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A SYSTEMATIC REVIEW: ENVIRONMENTAL OCCURRENCE OF *MYCOBACTERIUM TUBERCULOSIS* AND ITS ASSOCIATED RISK OF TUBERCULOSIS INFECTION AND DISEASE

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ABSTRACT
Tuberculosis (TB) remains a major public health concern, primarily caused by *Mycobacterium tuberculosis* and transmitted through the air. Although airborne transmission is well established, the occurrence of the bacterium in different environmental settings and its possible contribution to exposure and infection remain incompletely understood. This systematic review aimed to examine the environmental occurrence of *M. tuberculosis* and the *M. tuberculosis* complex (MTBC), identify the methods used for detection, and evaluate the evidence linking environmental occurrence to potential exposure, TB infection, and disease. Ten studies, consisting of seven primary studies and three reviews, were examined. The included studies covered healthcare facilities, schools, households, prison cells, public transportation, soil, and wastewater across South Africa, Indonesia, Brazil, discussed in global reviews. Detection methods included air filtration, surface sampling, polymerase chain reaction (PCR), droplet digital PCR (ddPCR), GeneXpert, DNA sequencing, and culture-based techniques. Findings indicated that MTBC DNA was detected in several environmental samples, including indoor air and surfaces in settings associated with potential exposure. Environmental conditions such as poor ventilation and overcrowding were also identified as factors that may increase transmission risk. However, six of the seven primary studies relied on DNA-based detection, which cannot confirm bacterial viability or prove environmental transmission to humans. The findings suggest that environmental monitoring may help identify locations with potential TB exposure risks. Further research using standardized sampling methods and bacterial viability testing is needed to clarify the role of environmental occurrence in TB transmission and inform effective prevention strategies.

Keywords:
Mycobacterium tuberculosis, Tuberculosis Transmission, Environmental Detection, Indoor Air, Systematic Review

INTRODUCTION
Tuberculosis (TB) remains a major global public health problem caused primarily by *Mycobacterium tuberculosis* (*M. tuberculosis*), a bacterium that mainly affects the lungs but can also affect other parts of the body. According to the World Health Organization (WHO, 2025), an estimated 10.7 million people developed TB in 2024, demonstrating the continuing need to improve its prevention and control. TB is primarily transmitted through the air when a person with infectious TB releases bacteria-containing particles that can be inhaled by others (WHO, 2025). Although airborne transmission is well established, understanding where *M. tuberculosis* occurs in the environment and whether environmental contamination contributes to human exposure remains an important area of research.

Recent studies have investigated the occurrence of *M. tuberculosis* in different environmental settings, including indoor air, room surfaces, soil, and wastewater. For example, Verma et al. (2022) detected *M. tuberculosis* DNA on surfaces in prison cells occupied by individuals with active TB, suggesting that environmental sampling may help identify locations associated with potential exposure. Similarly, research has investigated the presence and possible persistence of the bacterium in natural and built environments (Martinez et al., 2019). These findings suggest that environmental investigations may provide additional information about the distribution of *M.*

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tuberculosis beyond conventional patient-based testing. However, the detection of bacterial DNA does not necessarily confirm that viable bacteria are present or that environmental exposure has resulted in infection.
The relationship between environmental occurrence and the risk of TB infection and disease remains incompletely understood. Environmental conditions, such as ventilation, occupancy, and the characteristics of a particular setting, may influence exposure to airborne bacteria. However, the presence of *M. tuberculosis* in environmental samples does not automatically establish that the environment is an independent source of transmission. Furthermore, TB infection and active TB disease are different outcomes: not everyone who becomes infected develops the disease. Therefore, examining the available evidence is necessary to distinguish environmental detection, bacterial survival, potential exposure, and confirmed infection or disease.
This systematic review aims to examine the available scientific evidence on the environmental occurrence of *M. tuberculosis* and its associated risk of TB infection and disease. Specifically, it seeks to identify the environmental settings in which the bacterium has been detected, summarize the methods used for environmental detection, and evaluate the reported evidence linking environmental occurrence with potential exposure, infection, or disease. By synthesizing findings from existing studies, this review aims to identify current knowledge gaps and provide a clearer understanding of the possible public health implications of environmental *M. tuberculosis*.

OBJECTIVES

The main objective of the study is to conduct a systematic review and synthesize existing literature and research studies on the environmental occurrence of *Mycobacterium tuberculosis* and its associated risk of tuberculosis (TB) infection and disease. In addition, even with the increasing number of studies on environmental *M. tuberculosis*, the results are frequently limited to particular settings such as healthcare facilities, schools, households, prisons, soil, or wastewater, and rely on different detection methods. Six of the seven primary studies report the detection of bacterial DNA rather than viable bacteria or confirmed transmission, and none of these studies confirmed viability in the sampled environments. Therefore, this systematic review aims to identify the environmental settings in which *M. tuberculosis* and the *M. tuberculosis* complex have been detected, summarize the methods used for environmental detection, and evaluate the reported evidence linking environmental occurrence with potential exposure, infection, or disease by integrating evidence from multiple studies. Such understanding can help identify current knowledge gaps and inform more effective prevention and infection control strategies to reduce the risk of TB transmission.

METHODOLOGY

This systematic review will be conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The Input-Process-Output (IPO) model will also be used to guide this review. Inputs are the sources of information and will include literature that has already been published on this topic. The process involves the literature search, screening, selection and analysis methods. Outputs will include a summary of results, descriptions of the environmental settings, detection methods, public health implications, and what needs to be investigated further.
Scientific literature on the presence of *Mycobacterium tuberculosis* and the *M. tuberculosis* complex in the environment. To systematically search for literature, screen, select eligible studies based on pre-determined inclusion and exclusion criteria, extract and analyse data. A summary of the evidence, descriptions of the environmental settings and detection methods found, public health implications, and areas that need further investigation.

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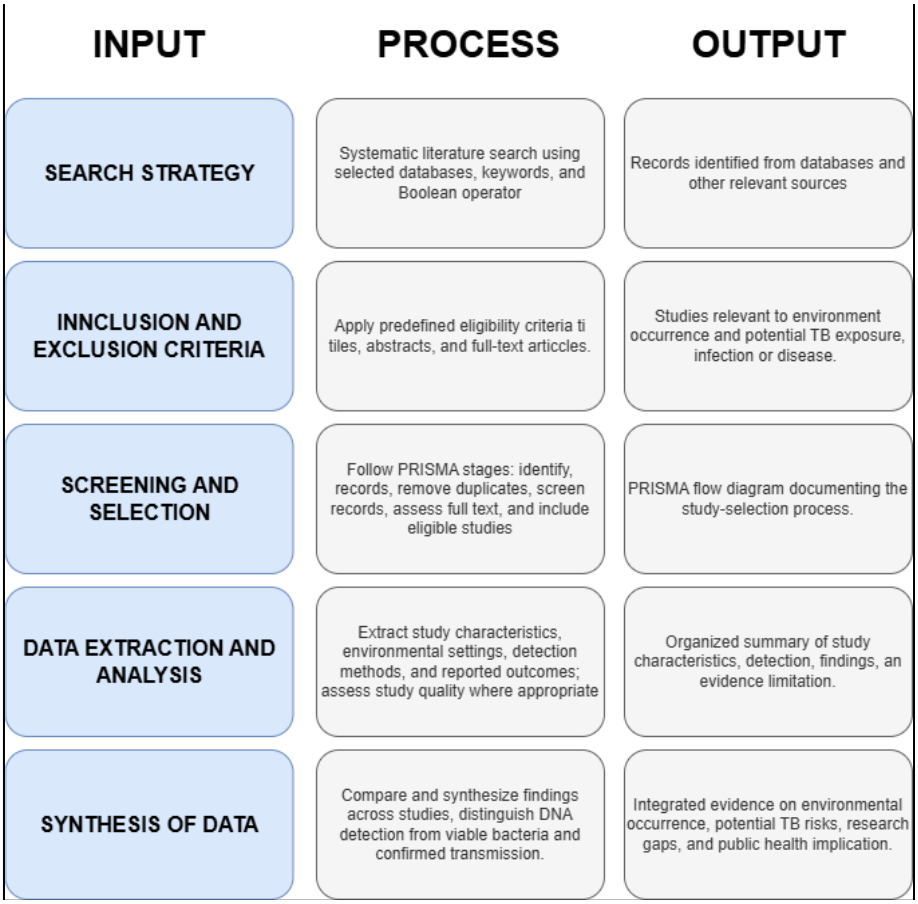


