, pus aspirate from 1 patient with a skin abscess, and blood cultures from 8 patients.

Antibiotic susceptibility testing revealed the following results:

- **Ceftazidime:** 10 (80%) isolates showed sensitivity, while 2 (20%) isolates were resistant.
- **Levofloxacin:** 3 (30%) isolates showed sensitivity.
- **Imipenem:** 7 (70%) isolates showed sensitivity.
- **Cotrimoxazole, minocycline, and meropenem:** All 10 (100%) isolates showed sensitivity.

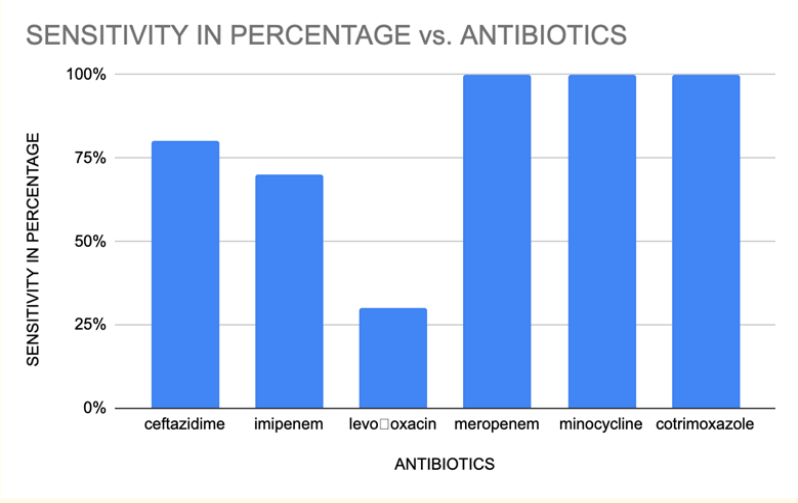

Figure 2: The antibiotic sensitivity pattern of the isolates.

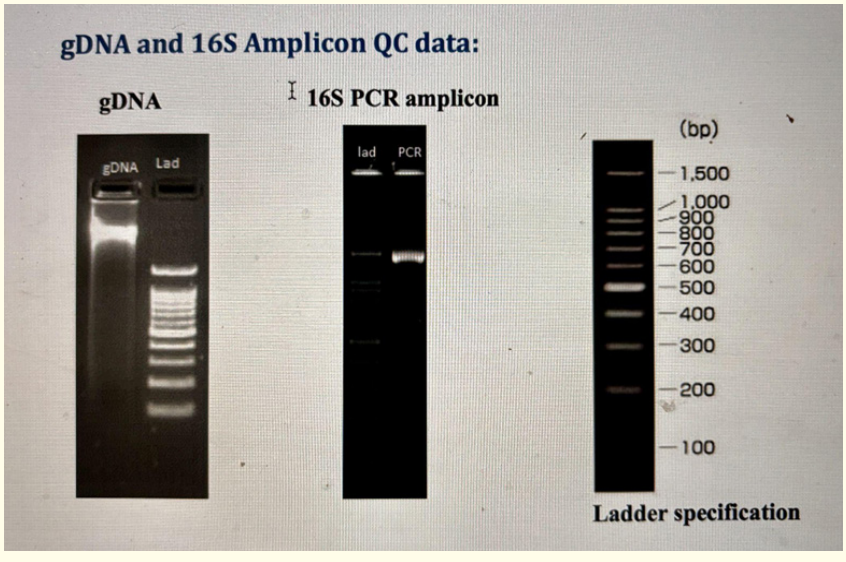

Figure 3: gDNA and 16S Amplicon QC data.

