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A Novel Air Sampling Device for the Identification of *Mycobacterium leprae* In Leprosy Care Facilities

Preethi R[1], P Agastian[2], P Ravi[3], Sanjith Kumar R[4], A Manimaran[5]

[1]Department of Advanced Zoology and Biotechnology, Government Arts College, Nandanam, Chennai 600 035, Tamil Nadu, India.
Email: *preethiravi107[at]gmail.com*

[2]Department of Plant Biology & Plant Biotechnology, Loyola College, Chennai 600 034, Tamil Nadu, India

[3]Department of Biochemistry, Central Leprosy Teaching and Research Institute (CLT&RI), Thirumani, Chengalpattu 603003, Tamil Nadu, India

[4]School of International Bio design, All India Institute of Medical Science (AIIMS), New Delhi 110016, India

[5]Department of Advanced Zoology and Biotechnology, Government Arts College, Nandanam, Chennai 600 035, Tamil Nadu, India.
Corresponding Author Email: *mailtomanimaraan[at]gmail.com*

Abstract: ***Leprosy, a chronic infectious disease caused by the bacterium Mycobacterium leprae, remains a significant public health concern in endemic regions, despite advances in treatment and early identification of patients. Airborne transmission routes have been suggested, but remain poorly studied partly due to the lack of affordable and specific sampling tools. Commercial air samplers are costly, complex, and not designed for detecting Mycobacterium species in air, which requires large-scale environmental surveillance. To address this gap, we developed a simple, low-cost, and easily sterilizable assembly using basic laboratory materials, suitable for air sampling. The assembly employed a suction pump with a flow rate of 15 L/min at <5 mm Hg hermeticity, ensuring sterility by preventing direct contact between the pump and the collection medium. Environmental air sampling was conducted in leprosy-endemic dwellings, slit-skin smear examination rooms, and experimental animal facilities. Air samples were bubbled through Hank's Balanced Salt Solution (HBSS) buffer to preserve microbial viability. Microscopic examination revealed the presence of acid-fast and alcohol-fast organisms resembling M. leprae in air samples. PCR amplification of the 16S rRNA gene detected a 227 bp fragment for* **Mycobacterium** *species and a 173 bp fragment specific for* **M. leprae***, confirmed by gel electrophoresis. This innovative device demonstrated efficiency, sterility, and versatility, offering other applications in microbial inoculation, harvesting, and environmental monitoring. This study highlights its potential as a practical tool for environmental surveillance in healthcare and endemic settings, supporting preventive approaches in leprosy research and occupational health safety.***

Keywords: Leprosy, *Mycobacterium leprae*, Air sampling, PCR detection, Healthcare biosafety

1. Introduction

Air is a significant medium for the transmission of many microbial infections, including those caused by mycobacteria, such as *Mycobacterium leprae*. Airborne microbial transmission is notably high, especially in healthcare environments and leprosy-endemic regions. In leprosy, transmission occurs predominantly through the inhalation of bacilli shed in upper respiratory secretions, with the nasal mucosa acting as the principal portal of both entry and exit for *M. leprae* [1,2]. Airborne mycobacteria are often associated with dust or particulate matter originating from water or other environmental sources. Consequently, microbial air surveillance has been emphasized, especially in healthcare environments, where the airborne bacterial load correlates with infection risks [1,3,4]. Since *M. leprae* cannot be cultivated *in vitro*, diagnosis relies mainly on clinical evaluation of the patient's skin and neurological status. Early detection is essential to ensure timely and appropriate treatment, which helps prevent complications and physical disabilities that affect both social and occupational life, and play a major role in perpetuating the stigma and discrimination associated with the disease. [1].

Dr. Hanks, renowned internationally as a leading leprologist and pioneering scientist, developed one of his key innovations, Hank's Balanced Salt Solution (HBSS), which has since become a standard tool in laboratory practice [5]. HBSS buffer helps preserve the viability of *M. leprae* during short-term transport from biopsy specimens (such as skin scrapings or tissue samples). Therefore, in leprosy research and diagnosis, *M. leprae* are often suspended in HBSS buffer for experimental transmission, storage, and animal inoculation. Up to the present, the bacteria have not been successfully cultivated in artificial media and can only be propagated *in vivo*, with animals such as the mouse (footpad) and armadillo (liver) being successful hosts [6]. This is the main reason why the HBSS buffer was also used in the air sampling device for collecting air samples into the buffer.