Figure 1. IPO diagram of the Study

a. Search Strategy

A systematic literature search will be conducted using Google Scholar, ResearchGate, PubMed, ScienceDirect, and other relevant academic sources to identify studies published from 2019 to 2026. The search will focus on the environmental occurrence of *Mycobacterium tuberculosis* and the *M. tuberculosis* complex, particularly their detection in air, soil, surfaces, wastewater, and other environmental settings, as well as their potential association with tuberculosis (TB) exposure, infection, and disease. Keywords will include "*Mycobacterium tuberculosis*," "environmental occurrence," "environmental detection," "airborne transmission," "environmental contamination," "soil," "wastewater," "TB infection," and "TB disease." Boolean operators such as AND and OR will be used to combine search terms and refine the search results. The identified records will be organized, and duplicate records will be removed before titles and abstracts are screened according to the predefined inclusion and exclusion criteria. Relevant full-text articles will then be assessed for eligibility, and the study selection process will be documented using the PRISMA flow diagram. The final search results will provide the basis for summarizing the environmental settings, detection methods, and reported evidence regarding the potential relationship between environmental occurrence and TB exposure, infection, or disease.

b. Inclusion/Exclusion Criteria

Predefined inclusion and exclusion criteria will be applied to ensure that the selected studies are relevant to the objectives of the systematic review. The criteria will focus on the environmental occurrence of *M. tuberculosis* and MTBC, the methods used to detect them, and the reported evidence concerning potential exposure, infection, or disease.

Studies will be included if they investigate the occurrence, detection, persistence, or survival of M. tuberculosis or MTBC in environmental samples or settings. Eligible environmental sources will include indoor air, healthcare facilities, schools, households, prisons, public transportation, environmental surfaces, soil, water, and wastewater. Original research articles and relevant review articles will be considered when they provide information related to the research objectives. Studies reporting molecular detection, culture-based detection, environmental monitoring, or other relevant methods will also be included.

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Studies will be excluded if they focus exclusively on clinical diagnosis or patient treatment without examining environmental occurrence or exposure. Publications unrelated to *M. tuberculosis* or MTBC, duplicate records, studies without sufficient information about their methods or findings, and articles whose full texts cannot be accessed for eligibility assessment will also be excluded. Studies concerning other mycobacterial species without relevant findings on MTBC will not be included in the synthesis of evidence specific to the review objectives
These criteria will guide the screening process and help maintain consistency in selecting studies for data extraction and analysis. Studies that violate these prerequisites were excluded to narrow the scope of this systematic review.

c. Study Selection Process

The study selection process will follow the stages outlined in the PRISMA guidelines: identification, screening, eligibility assessment, and inclusion. All records retrieved through the literature search will first be compiled, and duplicate publications will be removed. The remaining records will undergo title and abstract screening to identify studies potentially relevant to the environmental occurrence of *M. tuberculosis* and its associated TB risks.
The full texts of potentially eligible studies will then be examined using the predefined inclusion and exclusion criteria. Studies that do not meet the eligibility requirements will be excluded, and the reasons for exclusion will be documented. The remaining studies will be included in the systematic review and subjected to data extraction and analysis.
The number of records identified, duplicates removed, records screened, full-text articles assessed, studies excluded, and final studies included will be recorded. These numbers will be presented in a PRISMA flow diagram to provide a transparent account of the study selection process. The final set of included publications will serve as the basis for synthesizing the available evidence on environmental occurrence, detection methods, and potential TB exposure risks.

d. Study Area

This systematic review will cover environmental settings reported in the selected scientific literature rather than a single geographical study site. The geographical scope will be international, incorporating studies conducted in different countries and environmental conditions. The review will examine both natural environments and human-made settings where *M. tuberculosis* or MTBC has been investigated. The environmental settings will include healthcare facilities, schools, households, prisons, public transportation, indoor air, contaminated surfaces, soil, water, and wastewater treatment systems. These settings will be grouped according to the environmental source investigated in each publication.
The studies included in the review represent different geographical contexts, research designs, and sampling conditions. For example, the literature summarized in the review includes environmental investigations in healthcare facilities, schools, and public transportation in South Africa; prison environments in Brazil; household environments in Indonesia; and soil sampling in Kruger National Park, South Africa. Global reviews addressing multiple environmental sources will also be considered when they meet the eligibility criteria. Comparing these settings will help describe the range of environments investigated and identify areas where further research is needed.

e. Data Extraction and Analysis

Data extraction will be conducted using a structured table to organize relevant information from each included publication. The extracted information will include the author and year of publication, country, environmental setting, environmental source, study design, sampling procedures, detection methods, reported environmental occurrence, associated TB exposure or transmission risks, major findings, and identified limitations. Particular attention will be given to the methods used to detect M. tuberculosis and MTBC. These methods may include polymerase chain reaction (PCR), quantitative PCR (qPCR), droplet digital PCR (ddPCR), culture-based techniques, whole-genome sequencing, and environmental monitoring methods. Information on ventilation, occupancy, and other environmental conditions will also be extracted when reported by the included studies.
The extracted data will be analyzed descriptively to compare findings across environmental settings and detection methods. Reported detection results, including the number of positive samples and the total number of samples tested, will be recorded when available. Detection percentages will be compared using the values reported by the original studies, with consideration given to differences in sampling procedures, analytical methods, and study designs.
The analysis will distinguish between the detection of bacterial DNA, evidence of viable bacteria, experimental evidence of survival or infectious potential, and documented associations with TB infection or disease. This distinction is necessary because molecular detection alone does not establish bacterial viability or confirm that environmental exposure caused an infection. Differences in study methods, findings, and limitations will be documented to support a balanced interpretation of the available evidence.

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f. Synthesis of Data

The findings of the included studies will be synthesized using a narrative synthesis approach. This approach will allow the results of studies with different research designs, environmental sources, sampling procedures, and detection methods to be compared and interpreted systematically. A quantitative meta-analysis will not be conducted because the included studies examine diverse settings and use different approaches to measure environmental occurrence and potential exposure.

The synthesis will organize the findings into major environmental categories, including air, indoor environments, healthcare facilities, schools, households, prisons, environmental surfaces, soil, water, and wastewater. Within each category, the review will summarize the reported occurrence of *M. tuberculosis* or MTBC, the methods used for detection, the principal findings, and the potential public health implications.

The review will also compare the strength and limitations of the evidence linking environmental occurrence with TB exposure, infection, and disease. Studies reporting molecular detection will be distinguished from those providing evidence of bacterial viability or potential infectiousness. Reported environmental factors, such as ventilation, occupancy, and contamination conditions, will be considered when interpreting possible exposure risks.

Finally, the findings will be integrated to identify recurring patterns, inconsistencies, and research gaps. Particular attention will be given to limitations in confirming bacterial viability, determining the actual contribution of environmental reservoirs to human TB transmission, and establishing direct links between environmental detection and confirmed infection or disease. The resulting synthesis will provide an overview of current scientific evidence and identify areas for further investigation to improve understanding of environmental TB risks and inform infection prevention and control strategies. Important note before finalizing: The uploaded publication table contains 10 studies or reviews, including publications from 2019 to 2026. The methodology above follows that material, but the exact number of records identified, duplicates removed, full-text articles excluded, and studies finally included must be based on your actual search and screening results. Do not invent these numbers for the PRISMA flow diagram.