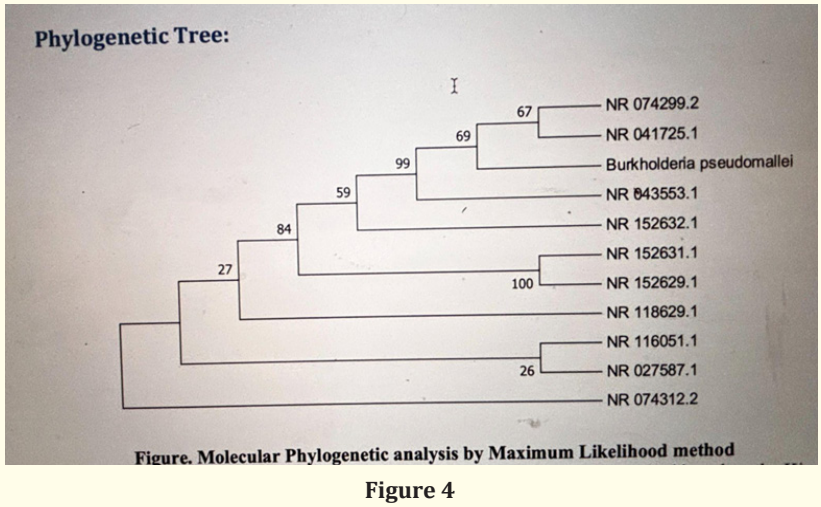

Figure 4

Sample which was labelled as *Burkholderia pseudomallei* showed high similarity with *Burkholderia pseudomallei* based on nucleotide homology and phylogenetic analysis

Serotyping of *Burkholderia pseudomallaie* using multiplex PCR.

Accession numbers obtained from NCBI after submission of gene sequences.

Sr. NO.	Isolate identified from Urine sample by VITEK2	% Similarity	Isolate identified as	Accession number (NCBI)
1	*Burkholderia pseudomallei*	99%	*Burkholderia pseudomallei*	PP109375

Table 1a: Isolate identification and accession number the best bacterial isolate from Urine Sample.

Name of the institute	Specification
EUROPHINE (National Centre for Cell Science)	16 S rRNA gene sequencing
NCBI (National Centre for Biotechnology Information)	Obtaining gene accession number

Table 1b: Laboratory facility used from other than host institute.

Study Area: Description of GOA region with maps.

Figure 5

Discussion

Melioidosis can be contracted through direct exposure to contaminated soil, ingestion of untreated water, or inhalation of dust or contaminated aerosols. Individuals with underlying conditions such as diabetes, renal or liver disease, and various forms of immunosuppression are particularly vulnerable. *B. pseudomallei*, the causative bacterium, is commonly found in the soil and stagnant water of paddy fields and wetlands in endemic regions, with contaminated soil and water being the primary sources of infection [7]. Studies have demonstrated an association between melioidosis and activities like paddy cultivation, as well as the presence of *B. pseudomallei* in drinking water sources [15].

The bacterium has been isolated from various environmental niches, including natural water environments and unchlorinated drinking water wells in endemic areas. Its remarkable ability to thrive in harsh environments contributes to its long-term persistence in such settings [16].

This study presents the initial evidence of *B. pseudomallei* presence in soil and natural water reservoirs in Goa India. Significantly, it underscores the importance of obtaining a comprehensive exposure history from patients to identify specific sites harboring the bacterium.

The detection of *B. pseudomallei* in natural water reservoirs in India, especially considering that less than half of households have access to piped and treated water. Given that household wells are commonly utilized for drinking, cooking, and bathing purposes, the prevalence of melioidosis cases presenting with parotid and abdominal abscesses in India may indicate transmission through ingestion of contaminated water [17].

Figure 6: Unchlorinated domestic wells and one each from still water in a paddy field, stagnant water from a garden and a perennial spring.

Natural water sources of *Burkholderia pseudomallei*. (A, B) unchlorinated domestic well (C) Stagnant water in paddy field (D) perennial spring "Bubula".

The widespread distribution of *B. pseudomallei* in the environmental settings of endemic regions and the constant exposure of agricultural communities present significant challenges for the prevention and management of melioidosis. At a community level, if the contamination of water reservoirs is verified through microbial culture, proactive interventions such as the chlorination of wells or the implementation of water filtration or boiling methods prior to consumption can be advised to mitigate the likelihood of melioidosis transmission [16].

