

***B. pseudomallei* isolate from soil adhering to underground pipeline repair in market place at Delhi highlights importance of improvising policies addressing environmental pathogens.**

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Abstract

Background: *Burkholderia pseudomallei* is a Gram –negative bacteria that can cause the fatal disease of Melioidosis with mortality ranging from 21-40% in different countries. Further, *Burkholderia pseudomallei* is a highly resilient environmental pathogen and can live as a free-living bacterium in soil, water and a parasite within amoeba, fungi, and animal cells. It is known to have developed adaptation to stressful environmental factors like salinity, iron levels, and oxidation. Widely this pathogen is associated with agricultural activities, construction activities and hence farmers, laborers being the important target groups. The other risk factor for this disease includes Diabetes mellitus and India having increasing incidence of diabetes cases is a matter of concern as for the prevalence of melioidosis. Since certain states and places in India have higher incidence of diabetes like Delhi, it is quite possible to have higher incidence of Melioidosis in these places.

Approach: Environmental Screening of urban village that has a convergence of vegetable market and urban households. **Results:** In this report, we demonstrate the presence of *Burkholderia pseudomallei* in a 3-4 feet deep pipeline repair pit with a rusted iron pipeline in a busy market area in Kotla, South Delhi.

Conclusion: *Burkholderia pseudomallei* being an environmental pathogen, it becomes essential to focus on policies that govern environmental pollution. Thus, the construction and demolition (C&D) policies that govern the dismantling of underground repair pipelines, especially in crowded areas, needed to be followed as it is not only dust that pollutes but also human pathogens.

Background

Melioidosis is a disease caused by the Gram-negative environmental bacterium *Burkholderia pseudomallei* that exists in soil, water and can infect through percutaneous, inhalation and ingestion routes [1,2]. It is a life threatening disease resulting in 89000 deaths worldwide in an year which is roughly equivalent to deaths caused by measles and more than the death caused by dengue and leptospirosis but yet to be recognised of equal public health importance by international health

organisation [3]. Melioidosis is a class III bio threat agent which should be of prime concern while isolated from any patient in the laboratory [3].

The disease primarily involves respiratory system (40-60%), followed by gastrointestinal system (10-30%), cardiovascular system (10-40%), urinary tract system (14-28%), skin (13-24%), musculoskeletal (4-14%), and central nervous system (1-5%) [3]. Relapse after many years is also known to occur with no clear knowledge of its occurrence [3]. The common targeted organs include lungs, liver, spleen, skin, genitourinary and bone and joints.

Burkholderiapseudomallei is known to survive in harsh environments for many years [4]. It has been shown that soil with 20% moisture can help harbor this pathogen for 439 days [5]. It has also been shown that *Burkholderiapseudomallei* is present in soil depths of >30cm and has poor nitrogen, carbon, and high water content [6]. In general, the presence of this bacterium is associated with rainfall since it adds to the soil moisture, and dark clouds are known to protect soil bacteria from the UV spectrum of sunlight [7]. To survive the harsh environment of soil, *Burkholderiapseudomallei* can be found to be associated with protozoans like *Acanthamoeba* spp, *Hartmannella* spp., and *Naegleria* spp [8]. It can even be associated with plant legumes [9]. Although *B. pseudomallei* can survive in soil with low iron content, high iron content is known to increase its persistence [4,10]. It is also known that *B. pseudomallei* is associated with soft bore water of acidic pH, high iron level, and low salinity.

The occupational risk factors are those with farming, construction, children with the tendency to play with soil, and the health factors that can result in higher risk include diabetes, Thalassemia, chronic renal disease [4], and cystic fibrosis patients who tend to have higher salt content in their lungs than normal [11]. The symptoms include widespread and non-specific, like fever, septicemia, and localized skin manifestation, pneumonia [4]. The route of transmission includes inhalation, ingestion, and through skin abrasions. In a study, this pathogen was found in slaughtered pigs brought to market areas in Uganda [12]. One other study showed that the ambient distribution of *Burkholderiapseudomallei* in Taiwan during typhoon season indicated its presence to be 6.94 copy/m³ [13]. In agricultural workers, infection through the skin is considered the main route, and in common people, inhalation and ingesting contaminated water is considered the primary route [14]. Further unconventional outbreaks involving lab personnel garment contamination have been reported in the USA [14]. Melioidosis has the involvement

of the lungs, liver, and the skin. Children with tendency to play in soil [15] and farmers have higher chances of getting exposed to this soil pathogen [16].