RESULTS AND DISCUSSION

Table 1: Summary of Reviewed Literature

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Study # - Environmental source	Country - Setting	Detection method	Environmental occurence	Associated TB Risk / Transmission	Findings	Author/ Year
1 - Air in healthcare facilities (district hospitals, TB hospitals, primary health clinics).	*South Africa - Public healthcare facilities: district hospitals, TB hospitals, primary health clinics*	Air filtration through 37 mm PTFE filters, then real-time quantitative PCR (LightCycler *Mycobacterium* Detection Kit, 16S rRNA gene). Personal samples (n = 264) were collected at 4 L/min and stationary samples (n = 327) at 20 L/min, placed 1.5 m high. Walkabout observations and an IPC questionnaire were also used.	13 of 591 samples (2.2%) were positive, with 1.08×10^4 to 3.55×10^7 DNA copies/m³. All positives were area samples (none personal) from medical wards, casualty and TB wards. No positives came from primary health clinics or in summer.	Airborne transmission to healthcare workers. Western Cape had higher odds of airborne MTB than Gauteng (OR 4.82). Mechanical ventilation had higher odds than natural ventilation (OR 4.77). Positives were more frequent where UVGI was absent (9 vs 4), but that was not statistically significant. Ventilation was 0–0.34 ACH, far below the 6 ACH recommended.	Airborne MTB is detectable in healthcare settings despite reported IPC training. Risk varied by province, facility type, area, season, UVGI and ventilation. The authors note qPCR can't distinguish viable from non-viable bacteria.	**Matuka et al. (2021)**
2 - Indoor air in congregate buildings (clinic, shops, churches, bars, hair salons) and public transport (minibus taxis, buses, trains).	*South Africa - Congregate buildings and public transport (Peri-urban township, Cape Town)*	Mtb was not directly detected. CO_2 dataloggers (every 5 min in buildings, every 30 s on transport) and manual people counts were used to estimate ventilation rates. A relative potential-transmission rate was then calculated as mean other people present ÷ ventilation rate.	Estimated ventilation ranged from 44–443 L/s in minibus taxis, 232–2469 L/s on trains, 671–1270 L/s in the clinic, and 24–425 L/s in bars. Mean occupancy was ≤25 people in most places, but reached 76 in the clinic and up to 132 on trains.	Airborne transmission driven by low ventilation and high occupancy. Minibus taxis were driven mainly by low ventilation, and trains mainly by crowding.	Risk was highest in minibus taxis (up to 9× the median rate on 12 of 43 journeys) and on trains, and lowest in salons, bars and shops. The clinic had good ventilation but high risk at times because of crowding. Public transport is a promising target for IPC interventions.	**Deol et al. (2022)**
3 - Soil (topsoil, 3 cm depth) collected 1 m from the water's edge at waterholes.	*South Africa - Kruger National Park (6 sites, sampled at 6 time points in June 2022; n = 180 samples).*	Direct soil DNA extraction (PowerSoil Pro kit), without culture. *Mycobacterium* genus-specific hsp65 PCR with Sanger amplicon sequencing,	hsp65 PCR was positive in 137 out of 180 (76%) samples. Sanger sequencing identified MTBC DNA in 3/180 (1.7%) samples, of which 2/3 were from site 3.	Indirect transmission through the environment: infected animals shed *M. bovis* in respiratory secretions, urine and faeces, and *M. bovis* is	First evidence of *M. bovis* DNA in Kruger soil, which may represent environmental contamination from infected wildlife. The following limitations should	**Matthews et al. (2025)**

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		GeneXpert MTB/RIF Ultra qPCR, and RD1/RD4 region-of-difference PCR to confirm *M. bovis*.	Non-tuberculous mycobacteria were identified in 21/44 (48%) samples. RD-PCR identified *M. bovis* in 2/3 (both from site 3) and the third one was not differentiated. GeneXpert Ultra detected MTBC in 0/180 samples.	endemic in the park, with African buffalo as maintenance hosts. The study examines animal TB and does not address human infection.	be noted: no cultures were obtained (due to regulatory restrictions on transport), therefore viability of the isolates cannot be determined; identification was based on conventional targets (hsp65 and RD regions) rather than whole genome sequencing; GeneXpert Ultra was not sensitive enough to detect paucibacillary soil samples (its limit of detection was 10 -3 ng/mL *M. bovis* DNA); and animals were not assayed.	
4. Indoor air and household physical conditions, including ventilation, humidity, temperature, lighting, and occupancy density	*Indonesia – Lubuk Alung, Padang Pariaman Regency; households with and without TB cases.*	Observational case-control study; household environmental assessment, air sampling, and PCR testing for MTB.	MTB was detected in 4 of 15 households. Ten households (66.7%) did not meet the study's overall healthy-home criteria	Poor ventilation, high humidity, overcrowding, and inadequate lighting may contribute to indoor exposure and TB transmission	The study reported an odds ratio of 2.667 for the association between inadequate overall household conditions and TB incidence. However, the small sample and wide confidence interval indicate considerable uncertainty.	**Marni et al. (2024)**
5 - Environmental surfaces in prison cells, including clothing, bedding, bathroom surfaces, utensils, floors, and walls	*Brazil – Prison facilities in Dourados and other study locations.*	Environmental surface swabs; GeneXpert MTB/RIF Ultra, quantitative PCR, and whole-genome sequencing of selected samples.	MTB DNA was detected in 55 of 340 swabs (16%) from cells occupied by people with active TB, compared with 0 of 49 control swabs.	Environmental surface contamination may help identify shared spaces associated with potential exposure to people with active TB.	Surface detection was associated with the sputum bacterial load of people with active TB. Genetic analysis identified antibiotic-resistance markers in some samples. However, a viable MTB was not confirmed.	**Verma et al. (2022)**

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6 - Indoor air in a primary-care clinic, including areas where patients may gather or receive TB-related care.	South Africa – Primary-care clinical setting.	High-flow air filtration, DNA extraction, droplet digital PCR (ddPCR), and carbon dioxide (CO_2) monitoring.	Among 74 clinic samples, the median detected quantity was 121 MTB DNA copies, compared with 3 copies among 47 control samples.	Air sampling may identify locations with potential airborne TB exposure and support investigations of indoor air quality.	The study demonstrated that airborne MTB DNA could be detected in a clinical environment. However, additional method development is necessary, and DNA detection alone cannot confirm infectiousness or actual transmission.	**Middelkoop et al. (2022)**
7 - Air in school classrooms and indoor spaces in public health clinics.	*South Africa – Two high schools and public health clinics.*	High-volume air filtration, droplet digital PCR (ddPCR), CO_2 monitoring, and student TB screening.	MTB DNA was detected in 13 of 72 classrooms (18.1%) and 4 of 39 clinic measurements (10.3%).	Shared indoor spaces may present opportunities for airborne exposure. Ventilation and occupancy conditions are important factors to investigate.	Classroom CO_2 concentrations were higher than those in clinics. The study demonstrated the feasibility of detecting airborne MTB DNA, but the detection of DNA does not establish the presence of viable bacteria or prove transmission.	**Bunyasi et al. (2022)**
8 - Wastewater: raw sewage, treated effluent, activated sludge, sewage sediment, and wastewater-impacted water and soil.	Global Review (sources include Poland, India, Kazakhstan, Russia, Germany, Hong Kong, Portugal, Iran, Pakistan, South Africa)	Reviews culture-based methods (chemical decontamination with NaOH or CPC, then 7H9/7H10/L-J media at 35–37 °C for 6–12 weeks), PCR-based methods (16S rRNA, IS6110), and high-throughput sequencing (Illumina metagenomics).	MTBC was detected in raw wastewater, treated wastewater, activated sludge, soil, and water. Up to 5.5×10^5 ($\pm 3.9 \times 10^5$) copies/L were reported in untreated wastewater. Primary and secondary treatment removed 98.6 (±2.7)%. MTBC was still detected in treated effluent (7 of 13 samples; $0.74 \times 10^4 \pm 1.40 \times 10^4$ copies/L).	Aerosols formed during wastewater treatment are probably the major route of infection, exposing WWTP workers and the communities around them. The faecal-oral route is plausible since 20% of cases of pulmonary TB may present with extra-pulmonary manifestations such as GITB, leading to shedding in the stools and urine. In the cited study, about 21% of the 104 sewage workers interviewed were	The presence of viable MTBC in treated or untreated wastewater can cause infection. The survival of these microorganisms in wastewater has not been experimentally demonstrated. It was inferred from their survival in other environments, such as water. For example, *mycobacteria* can withstand chlorine concentrations 100-330 times higher than E. coli and 2-10 times higher UV exposure than E.	**Mtetwa et al. (2022)**

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				TB sufferers.	coli. No study has demonstrated the presence of MTBC in wastewater using RT-PCR. The article recommends that sewage workers use respiratory protection and continued research on the risk of infection through wastewater.	
9 - Detection, survival and infectious potential of *Mycobacterium tuberculosis* in the environment: a review of the evidence and epidemiological implications	Global Review	Reviews environmental culture, molecular detection methods (including PCR), air sampling, and animal experiments used to assess bacterial viability and infectious potential.	*M. tuberculosis* has been detected in soil, water, air samples, dust, and other contaminated materials. Experimental studies found that it could remain viable in soil for months or longer.	Environmental bacteria may pose a potential exposure risk if they remain infectious and become airborne again. However, direct evidence that environmental reservoirs are an important source of human TB transmission remains limited.	Historical and modern studies indicate that *M. tuberculosis* can survive in natural and indoor environments. Animal experiments support its potential infectiousness, but further research is needed to establish the actual contribution of environmental exposure to human TB cases.	**Martinez et al. (2019)**
10 - The impact of *Mycobacterium tuberculosis* complex in the environment on one health approach	Global Review	Reviews microscopic examination, culture-based methods, molecular methods (such as PCR), and whole-genome sequencing (WGS).	MTBC has been reported in soil, water, pasture, air, dust, animal feces, and contaminated household or healthcare environments.	Environmental exposure may contribute to infection in humans and animals. Contaminated materials or dust may become airborne, but the importance of environmental transmission in human TB remains uncertain.	MTBC can persist in environmental conditions and circulate among humans, animals, and their surroundings. The authors emphasize improved detection methods and a One Health approach integrating human, animal, and environmental health.	**Zhang et al. (2022)**

(a) Airborne detection in healthcare facilities.