Conclusion

This study provides evidence of the presence of *B. pseudomallei* in soil and natural water sources in Goa India, highlighting the potential risk of melioidosis among populations utilizing untreated water from these sources. While there is currently no vaccine available for melioidosis, public education regarding the hazards of consuming untreated water and increased awareness among clinicians to consider melioidosis in the differential diagnosis of febrile illnesses in regions lacking treated water supplies could aid in reducing transmission and facilitating early diagnosis. Furthermore, identifying specific environmental sites where *B. pseudomallei* is present may enable targeted soil and water remediation efforts to decrease the bacterial burden and mitigate risk.

Melioidosis is increasingly recognized as a significant bacterial infection and public health concern, presenting with a wide range of clinical manifestations from asymptomatic to potentially fatal septicemia, often with a prolonged incubation period. The emergence of ceftazidime-resistant strains underscores the importance of vigilance among treating clinicians and microbiologists. Early clinical suspicion, prompt sampling and culture, and heightened awareness among healthcare providers are crucial for effective infection control measures.

Acknowledgements

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Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

Ethical Approval

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

Competing Interests

The authors have declared that no competing interests exist.

Bibliography

1. Samy R P., *et al*. "Melioidosis: Clinical impact and public health threat in the tropics". *PLOS Neglected Tropical Diseases* 11.5 (2017): e0004738.

2. Mukhopadhyay C., *et al*. "Melioidosis in South Asia (India, Nepal, Pakistan)".

3. Brosh-Nissimov T., *et al*. "Case Report: Imported Melioidosis from Goa, India to Israel, 2018". *The American Journal of Tropical Medicine and Hygiene* 101.3 (2019): 580-584.

4. Limmathurotsakul D., *et al*. "Predicted global distribution of Burkholderia pseudomallei and burden of melioidosis". *Nature Microbiology* (2016).

5. Corea E., *et al*. "Melioidosis in Sri Lanka: An emerging infection". *Sri Lankan Journal of Infectious Diseases* (2012).

6. Sarovich D S., *et al*. "Development of ceftazidime resistance in an acute Burkholderia pseudomallei infection". (2012).

7. Gassiep I., *et al*. "Human melioidosis". *Clinical Microbiology Review* 33.2(2020):e00006-19.

8. Princess I., *et al*. "Melioidosis: An Emerging Infection with Fatal Outcomes". (2017).

9. Behera B., *et al*. "Ceftazidime resistance in Burkholderia pseudomallei: First report from India (2012).

10. Mc S B., *et al*. "Melioidosis: An emerging infection in India" (2017).

11. Dance D. "Treatment and prophylaxis of melioidosis". *International Journal of Antimicrobial Agents* 43.4 (2014): 310-318.

12. Hassan M R A., *et al*. "Antimicrobial susceptibility patterns of Burkholderia pseudomallei among melioidosis cases in Kedah, Malaysia". (2014).

13. Zimmermann R E., *et al*. "Rivers as carriers and potential sentinels for Burkholderia pseudomallei in Laos". *Scientific Reports* (2018).

14. Jayasinghearachchi H S., *et al*. "Biogeography and genetic diversity of clinical isolates of Burkholderia pseudomallei in Sri Lanka". (2021).

15. McRobb E., *et al*. "Tracing melioidosis back to the source: using whole-genome sequencing to investigate an outbreak originating from a contaminated domestic water supply". *Journal of Clinical Microbiology* 53.4 (2015): 1144-1148.

16. Limmathurotsakul D., *et al*. "Activities of daily living associated with acquisition of melioidosis in northeast Thailand: a matched case-control study". (2013).

17. Sreenidhi H C., *et al*. "STUDY OF EPIDEMIOLOGICAL FACTORS, CLINICAL SPECTRUM AND ANTIBIOTIC SUSCEPTIBILITY OF MELLIODOSIS IN GOA MEDICAL COLLEGE". *International Journal of Scientific Research* 8.2 (2019): 2277-8179.