Importance of Melioidosis surveillance in India

India having larger incidence of diabetes and large population involved in rural agricultural activity [17], places it high risk population for melioidosis. The predicted burden of melioidosis in India is about 52000 cases and 31,425 deaths annually [14]. Due to misdiagnosis with pseudomonas infection and tuberculosis, melioidosis gets under reported [16]. Such factors results in higher rate of death. The key intervention for melioidosis includes avoiding contact with loose, muddy soil in endemic areas and access to safe drinking water. Further it has been shown that agriculture, gardening, fishing and road construction in which exposure to wet soil occurs act as risk factors for melioidosis infection [18]. In low and middle income countries including India, large unregulated vulnerable workforce exists in construction and demolition activities and hence risk mitigation is required [19]. Moreover the lack of awareness about melioidosis even among literate people augments the problem. Amidst the illiterate workers, it is a common scene in India to notice workers sitting on road pavements or amidst construction materials to consume their food that can result in ingestion of dust harbouring the pathogen.

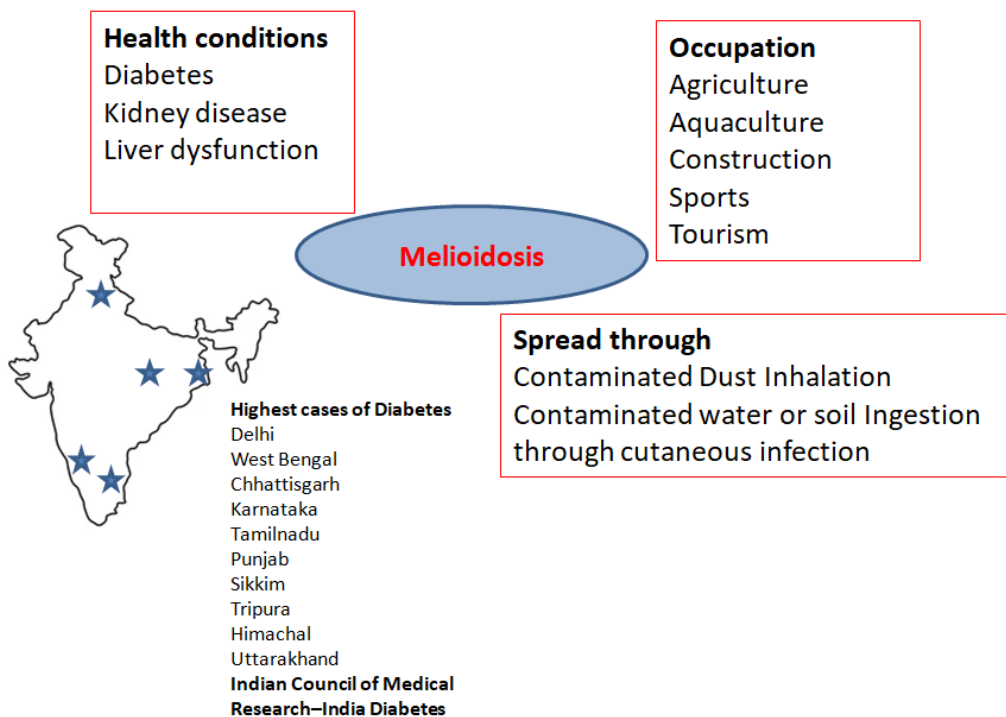


Figure 1 Risk factors of Melioidosis

India considered to be the diabetes capital of the world has higher incidence of diabetes in Delhi, West Bengal, Chhattisgarh, Karnataka, Tamilnadu, Punjab, Sikkim, Tripura, Himachal, Uttarakhand. The risk factors for Melioidosis include diabetes and occupation like agriculture, construction activities, sports, tourism in which exposure to dust is high.

Gap Analysis

Currently there are no policies that directly address the environmental spread of *B. pseudomallei* in India. Since *B. pseudomallei* being spread through the environment and the favorable environmental conditions for the pathogen could be addressed upon, gap analysis was done to analyse possible shortcomings.

a. Current knowledge about the environmental presence of *B. pseudomallei*

It is known that the following factors favor *B. pseudomallei* [20] that includes Anthrosol and Acrisol soil (paddy farmlands), Residual crop burning leading to soil nutrient depletion, Global warming, Rise in temperature and unplanned urbanization can lead to spread of this pathogen. It is further known that the following environments favor the pathogen [21] which includes surface water of rice paddies, fields newly planted with oil palm, drains, gardens and playgrounds in endemic areas.

b. Possible gaps in existing policies

This brief report brings out other potential sources next to human occupation. One such is repair works and demolition works in open market areas and transporting them in open rickshaws. Although there are existing policies in India as stated in environmental clearance.nic.in that state, vehicles carrying construction and demolition wastes (C&D Wastes) need to be covered with plastic sheets to prevent dust emissions, it does not state that it can't be dismantled in public places as it considers only dust emission but not pathogenic bacteria [22].

c. Important facts as highlighted in policies outside India

US Environmental protecting agency [23] states the distance between drinking water pipe and sewer should be 10ft. Further, in saturated soil conditions, microbes can move several meters in quickly [24] and this transport could be aided by water flowing out of the sewer (exfiltration). Hence, it could be dangerous to have sewers and drinking lines with leakage adjacent to one another as it can contaminate.