Matuka et al. (2021) investigated airborne *M. tuberculosis* in public healthcare facilities in South Africa, covering district hospitals, TB hospitals, and primary health clinics. Using air filtration through 37 mm PTFE filters followed by real-time quantitative PCR, they collected 591 air samples, of which 13 (2.2%) were positive, with DNA concentrations ranging from 1.08×10^4 to 3.55×10^7 copies/m³. Notably, all positive samples were stationary area samples taken from medical wards, casualty, and TB wards, while none of the 264 personal samples tested positive, and no positives were recorded in primary health clinics or during summer. The odds of airborne detection were higher in the Western Cape than in Gauteng (OR = 4.82) and with mechanical ventilation compared

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to natural ventilation (OR = 4.82 and OR = 4.77, respectively), and detection was more frequent where UVGI was absent (9 vs 4 positives), although the latter was not statistically significant. Measured ventilation ranged from only 0 to 0.34 ACH, far below the 6 ACH recommended for airborne infection control. These results demonstrate that airborne *M. tuberculosis* is detectable in healthcare settings despite reported IPC training, but because qPCR cannot distinguish viable from non-viable bacteria, the findings confirm the presence of genetic material rather than infectious organisms.

(b) Ventilation and occupancy in congregate settings and public transport.

In the study by Deol et al. (2022), *M. tuberculosis* was not directly detected. Instead, CO_2 dataloggers and manual occupancy counts were used to estimate ventilation rates and derive a relative potential-transmission rate, calculated as the mean number of other people present divided by the ventilation rate. Estimated ventilation ranged from 44 to 443 L/s in minibus taxis, 232 to 2469 L/s on trains, 671 to 1270 L/s in the clinic, and 24 to 425 L/s in bars, while mean occupancy was at most 25 people in most settings but reached 76 in the clinic and up to 132 on trains. Risk was highest in minibus taxis, where potential-transmission rates reached up to nine times the median on 12 of 43 journeys, driven mainly by low ventilation, whereas risk on trains was driven mainly by crowding. Risk was lowest in salons, bars, and shops. Since the rate is derived from occupancy relative to ventilation, the low risk in bars, despite ventilation as low as 24 L/s, is consistent with the small number of occupants typically present. The clinic, despite its good ventilation, experienced periods of elevated risk owing to high occupancy. These findings identify public transport and crowded congregate settings as promising targets for infection prevention and control interventions, although conditions favoring exposure are described rather than actual bacterial occurrence or transmission being demonstrated.

(c) Soil and environmental occurrence of *M. bovis*.

Matthews et al. (2025) collected 180 topsoil samples (3 cm depth) at 1 m from the water's edge at six waterholes in Kruger National Park, sampled at six time points in June 2022. Using direct soil DNA extraction without culture, genus-specific hsp65 PCR, Sanger sequencing, GeneXpert MTB/RIF Ultra, and RD1/RD4 PCR, they found the hsp65 assay positive in 137 of 180 samples (76%), with MTBC DNA identified in 3 of 180 samples (1.7%), of which 2 of 3 were confirmed as *M. bovis*, both from site 3. GeneXpert Ultra detected MTBC in 0 of 180 samples, indicating its limit of detection was insufficient for paucibacillary soil samples, while non-tuberculous mycobacteria were identified in 21 of 44 samples (48%). Because *M. bovis* is endemic in the park, with African buffalo as maintenance hosts shedding the organism in respiratory secretions, urine, and faeces, this finding represents environmental contamination from animal tuberculosis rather than direct evidence of environmental *M. tuberculosis*. The absence of cultures, due to regulatory restrictions on sample transport, means viability could not be determined, so the finding should be read as molecular evidence of environmental MTBC presence only.

(d) Household conditions and TB incidence.

Marni et al. (2024) conducted an observational case-control study in Lubuk Alung, Padang Pariaman Regency, Indonesia, comparing households with and without TB cases using household environmental assessment, air sampling, and PCR testing for MTB. MTB was detected in 4 of 15 households, and ten households (66.7%) failed to meet the study's overall healthy-home criteria. The study reported an odds ratio of 2.667 for the association between inadequate overall household conditions and TB incidence. Poor ventilation, high humidity, overcrowding, and inadequate lighting may therefore contribute to indoor exposure and TB transmission. However, the small sample size and the wide confidence interval indicate considerable uncertainty, so the association supports further investigation of household conditions but does not establish that any single environmental factor independently caused TB infection.

(e) Surface contamination in prison cells.

Verma et al. (2022) collected environmental surface swabs from prison facilities in Dourados, Brazil, and other study locations, covering clothing, bedding, bathroom surfaces, utensils, floors, and walls, and analyzed them with GeneXpert MTB/RIF Ultra, quantitative PCR, and whole-genome sequencing of selected samples. *M. tuberculosis* DNA was detected in 55 of 340 swabs (16%) from cells occupied by people with active TB, compared with 0 of 49 control swabs. Surface detection was associated with the sputum bacterial load of the occupants, and genetic analysis identified antibiotic-resistance markers in some samples, which suggests that surface sampling can help identify shared spaces associated with potential exposure to people with active disease. Nevertheless, viable *M. tuberculosis* was not confirmed in any sample, so the findings locate potential contamination without establishing that surface contact caused infection.

(f) Clinic and school air detection.

Middelkoop et al. (2022) used high-flow air filtration, DNA extraction, droplet digital PCR, and CO_2 monitoring in a South African primary-care clinic, quantifying a median of 121 *M. tuberculosis* DNA copies among 74 clinic

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samples compared with 3 copies among 47 control samples. Bunyasi et al. (2022) applied a similar approach in two high schools and public health clinics, combining high-volume air filtration, ddPCR, CO_2 monitoring, and student TB screening, and detected airborne DNA in 13 of 72 classrooms (18.1%) and 4 of 39 clinic measurements (10.3%), with classroom CO_2 concentrations exceeding those measured in clinics. Together, these studies demonstrate that molecular air sampling is feasible in both clinical and educational settings and that shared indoor spaces present opportunities for airborne exposure. However, neither study established infectiousness or actual transmission, and the classroom CO_2 finding highlights ventilation and occupancy as factors requiring investigation.

(g) Review articles on wastewater, survival, and One Health.

The three reviews extend the evidence beyond occupied buildings. Mtetwa et al. (2022) reported MTBC in raw wastewater, treated effluent, activated sludge, sewage sediment, and wastewater-affected water and soil, with concentrations up to 5.5×10^5 ($\pm 3.9 \times 10^5$) copies/L in untreated wastewater and detection persisting in 7 of 13 treated-effluent samples ($0.74 \times 10^4 \pm 1.40 \times 10^4$ copies/L) after primary and secondary treatment removed 98.6 (±2.7)% of organisms; notably, about 21% of the 104 sewage workers interviewed in a cited study were TB sufferers. Martinez et al. (2019) compiled historical and modern evidence that *M. tuberculosis* can survive in natural and indoor environments, remaining viable in soil for months or longer, with animal experiments supporting its potential infectiousness. Zhang et al. (2022) reported MTBC in soil, water, pasture, air, dust, and animal feces, emphasizing a One Health approach integrating human, animal, and environmental health. Collectively, the reviews establish that environmental persistence is plausible and exposure routes exist, but the actual contribution of environmental reservoirs to human TB transmission remains insufficiently established.

(h) Geographic and evidential distribution.