Numerous studies have highlighted the efficacy of air sampling devices and techniques for environmental monitoring, as well as considerations regarding the cost and practicality of these devices [7-12]. Health and environmental safety require monitoring airborne microbes to determine the degree and source of contamination, ensuring reliable results for both preventive measures and occupational safety [3]. Recent developments in air sampling assemblies have enhanced the sensitivity and specificity for detecting *M. leprae*, capable of identifying as little as 10 femtograms (fg) of purified bacillary DNA [13]. Monitoring air for the presence of *M. leprae* is therefore critical to understand the environmental reservoirs of infection, to assess transmission risks, and to support strategies for infection control in both

hospital and endemic community settings. This study aims to design, implement, and evaluate a cost-effective air sampling device for detecting *Mycobacterium leprae* in clinical and endemic settings, thereby contributing to environmental surveillance strategies in leprosy care.

2. Literature Survey

The nose and skin serve as the principal routes of *M. leprae* infection and transmission [14,15], with nasal mucosa involvement often occurring early, even prior to the appearance of lesions at other sites [16]. Active air sampling using the Surface Air System Sampler (SAS, International PBI, Milan, Italy) at 180 L/min beside Inhibitory Mold Agar (IMA) plates has been employed, though ISPESL and ISO guidelines lack precise recommendations for sampling volume or duration [17].

Impingers, bubble tubes collecting airborne matter into liquid media, capture or react with target particles. For sampling, a new impinger with the recommended liquid is used; in area sampling, it is placed with the pump and trap in the target site, and in personal sampling, mounted at the worker's breathing zone with the pump on the belt. Glass impingers and liquids require careful handling, and sampling start time and details are recorded [18].

A relative centrifugal force (RCF) of 1,800 – 2,400 × g for 15–30 minutes is recommended for the recovery of *Mycobacteria* [19]. Lowenstein-Jensen medium with added antimicrobials supports mycobacterial growth and niacin testing [20]. Acid-fast bacilli (AFB) staining, developed by Ziehl and modified by Neelsen, differentiates acid-fast from non–acid-fast bacteria [21]. PCR studies have detected *M. leprae* DNA in nasal swabs and mucosa biopsies of healthy individuals in endemic areas [22-24].

3. Materials and Methods

Study Area and Sampling Site

The study was conducted at the Central Leprosy Teaching and Research Institute (CLT&RI), Thirumani, Chengalpattu, Tamil Nadu, India. This premier institution is under the Ministry of Health and Family Welfare, Government of India. Air sampling was conducted in targeted hospital zones to assess microbial load. During each sampling event, ambient temperature and relative humidity were recorded and maintained within a limit. This study was conducted with the permission from the head of the institution.

Air Sampling Apparatus

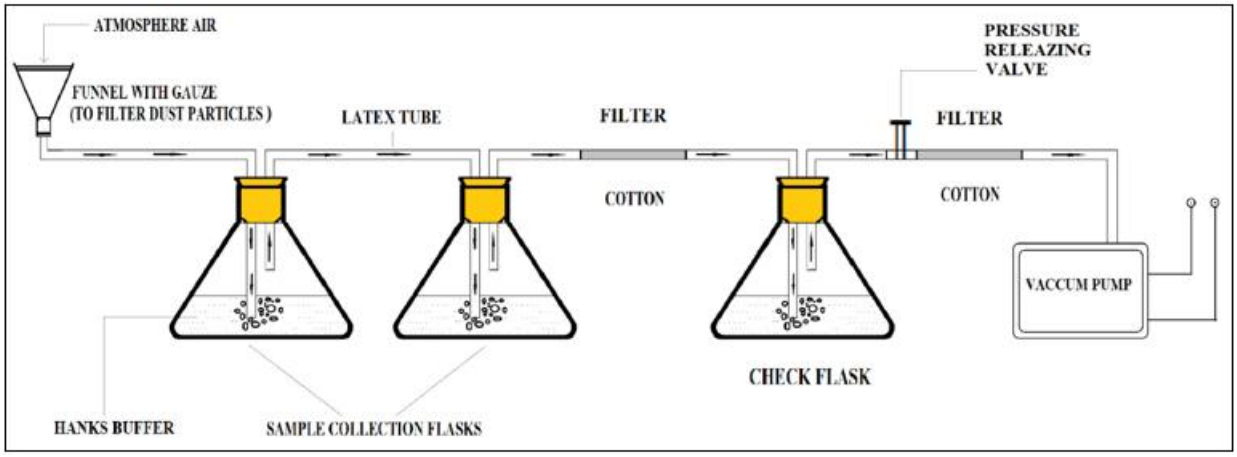

Figure 1: Detachable, sterilizable air sampling setup using simple lab glassware.