Methodology

This work was conducted as part of the Indian Council of Medical Research funded project on Melioidosis. The coordinating Centre from Delhi is ICMR Headquarters and the main Referral Centre for laboratory analysis for culturing and identification of this pathogen is Department of Microbiology, Kasturba Medical College which is authorized to handle this pathogen as it is certified with IBSC. Samples from this project sites as well as samples sent from other institutes in India are analyzed at KMC, Manipal for detection of *B. pseudomallei*. This soil sample from the Kotla market area in South Delhi was collected in March 2023 while there was rainfall for few days from a freshly dug pipeline repair pit. The analysis of the sample was performed at the department of microbiology at KMC, Manipal.

Results

Soil adhered to underground pipeline under repair (Figure 2) at the geo location 28.57146135895692, 77.22482533623553 tested positive for the pathogen *Burkholderiapseudomallei*.



Figure 2 Pictures from pipeline repair pit from which the soil tested positive for the soil pathogen *B. pseudomallei*

A. Pipeline repair pit. B. Pipeline repair pit filled with leakage water. C. Scrapped rusted metal pipeline transported in cycle rickshaw without cover. D. Pipeline leakage filled pit with intersectional pipes. E. Water collection from intersectional pipes. F. Food vendor near pipeline-repair, selling rotis that can lead to ingestion of soil dust particles that can harbor the pathogen.

Recommendations

- Dismantling and breaking of underground pipelines in crowded market areas should be prevented since underground pipelines could harbor *B. pseudomallei* and could easily result in spread of this pathogen. Instead, such pipelines could be removed intact and taken to a remote place for scrapping.
- Also, if such repairs of municipal pipelines come under construction and demolition act is still being determined. That could be recommended if such municipal repairs are not listed in the C & D Act,
- Another recommendation is apart from dust emission, there is also the presence of pathogenic bacteria from C&D waste and hence, can't be scrapped in public places.
- Eateries located next to a demolition site could be notified not to operate while the demolition is on-going as dust from demolition could contaminate the eateries.

Discussion: Since the pathogen is known to have increased persistence with soil of higher iron content, rusted iron pipes that are underground for transport of human sewerage could be hypothesized as potential sources for this pathogen. In urban areas, additional risk factors like population with higher incidence of diabetes could be used as factors for shortlisting areas that could have the environmental presence of this pathogen, possibly introduced through human sewerage to the environment. Delhi is one of the places in India that has higher population of people with diabetes as indicated in a recent study by ICMR [25]. Hence isolating *B. pseudomallei* from soil next to a pipeline repair in a crowded market place in Delhi could be attributed to higher population of diabetes that could include positive cases and the leaked sewerage causing an environmental contamination. The pathogen could have survived in the leaked pipeline soil due to favorable factors [5,6,7] like higher moisture, higher iron content from leached pipeline. Further since the repair pipeline was 3-4 feet beneath the soil surface, it would have protected the isolate from sunlight, thus enabling its detection.

Importance to Society: The presence of such pathogen in underground pipeline repair pits and transport of demolished pipeline metals in open hand pulled vehicle through crowded market place could result in environmental spread of this pathogen. It has been shown in literature that dust can harbor microbes including 3.8×10^2 – 1.6×10^8 CFU/g bacteria and 1.5×10^2 – 6.5×10^6 CFU/g fungi that included *Bacillus*, *Pseudomonas*, *Actinomyces*, *Corynebacterium*, *Clostridium*, *Rickettsia*, *Penicillium*, *Aspergillus*, *Alternaria*[26]. Hence it becomes important to follow policies that govern construction and demolition in which scrapped or demolished materials should be transported not in open and not to generate dust by breaking of such materials in crowded areas. Moreover inadequacy in environmental public health related policies has been addressed in India [27]. Taking into account all the above mentioned factors, it becomes essential to work upon policies related to environmental public health that involves dealing with dust since dust not only results in non communicable diseases like cancer, asthma but can act as a carrier for respiratory pathogen.

Conclusion

B. pseudomallei is an important environmental pathogen and it being isolated from urban villages indicates that importance should be given to existing policies related to environmental pollution and that on construction and demolition. Further new policies could be introduced to curb the spread of this pathogen.

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