The ten publications collectively show that environmental investigations can locate mycobacterial material across air, surfaces, soil, wastewater, and occupied settings, with South Africa contributing the largest share of primary studies, covering healthcare facilities, schools, indoor air, and soil, and Indonesia and Brazil each contributing one study on households and prison cells, respectively. This concentration means the table describes the reviewed literature rather than the global distribution of environmental *M. tuberculosis*, and the evidence is uneven across settings. Furthermore, the soil study targeted *M. bovis* rather than *M. tuberculosis* specifically, so findings from different members of the MTBC must be interpreted according to the organism investigated. Overall, the publications show that environmental investigations can identify locations where mycobacterial material is present and where potential exposure deserves assessment, but they vary considerably in their ability to demonstrate viable bacteria and to connect environmental contamination directly with human transmission.

I. Study Characteristics and Geographic Distribution of Environmental Mycobacterium tuberculosis

The reviewed literature consisted of ten publications examining the environmental occurrence of *Mycobacterium tuberculosis* and the *M. tuberculosis* complex (MTBC), including original research studies and review articles. These publications investigated different environmental sources, detection methods, and possible relationships between environmental contamination and tuberculosis (TB) exposure, infection, or disease. The environmental settings covered healthcare facilities, schools, households, prisons, public transportation, environmental surfaces, soil, water, and wastewater treatment systems.

The geographic distribution of the reviewed literature showed that five of the seven primary studies were conducted in South Africa (Matuka et al., 2021; Deol et al., 2022; Matthews et al., 2025; Middelkoop et al., 2022; Bunyasi et al., 2022). Middelkoop et al. (2022) investigated airborne bacterial DNA in a primary-care clinic, and Bunyasi et al. (2022) examined school classrooms and public health clinics. These studies demonstrated the importance of investigating indoor environments where people gather and where ventilation and occupancy conditions can influence potential exposure.

Other original studies examined different environmental settings. Verma et al. (2022) investigated environmental surface contamination in prison facilities in Brazil, while Marni et al. (2024) examined household conditions associated with TB incidence in Indonesia. Matthews et al. (2025) investigated the detection of *M. bovis* DNA in soil from Kruger National Park, South Africa. Meanwhile, review articles by Martinez et al. (2019), Mtetwa et al. (2022), and Zhang et al. (2022) addressed environmental occurrence, bacterial persistence, wastewater contamination, and the broader One Health perspective.

The geographic and environmental distribution of the publications indicates that research on environmental mycobacteria has covered both human-occupied environments and natural settings. However, the number of studies represented in the reviewed table does not establish the global distribution of environmental *M. tuberculosis*. The evidence is also uneven across settings, with four of the seven primary studies focusing on South African healthcare facilities, schools, or public transport. Furthermore, the soil investigation by Matthews et al. focused on *M. bovis*, a member of MTBC, rather than specifically demonstrating environmental occurrence of *M.*

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tuberculosis. Therefore, findings from different members of the complex must be interpreted according to the organism investigated in each study. Overall, the reviewed publications provide evidence that environmental investigations can identify locations where mycobacterial material is present and where potential exposure deserves further assessment. Nevertheless, the available evidence varies in its ability to demonstrate viable bacteria and establish a direct connection between environmental contamination and human TB transmission.

Table 2. Characteristics of the Reviewed Publications

Setting	Environmental Source	Study focus / evidence reported	Author/year
South Africa	Healthcare-facility air	*M. tuberculosis* DNA detected in stationary air samples; ventilation and facility characteristics examined.	Matuka et al. (2021)
South Africa	Congregate settings and public transport	Ventilation and occupancy used to estimate potential airborne transmission conditions; no direct environmental detection.	Deol et al. (2022)
South Africa	Soil near Waterholes	MTBC DNA detected in soil; study focused on *M. bovis*. Publication year should be checked against the reference list.	Matthews et al. (2025)
Indonesia	Household environments	Household physical conditions and their association with TB incidence examined.	Marni et al. (2024)
Brazil	Prison-cell surfaces	*M. tuberculosis* DNA detected on environmental swabs; viability was not confirmed.	Verma et al. (2022)
South Africa	Primary-care clinic air	Airborne *M. tuberculosis* DNA quantified in clinic and control samples; year differs from the reference list.	Middelkoop et al. (2022)
South Africa	School classrooms and clinic air	*M. tuberculosis* DNA detected in classroom and clinic air measurements.	Bunyasi et al. (2022)
Multiple settings	Wastewater	Review of MTBC evidence in wastewater and related environments.	Mtetwa et al. (2022)
Multiple settings	Wastewater	Review of environmental detection, survival, and persistence.	Martinez et al. (2019)
Global	One Health scope	Review of MTBC across human, animal, and environmental contexts.	Zhang et al. (2022)

Table 2 presents the features of the ten included publications, namely the setting, the environmental source, the study focus, and the authors and year. Seven primary studies were included in this review. These examined air in healthcare facilities (Matuka et al., 2021), congregate settings and public transport (Deol et al., 2022), soil around waterholes (Matthews et al., 2025), household settings in Indonesia (Marni et al., 2024), surfaces in prison cells in Brazil (Verma et al., 2022), air in a primary-care clinic (Middelkoop et al., 2022), and air in school classrooms and clinics (Bunyasi et al., 2022). Three review articles were also included, addressing M. tuberculosis in wastewater and related environments (Mtetwa et al., 2022), environmental detection, survival, and persistence (Martinez et al., 2019), and MTBC across human, animal, and environmental contexts under a One Health

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framework (Zhang et al., 2022). Of the seven primary studies, five reported *M. tuberculosis* DNA in air, household, or surface samples (Matuka et al., 2021; Marni et al., 2024; Verma et al., 2022; Middelkoop et al., 2022; Bunyasi et al., 2022). Among these, Marni et al. (2024) examined household physical conditions in relation to TB incidence and also combined air sampling with PCR testing, with *M. tuberculosis* detected in 4 of 15 households. Matthews et al. (2025) reported MTBC DNA in soil that was specifically identified as *M. bovis*. Deol et al. (2022) stands alone in that direct detection of the bacterium was not attempted, with only ventilation and occupancy being examined as potential airborne transmission conditions.

(a) Primary studies from South Africa.

Five of the seven primary studies were conducted in South Africa: Matuka et al. (2021) on healthcare-facility air, Deol et al. (2022) on congregate settings and public transport, Matthews et al. (2025) on soil near waterholes, Middelkoop et al. (2022) on primary-care clinic air, and Bunyasi et al. (2022) on school classrooms and clinic air. This clustering means that the majority of direct-detection evidence in the review, 5 of 7 primary studies (71%), comes from South African settings, which strengthens internal consistency across studies that share similar environmental and health-system conditions but limits the geographic generalizability of the pooled findings. It also reflects the concentration of environmental TB research in a country with a high TB burden, leaving large regions of the world unrepresented in the direct-detection evidence base.

(b) Primary studies from Indonesia and Brazil.

The remaining two primary studies broaden the settings: Marni et al. (2024) examined household physical conditions and their association with TB incidence in Indonesia, and Verma et al. (2022) detected *M. tuberculosis* DNA on prison-cell surfaces in Brazil, with bacterial viability remaining unconfirmed. These studies extend the evidence to lower-resource household and custodial environments that differ substantially from the South African healthcare and school settings, and together they account for 2 of the 7 primary studies (29%). However, each represents a single study from its country, so neither can be treated as regionally representative, and their findings should be read as setting-specific evidence rather than as national estimates. The environmental conditions considered include household physical conditions and custodial environments, although specific factors such as ventilation, temperature, and humidity were not identified in the descriptions provided.

(c) Review articles.

The three review articles synthesize evidence across multiple countries rather than generating new environmental observations: Mtetwa et al. (2022) reviewed MTBC in wastewater and related environments, Martinez et al. (2019) reviewed environmental detection, survival, and persistence, and Zhang et al. (2022) reviewed MTBC across human, animal, and environmental contexts under the One Health framework. Their inclusion means 3 of the 10 included publications (30%) are reviews, and the remaining 7 (70%) are primary studies. Given the potential overlap between the evidence summarized in the reviews and the primary studies included in the same table, the ten publications should not be counted as ten equivalent independent experiments, and the reviews should be used to contextualize rather than to multiply the primary.

(d) What the table does and does not show.