This air sampler works on the principle of an impingement sampler. This indigenously designed and developed air sampling assembly was employed for the collection of airborne microorganisms (Figure 1). The apparatus consisted of a funnel fitted with sterile gauze to filter coarse particles in the air. The funnel was connected through sterile latex tubing into two 500 mL rubber corked collection flasks containing sterilized Hanks Balanced Salt Solution (HBSS). HBSS is available commercially, containing 1×W/Sodium Bicarbonate with Phenol red. A downstream check flask was incorporated to prevent backflow of air contaminants. Cotton plugs were placed at connecting points to filter contaminants' entry into the sample flask. A pressure-releasing valve was incorporated in the sampling assembly to regulate even airflow, ensuring uniform suction throughout the sampling process. The system was powered by a 12V DC, 15 L/min 555 vacuum pump, to maintain a constant air suction rate. A device was fabricated using simple laboratory wares, including autoclavable latex rubber tubing, 500 mL conical flasks, a glass funnel, and rubber corks with two bores. As the entire unit is made of autoclavable materials with cotton filters incorporated at critical points, to ensure a complete aseptic operation. The device is also reusable for multiple sampling events. This cost-effective and multipurpose device design provides a valuable support tool for the basic research activities in microbiology and related biological sampling processes.

Sampling Procedure

Air samples were collected at 15 L/min for 30 minutes at each site, ensuring uniformity in the total volume of air collected across all sampling locations. The airborne microorganisms were trapped completely in the HBSS buffer solution contained in the sterile collection flasks. Immediately after sampling, flasks were sealed, labeled, and transported to the microbiology laboratory under cold-chain conditions. Before assembly, all components of the air sampler, including HBSS-containing flasks, rubber corks fitted with glass tubes, latex

rubber tubing, glass connectors, filters, and funnels, were individually wrapped in aluminium foil and sterilized by autoclaving at 121 °C for 20 minutes at 15 pounds per square inch of pressure (psi). Following sterilization, the apparatus was aseptically assembled in a laminar airflow chamber. During this assembling operation, a sterile filter unit placed between the HBSS containers and the pump served as a safety barrier to prevent contamination of the instrument, operator, and surrounding environment.

Air Sample Characterization

Environmental air samples collected in HBSS buffer were centrifuged at 5,000 × g for 20 minutes at 25 °C. The supernatant was discarded, and the sediment was inoculated on Nutrient Agar (NA), MacConkey Agar (MCA), and Löwenstein–Jensen (LJ) media for the cultivation of *Mycobacterium* species and other bacteria. NA and MCA plates were incubated at 37°C for 24–48 hours, while LJ slants were maintained at 36°C and examined regularly for up to eight weeks. Growth obtained on the media was recorded, and representative isolates were subjected to Gram staining, Ziehl–Neelsen (ZN) staining, and biochemical characterization. Biochemical tests included the catalase test, which detects the enzyme that breaks down hydrogen peroxide into water and oxygen, and the coagulase test, which differentiates *Staphylococcus aureus* from coagulase-negative staphylococci by their ability to clot plasma. For molecular confirmation, genomic DNA was extracted from representative isolates using the CTAB–chloroform method. Samples were then analysed by PCR amplification targeting the 16S rRNA gene using two sets of primers: a 227 bp fragment specific for the amplification of *Mycobacterium* species (including *M. leprae*) and a 173 bp fragment specific for *M. leprae*. PCR products were resolved on agarose gel electrophoresis and compared with a 100 bp DNA ladder to confirm the presence and size of the expected amplicons. Positive bands were recorded and used for subsequent molecular identification.

4. Results and Discussion

Air Sample Culture Findings

Air samples collected from five distinct sites within CLT&RI, namely the Skin Smear Room, Animal House, Women's Block, OPD Block, and Sick Room, exhibited variable microbial growth profiles. The Skin Smear Room and Women's Block showed the highest prevalence of Gram-positive cocci, while the OPD Block demonstrated no detectable growth on either medium, likely due to frequent cleaning practices and superior ventilation. The predominance of Gram-positive cocci, particularly *Staphylococcus* and *Micrococcus* species, is consistent with the well-documented hospital air microbiota. Notably, the Sick Room, housing severely affected patients, displayed microbial patterns suggestive of airborne opportunistic pathogens in confined spaces (Table 1).