Table 2 identifies each publication's setting, environmental source, study focus, and authorship, and most of the primary studies, specifically those on air, soil, and surfaces, reported detection of *M. tuberculosis* DNA in their respective samples, while others examined only conditions such as ventilation, occupancy, and household factors. The table does not establish whether detected *M. tuberculosis* was viable or capable of transmission, which is the central interpretive limitation of the included evidence. Readers should therefore treat the listed characteristics as describing what each study investigated, not what each study proved about infection risk, and the distinction between direct-detection studies and condition-assessment studies should be maintained when weighing the overall evidence. Others examined only conditions such as ventilation, occupancy, and household factors. The table does not show whether detected *M. tuberculosis* was viable or capable of transmission, which is the central interpretive limitation of the included evidence. Readers should therefore treat the listed characteristics as describing what each study investigated, not what each study proved about infection risk, and the distinction between direct detection studies and condition-assessment studies should be maintained when weighing the overall evidence.

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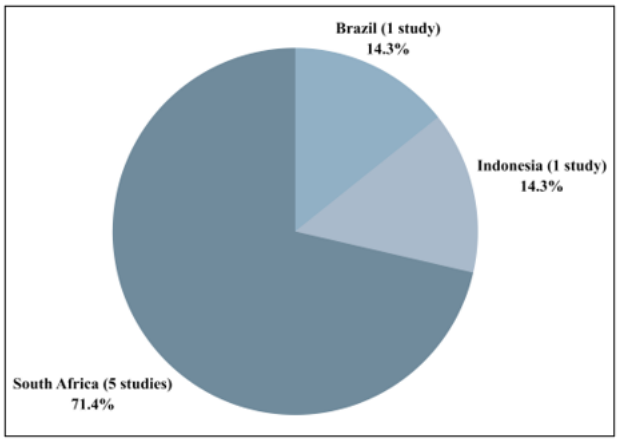


Figure 2. Geographic Distribution of Primary studies

This figure shows the geographic distribution of the seven primary studies included in the review. South Africa contributed the highest number, with five studies examining different environmental settings, including healthcare facilities, schools, indoor air, and soil. Meanwhile, Indonesia and Brazil each contributed one study, focusing on household environmental conditions and prison-cell surfaces, respectively.

Overall, the distribution indicates that research on the environmental occurrence of *Mycobacterium tuberculosis* and the *M. tuberculosis* complex is concentrated in South Africa, with limited representation from other countries. This highlights the need for further studies in different geographic regions to provide a broader understanding of environmental occurrence and its potential association with TB exposure, infection, and disease.

II. Environmental Occurrence and Detection Methods Across Different Sources and Settings

The reviewed studies reported the occurrence of *M. tuberculosis* or MTBC in several environmental sources, including air, indoor surfaces, soil, and wastewater. To investigate these settings, the publications used different sampling procedures and laboratory methods, including air filtration, environmental surface swabbing, soil DNA extraction, polymerase chain reaction (PCR), quantitative PCR (qPCR), droplet digital PCR (ddPCR), culture-based techniques, and whole-genome sequencing. One study, Deol et al. (2022), examined ventilation and occupancy to estimate potential airborne transmission conditions rather than directly detecting the bacterium. The findings demonstrate that environmental investigations can detect mycobacterial material in different settings, although the meaning of these detections depends on the organism identified and the method used.

Air and indoor environments. Matuka et al. (2021) used air filtration followed by real-time quantitative PCR to investigate airborne *M. tuberculosis* DNA in public healthcare facilities in South Africa, detecting bacterial DNA in 13 of 591 air samples (2.2%). All positive samples were stationary area samples, while none of the personal air samples tested positive. Middlekoop et al. (2022) used high-flow air filtration, DNA extraction, ddPCR, and carbon dioxide monitoring to examine airborne bacterial material and indoor conditions, reporting a median of 121 *M. tuberculosis* DNA copies in 74 clinic air samples, compared with three copies among 47 control samples. Bunyasi et al. (2022) similarly used air filtration and ddPCR, together with carbon dioxide monitoring and student TB screening, and detected airborne *M. tuberculosis* DNA in 13 of 72 classrooms (18.1%) and in 4 of 39 clinic measurements (10.3%). These findings demonstrate that molecular techniques, combined with environmental monitoring, can identify bacterial DNA in indoor air, although the results do not independently confirm viable bacteria or actual transmission.

Environmental surfaces. Verma et al. (2022) collected environmental surface swabs from prison cells occupied by individuals with active TB and used GeneXpert MTB/RIF Ultra, quantitative PCR, and whole-genome sequencing on selected samples. The study detected *M. tuberculosis* DNA in 55 of 340 environmental swabs (16%), compared with zero positive results among 49 control swabs, with positive samples including surfaces associated with clothing, bedding, bathrooms, utensils, floors, and walls. This finding indicates that environmental surface sampling can identify locations associated with people who have active TB. However, the detection of bacterial DNA on these surfaces does not establish that the bacteria remained viable or that contact with the surfaces caused infection.

Soil and natural environments. Matthews et al. (2025) used direct soil DNA extraction, genus-specific PCR, Sanger sequencing, and additional molecular assays to investigate the presence of mycobacteria in soil samples collected near waterholes in Kruger National Park, South Africa. The study reported positive results for MTBC

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DNA in three of 180 soil samples (1.7%), while the broader *Mycobacterium* genus-specific assay detected bacterial DNA in a larger number of samples. The investigation specifically focused on *M. bovis*, which is associated with animal tuberculosis, and therefore should not be interpreted as direct evidence of environmental *M. tuberculosis* contamination. Nevertheless, the findings illustrate how environmental sampling can investigate the occurrence of members of MTBC in natural settings.

Wastewater and water-related environments. The wastewater review by Mtetwa et al. (2022) described culture-based approaches and molecular methods, including PCR-based detection and high-throughput sequencing, and summarized evidence concerning MTBC in raw wastewater, treated effluent, activated sludge, sewage sediment, and wastewater-affected environments. The review reported detection in both untreated and treated wastewater, including a reported concentration of up to $$5.5 \times 10^5$$ DNA copies/L in untreated wastewater, and MTBC was detected in seven of 13 treated-effluent samples. These findings indicate that wastewater systems can contain detectable mycobacterial material. However, the presence of DNA in wastewater does not establish that viable, infectious bacteria remain after treatment.

In addition, Deol et al. (2022) estimated ventilation rates and relative potential-transmission rates using carbon dioxide measurements and occupancy counts in congregate buildings and public transport. As direct detection of M. tuberculosis was not performed in the sampled environments, its findings provide information about conditions that may affect airborne exposure rather than direct evidence of environmental bacterial occurrence. These conditions include ventilation rates, occupancy levels, and air exchange, which may influence the accumulation and dilution of airborne particles.*losis* in the sampled environments, its findings provide information about conditions that may affect airborne exposure rather than direct evidence of environmental bacterial occurrence.

The variability in detection methods is important when comparing reported results. Studies differ in their sampling locations, sample numbers, collection procedures, laboratory assays, and analytical sensitivity. For example, the detection of bacterial DNA in 2.2% of air samples in Matuka et al. (2021) and in 18.1% of classrooms in Bunyasi et al. (2022) cannot be interpreted as a direct difference in environmental contamination or transmission risk, because the studies examined different populations, environments, and sampling conditions. These differences demonstrate the importance of interpreting each study according to its methods and objectives. A positive molecular result establishes that the targeted genetic material was detected within the limitations of the assay, but it does not necessarily demonstrate bacterial viability. Similarly, ventilation measurements and environmental conditions can help characterize possible exposure but cannot independently confirm the presence of infectious bacteria. Consistent sampling procedures, clearly reported detection limits, and complementary methods would improve comparisons among future studies.