Staining and Colony Morphology

Gram staining confirmed the presence of both Gram-positive and Gram-negative cocci. Morphological diversity indicated a mixed microbial environment in all the sample sites except the OPD block. The findings show dominance of Gram-positive organisms in patient dwelling areas, while Gram-negative species were more prevalent in the Animal House and Sick Room (Table 1). Based on the microscopic examination, supported by ZN stain, the presence of *M. leprae* in the air samples was confirmed. This may be due to poor sanitation and through lab animals. This diversity highlights the potential risk of both environmental and zoonotic transmission routes.

Biochemical Characterization

Catalase and coagulase assays corroborated the presence of *Staphylococcus aureus* in the Skin Smear Room and Women's Block samples, consistent with Gram-positive cocci growth observed on culture. The catalase-positive reaction differentiated *Staphylococcus* from *Streptococcus* spp., while coagulase positivity confirmed pathogenic *S. aureus* strains capable of causing healthcare-associated infections. No other sites yielded coagulase-positive organisms, suggesting a comparatively lower risk of staphylococcal exposure in those areas (Table 1).

Molecular Detection of *Mycobacterium leprae*

PCR analysis targeting the 16S rRNA gene demonstrated amplification at 227 bp (general *Mycobacterium*) and 173 bp (specific for *M. leprae*). Positive bands were observed in samples from the Skin Smear Room and Animal House, confirming the presence of *M. leprae* at the genetic level (Figure 2). The detection in the Skin Smear Room is attributable to patient activity and active sample handling, while positivity in the Animal House aligns with mouse footpad inoculation and harvesting activities, highlighting potential occupational and zoonotic exposure risks. The detection of *M. leprae* DNA in these environments underscores the importance of airborne transmission, with the nasal mucosa acting as the principal portal of entry [25,26]. These findings emphasize the need for strict biosafety practices, continuous air quality monitoring, and improved infection-control strategies in leprosy hospitals.

Table 1: Microbial Analysis, Biochemical Characterization, and Molecular Detection of *Mycobacterium leprae* in Air Samples from Leprosy-Endemic and Hospital Areas

Sample Site	Nutrient Agar Growth	MacConkey Agar Growth	Colony Morphology	Gram Stain	Catalase Test	Coagulase Test	*M. leprae* PCR (16S rRNA, 227 bp)
Skin Smear Room	Gram-positive cocci Micrococci	Gram-positive cocci	Smooth, round	Gram-positive cocci	*S. aureus*	*S. aureus*	*M. leprae*
Animal House	Gram-negative cocci	—	Small, moist	Gram-negative cocci	—	—	*M. leprae*
Women's Block	Gram-positive cocci	Gram-positive cocci	Circular, raised	Gram-positive cocci	*S. aureus*	*S. aureus*	Negative
OPD Block	—	—	—	—	—	—	Negative
Sick Room (IP Ward)	Gram-negative cocci pairs	No Growth	Pinpoint colonies	Gram-negative cocci pairs	—	—	Negative

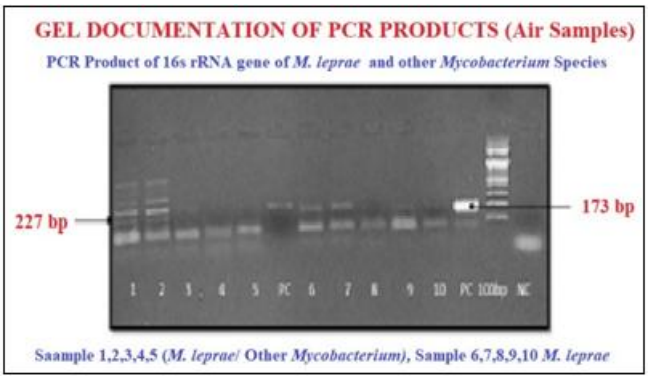

Figure 2: Agarose Gel Electrophoresis Showing PCR Amplification of *M. leprae* DNA

5. Conclusion

This study demonstrates the presence of *Mycobacterium leprae* in air samples collected from high-risk zones of the leprosy hospital, notably the slit-skin smear collection area and the animal house where bacilli were inoculated and maintained in laboratory mice. Developing a simple, low-cost, and reliable air sampling strategy was crucial, particularly given that *M. leprae* cannot be cultured *in vitro*. Careful consideration of parameters such as suction pressure, sterility, temperature, and transport conditions, together with the use of Hanks' Balanced Salt Solution (HBSS) as a stabilizing buffer, ensured the recovery and preservation of viable organisms for downstream analysis. In contrast, lower contamination levels observed in general patient wards suggest that exposure is highly localized to specific operational areas. These findings underscore the need for routine environmental surveillance and strong biosafety protocols to protect healthcare workers, reduce the risk of airborne transmission, and provide a foundation for future surveillance strategies in leprosy-endemic settings.

By enabling low-cost, in situ detection of airborne *M. leprae*, this study offers a valuable surveillance tool that can improve infection control practices and public health preparedness in leprosy-affected regions.

6. Future Scope

This study provides evidence for the environmental presence of *Mycobacterium* species, including *M. leprae*, in air samples collected from hospital and endemic settings, thereby opening multiple avenues for future investigation. The air sampling device developed here can be further refined to improve sensitivity, portability, and ease of use, while advanced molecular tools such as quantitative PCR, metagenomics, and next-generation sequencing could be incorporated to provide more comprehensive insights into pathogen diversity and viability. Large-scale surveillance across varied geographical regions and healthcare environments would help generate valuable epidemiological data and strengthen public health strategies aimed at controlling transmission. Beyond leprosy hospitals, the devices and methodologies employed in this work may also be adapted for monitoring airborne pathogens in other clinical and industrial environments, thereby extending their relevance to broader biosafety applications. Ultimately, the study lays the groundwork for developing improved infection control measures, community awareness programs, and environmental monitoring protocols that can reduce the risk of exposure to vulnerable populations. In addition to air sampling, the assembly can also be adapted for other microbiological applications, such as bottling of biological preparations in small volume aliquots of viable cultures under aseptic conditions. Inoculating bacterial seed cultures into growth media and also harvesting microbial suspensions, etc., can be performed under sterile conditions by reversing the vacuum or suction connection. This inexpensive autoclavable setup is a vital tool for university-level biology students and biotechnologists. The findings of non-mycobacterial species in the air samples will be analysed and presented in my future study.

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Author Profile

Ms. Preethi R (Author) B.E., M. Tech. (Biotechnology), Research Scholar, Department of Advanced Zoology and Biotechnology. She holds research interests in innovative biotechnology applications. She was awarded *Best Innovation of 2023* by Aarupadai Veedu Institute of Technology, Vinayaka Mission's Research Foundation, Chennai, Tamil Nadu, India. She also received funding support from the Tamil Nadu State Council for Science and Technology, Directorate of Technical Education, Guindy, Chennai, under the Student Project Scheme 2019–2020 (Science Stream).

Dr. A. Manimaran (Corresponding Author), M.Sc., M.Phil., Ph.D. (Zoology), Assistant Professor, Department of Advanced Zoology and Biotechnology. He has 20 years of teaching and research experience. He has published more than 20 research papers in reputed journals, with 217 citations and an h-index of 8. He is actively engaged in advancing knowledge in his field and, with a strong academic background, is committed to guiding students, promoting quality research, and contributing to academic excellence.

Dr. P. Agastian S. Theoder M.Phil., Ph.D., (Biotechnology), Associate Professor, Department of Plant Biology & Biotechnology. He has nearly 28 years of teaching and research experience in plant biology, biotechnology, and industrial microbiology. He has published extensively with over 5,600 citations and an h-index of 40. A recipient of the INSA Teachers Award (2021), he has also served as Head of the Department and holds editorial roles in reputed journals.

Mr. P Ravi M.Sc. MBA (Hospital Management), Founder, Sarvamangala Group of Laboratories, Kanchipuram. He holds 33 years of service at the Central Leprosy Teaching and Research Institute. In addition, he served 4 years in other government institutions in India and abroad. Altogether, he has nearly 40 years of experience in laboratory science.

Mr. Sanjith Kumar R B.Des., (Industrial Design), Innovation Fellow, School of International Biodesign, AIIMS Delhi. He has 2 years of experience working with medical technology startups and is currently an Industrial Designer and Innovation Fellow at the School of International Biodesign, AIIMS Delhi. His innovative project has been featured in Yanko Design, reflecting his creative skill in design and development of products.