Table 3. Summary of Environmental Detection Findings and Methods by Study

Setting	Environmental Source	Sampling and detection method(s)	What the method can show	Reported finding	Main limitation / interpretation	Author/ year
South Africa	Healthcare-facility air; public healthcare facilities	Air filtration; real-time qPCR (16S rRNA gene)	Collects airborne particles for laboratory analysis; detects and quantifies target DNA	13 of 591 samples (2.2%) positive (1.08×10^4 to 3.55×10^7 copies/m³); all positives were stationary area samples	Results depend on sampling conditions and collection procedures; DNA detection does not confirm viability or transmission	Matuka et al. (2021)
South Africa	Congregate buildings and public transport (minibus taxis, buses, trains); peri-urban township, Cape Town	CO_2 monitoring and occupancy counts; ventilation-rate estimation	Estimates ventilation conditions and potential exposure	Estimated ventilation 44 to 443 L/s in minibus taxis and 24 to 425 L/s in bars; potential-transmission rates up to nine times the median	Does not directly detect *M. tuberculosis* or confirm transmission; describes conditions that may affect	Deol et al. (2022)

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				on 12 of 43 taxi journeys	airborne exposure only	
South Africa	Clinic air; primary-care clinic	High-flow air filtration; ddPCR; CO_2 monitoring	Detects and quantifies *M. tuberculosis* DNA in air samples	Median 121 DNA copies in 74 clinic samples versus 3 copies in 47 control samples	Does not independently confirm infectiousness	Middelkoop et al. (2022)
South Africa	School classrooms and clinic air	Air filtration; ddPCR; CO_2 monitoring; student TB screening	Detects and quantifies *M. tuberculosis* DNA in air samples	13 of 72 classrooms (18.1%) and 4 of 39 clinic measurements (10.3%) positive	Airborne DNA was detected; infectiousness was not established	Bunyasi et al. (2022)
Brazil	Prison-cell surfaces	Environmental surface swabbing; GeneXpert MTB/RIF Ultra; qPCR; whole-genome sequencing	Samples surfaces for targeted mycobacterial DNA; detects and quantifies target DNA; characterizes or identifies detected genetic material	55 of 340 swabs (16%) positive; 0 of 49 control swabs positive	DNA detection does not confirm viable bacteria; surface-mediated transmission not confirmed; sensitivity limited by sample characteristics and assay detection limits; sequencing interpretation depends on target selection	Verma et al. (2022)
Kruger National Park, South Africa	Soil near waterholes	Direct soil DNA extraction; genus-specific hsp65 PCR with Sanger sequencing; GeneXpert Ultra; RD1/RD4 PCR	Investigates mycobacterial genetic material in soil; characterizes or identifies detected genetic material	MTBC DNA in 3 of 180 samples (1.7%); M. bovis confirmed in 2 of 3; GeneXpert Ultra positive in 0 of 180	Molecular detection alone cannot establish viability; study identified M. bovis, so it is not direct evidence of environmental *M. tuberculosis*	Matthews et al. (2025)
Durban, South Africa (review)	Wastewater and water-related environments	Culture-based methods; PCR-based detection; high-throughput sequencing	Culture can provide evidence of viable organisms when growth is recovered	Up to 5.5×10^5 copies/L in untreated wastewater; 7 of 13 treated-effluent samples positive	Detection does not by itself confirm infectious aerosols; recovery may be affected by sample quality and laboratory constraints	Mtetwa et al. (2022)

Table 3 provides a summary of studies investigating the presence of *Mycobacterium tuberculosis* in a range of environments including healthcare facility/clinic air, school classrooms, public transport/congregate settings, prison cell surfaces, soil around water holes and wastewater. The table includes information on the sampling method and detection method used such as air filtration, real time qPCR, ddPCR, CO 2 monitoring, surface

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swabbing, GeneXpert, sequencing and culture. Information such as what the method is able to demonstrate, what was found, limitations or what it indicates and who conducted the study and when. Findings indicated the presence of DNA in clinic and school classrooms, positive swabs from prison cells, *M. bovis* DNA in soil samples and high levels of DNA in untreated wastewater. Overall, there is evidence for the presence of genetic material from Mycobacterium; however, the limitations section of the table consistently states that this does not indicate viability, infectiousness or transmission, therefore more evidence is required to determine if there would have been significant human exposure.

(a) Air filtration with molecular detection in healthcare and clinical settings.

Matuka et al. (2021) used air filtration followed by real-time qPCR targeting the 16S rRNA gene and detected *M. tuberculosis* DNA in 13 of 591 samples (2.2%), with concentrations ranging from 1.08×10^4 to 3.55×10^7 copies/m³, all of which were obtained from stationary area samples. Middelkoop et al. (2022) used high-flow air filtration with ddPCR and CO_2 monitoring, quantifying a median of 121 DNA copies in 74 clinic samples compared with 3 copies in 47 control samples. Air filtration has been demonstrated to collect airborne particles and detect and quantify target DNA. However, detection efficiency may be influenced by environmental conditions, including airflow, ventilation rates, temperature, humidity, and particle distribution, as well as sampling duration and collection procedures. Furthermore, the presence of genetic material cannot be interpreted as evidence of bacterial viability or actual transmission, particularly when DNA-based detection is performed without complementary viability testing

(b) Air detection in schools.

Bunyasi et al. (2022) applied air filtration with ddPCR, CO_2 monitoring, and student TB screening in school classrooms and clinics, detecting DNA in 13 of 72 classrooms (18.1%) and 4 of 39 clinic measurements (10.3%). The classroom detection rate was therefore nearly twice that of the clinic measurements, while higher CO_2 concentrations were recorded in classrooms, suggesting comparatively poorer ventilation. The application of this detection approach across different indoor environments demonstrates its feasibility in identifying airborne mycobacterial DNA. However, the detection of genetic material alone cannot establish bacterial viability or infectiousness. Detection levels may also be influenced by differences in ventilation, occupancy, air circulation, and indoor environmental conditions, including temperature and humidity. Consequently, the findings demonstrate the feasibility of environmental detection rather than confirmed airborne transmission.fore nearly double that of the clinic measurements, and classroom CO_2 concentrations were higher than those in clinics, suggesting poorer ventilation in classrooms. This confirms that the same detection approach transfers across indoor settings, but airborne DNA was detected without establishing infectiousness, so the findings demonstrate feasibility rather than transmission risk.

(c) Surface swabbing with GeneXpert, qPCR, and sequencing.

Verma et al. (2022) collected 340 environmental surface swabs from prison cells occupied by people with active TB and analyzed them with GeneXpert MTB/RIF Ultra, qPCR, and whole-genome sequencing, detecting DNA in 55 of 340 swabs (16%) with 0 of 49 control swabs positive. Surface swabbing can therefore both detect and characterize detected genetic material, including resistance markers, and the contrast between 16% positives and zero controls provides a clear internal comparison. However, DNA detection does not confirm viable bacteria, surface-mediated transmission was not confirmed, and sensitivity was limited by sample characteristics and assay detection limits, with sequencing interpretation dependent on target selection. swabs positive. Surface swabbing can therefore both detect and characterize detected genetic material, including resistance markers, and the contrast between 16% positives and zero controls provides a clear internal comparison. However, DNA detection does not confirm viable bacteria, surface-mediated transmission was not confirmed, and sensitivity was limited by sample characteristics and assay detection limits, with sequencing interpretation dependent on target selection.

(d) Direct soil DNA extraction.

Direct soil DNA extraction was applied by Matthews et al. (2025) to 180 soil samples from Kruger National Park, and genus-specific hsp65 PCR, Sanger sequencing, GeneXpert Ultra, and RD1/RD4 PCR were used for analysis. MTBC DNA was detected in 3 of 180 samples (1.7%), and *M. bovis* was confirmed in 2 of these 3 positives, while the third positive sample was not differentiated to species level. GeneXpert Ultra returned no positive results among the 180 samples. The RD1/RD4 confirmation of *M. bovis* shows that the soil assay pipeline can resolve which MTBC member is present. However, bacterial viability cannot be established by molecular detection alone, and the identification of *M. bovis* in 2 of the 3 positive samples falls short of direct evidence for environmental *M. tuberculosis*. Detection may also be influenced by soil moisture, temperature, pH, and organic matter content, all of which can affect DNA preservation and extraction efficiency.

(e) Culture-based and molecular methods in wastewater.

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Mtetwa et al. (2022) summarized culture-based methods, chemical decontamination with NaOH or CPC followed by growth on 7H9/7H10/L-J media at 35–37 °C for 6 to 12 weeks, alongside PCR-based detection (16S rRNA, IS6110) and Illumina metagenomic sequencing. Reported concentrations reached up to 5.5×10^5 copies/L in untreated wastewater, and MTBC was still detected in 7 of 13 treated-effluent samples ($0.74 \times 10^4 \pm 1.40 \times 10^4$ copies/L) after treatment removed 98.6 (±2.7)% of organisms. Culture is the only method in the table that can provide evidence of viable organisms when growth is recovered, which makes it the critical complement to DNA-based detection, although recovery remains constrained by sample quality and laboratory constraints, and detection alone cannot confirm the presence of infectious aerosols. Recovery may also be affected by wastewater pH, temperature, organic matter, chemical composition, and sample storage conditions, which may influence bacterial survival and culture recovery.

(f) Ventilation estimation without direct detection.

Deol et al. (2022) used CO_2 monitoring and occupancy counts rather than bacterial assays, estimating ventilation of 44 to 443 L/s in minibus taxis and 24 to 425 L/s in bars, and deriving potential-transmission rates up to nine times the median on 12 of 43 taxi journeys. This method estimates ventilation conditions and potential exposure, but direct detection of *M. tuberculosis* and confirmation of transmission were not established, so it occupies a distinct evidential category from the detection-based rows of the table. Its contribution is to characterize where airborne exposure conditions are most unfavorable, which complements rather than substitutes for the molecular detection rows. Ventilation may also be influenced by outdoor temperature, wind conditions, occupancy, and the opening or closing of windows and doors, which can affect natural air exchange.

(g) Overall interpretation of methodological differences.

Across the table, the limitation column consistently states that DNA detection cannot establish bacterial viability, infectiousness, or transmission, and the studies differ substantially in sampling locations, sample sizes, collection techniques, assays, and detection limits. For example, detection rates ranged from 1.7% in soil and 2.2% in healthcare-facility air to 16% on prison surfaces and 18.1% in classrooms, but these figures reflect different environments, sample numbers, and analytical sensitivities rather than a single comparable contamination rate. These variations may also be influenced by environmental conditions, such as temperature, humidity, soil moisture, and ventilation, which can affect DNA persistence and sample recovery. Stronger conclusions about human exposure therefore require standardized sampling protocols combined with viability assessment, since the current evidence establishes only the presence of mycobacterial genetic material in these settings

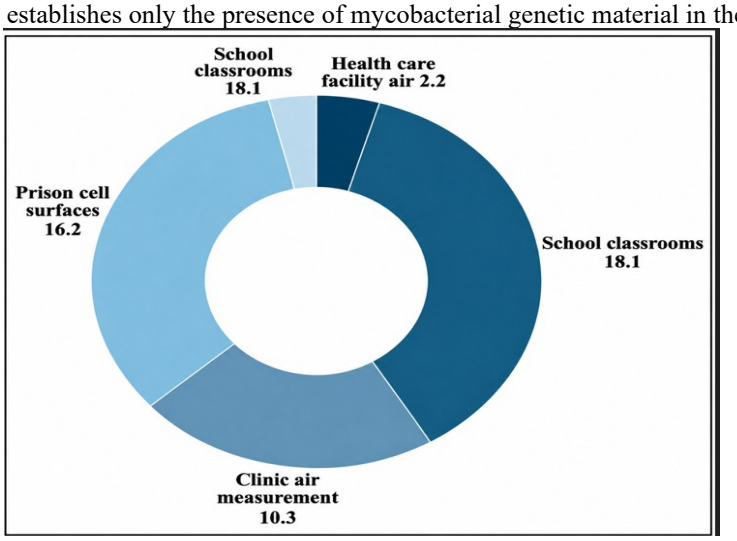


Figure 3. Distribution of Reported Environmental Detection of Mycobacterium tuberculosis Across Different SettingsDistribution of reported Environmental detection of M. tuberculosis across different settings

This figure shows the distribution of reported findings across different environmental settings. School classrooms had the highest percentage at 18.1%, followed by prison-cell surfaces at 16.2% and clinic air measurements at 10.3%. Healthcare-facility air and school classrooms were also reported at 2.2% and 8.1%, respectively.

Overall, the figure indicates that environmental investigations have examined several settings, particularly schools, clinics, and prisons. These findings highlight the importance of monitoring different environments for *Mycobacterium tuberculosis*. However, the percentages should be interpreted carefully because the studies used

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different sampling methods and conditions, and detection does not necessarily confirm the presence of viable bacteria or actual TB transmission.

III. Environmental Occurrence, Potential Exposure, Transmission Risks, and Limitations of DNA-Based Detection

The reviewed literature identified several environmental conditions that may be relevant to potential TB exposure, particularly in crowded indoor environments and locations occupied by individuals with active TB. However, the strength of the evidence connecting environmental occurrence with actual transmission differed among the studies. An important issue across the reviewed literature is the difference between detecting mycobacterial DNA and demonstrating the presence of viable or infectious bacteria: molecular techniques can detect genetic material in environmental samples, but positive results do not automatically establish that the organisms are alive, capable of replication, or able to cause infection.

Matuka et al. (2021) reported differences in airborne *M. tuberculosis* detection according to healthcare facility characteristics. Mechanical ventilation was associated with higher odds of airborne detection than natural ventilation in the study, with an odds ratio of 4.77. The authors also reported higher odds of detection in the Western Cape than in Gauteng, with an odds ratio of 4.82. These associations should not be interpreted as evidence that mechanical ventilation itself causes increased transmission. Differences in facility conditions, ventilation performance, and other factors may influence the observed results.

Deel et al. (2022) investigated ventilation and occupancy in congregate environments and public transportation. Their findings indicated that minibus taxis experienced particularly high relative potential-transmission rates under certain conditions, reaching up to nine times the median rate on 12 of 43 journeys. Trains also presented concerns related to crowding, while a clinic with generally good ventilation experienced periods of increased potential risk because of high occupancy. These results highlight the importance of considering both ventilation and the number of people sharing an indoor space. However, the study estimated potential transmission conditions rather than directly measuring environmental *M. tuberculosis* occurrence or confirmed transmission events.

Marni et al. (2024) examined household physical conditions, including ventilation, humidity, temperature, lighting, and occupancy density, and reported an odds ratio of 2.667 for the association between inadequate overall household conditions and TB incidence. However, the small sample size and wide confidence interval limited the certainty of this association. The findings support further investigation of household conditions but fall short of establishing that any particular factor independently caused TB infection. Surface detection likewise provides information about potential exposure locations. Verma et al. (2022) found *M. tuberculosis* DNA on surfaces in prison cells occupied by individuals with active TB and reported an association between surface detection and the sputum bacterial load of those individuals. These findings suggest that surface sampling can help identify areas of potential contamination. Nevertheless, viable bacteria on the sampled surfaces were not confirmed, nor was it established that surface contact resulted in infection.

Table 4. Environmental Factors Associated With Potential TB Exposure

Environmental factor / finding	Reported evidence	Interpretation	Author/year
Healthcare ventilation and facility characteristics	Higher odds of airborne detection with mechanical versus natural ventilation (OR = 4.77); higher odds in Western Cape than Gauteng (OR = 4.82).	Associations do not establish causation or prove increased transmission.	Matuka et al. (2021)
Ventilation and occupancy	Some minibus taxi journeys had estimated potential-transmission rates up to nine times the median on 12 of 43 journeys.	Crowding and ventilation may influence potential airborne exposure; the study did not directly detect *M. tuberculosis*.	Deol et al. (2022)
Household physical conditions	OR = 2.667 for the association between	Interpret cautiously because the sample was	Marni et al. (2024)

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	inadequate overall household conditions and TB incidence.	small and the confidence interval was wide.	
Environmental surfaces in prison cells	*M. tuberculosis* DNA detected in 16% of swabs from cells occupied by people with active TB.	May identify potentially contaminated locations but does not prove surface-mediated transmission.	Verma et al. (2022)

Table 4 provides a summary of studies investigating environmental factors associated with potential TB exposure in a range of settings including healthcare facilities, congregate buildings and public transport, households, and prison cells. The table includes information on the environmental factor or finding reported, such as healthcare ventilation and facility characteristics, ventilation and occupancy conditions, household physical conditions, and the presence of bacterial material on environmental surfaces. Information such as the reported evidence for each factor, what the evidence indicates or its limitations, and who conducted the study and when. Findings indicated higher odds of airborne detection with mechanical ventilation and in the Western Cape, high estimated potential-transmission rates in minibus taxis, an association between inadequate household conditions and TB incidence, and *M. tuberculosis* DNA detected on prison-cell surfaces. Overall, there is evidence that several environmental conditions are relevant to potential exposure; however, the interpretation section of the table consistently states that these associations do not establish causation, direct detection, or confirmed transmission, therefore more evidence is required to determine the actual contribution of these environmental factors to TB infection and disease.

(a) Healthcare ventilation and facility characteristics.

Matuka et al. (2021) reported higher odds of airborne detection with mechanical versus natural ventilation (OR = 4.77) and higher odds in the Western Cape than in Gauteng (OR = 4.82), alongside more frequent detection where UVGI was absent (9 vs 4 positives, not statistically significant) and measured ventilation of only 0 to 0.34 ACH against the recommended 6 ACH. These associations identify facility-level conditions linked to airborne detection, but they do not establish causation or prove increased transmission, since differences in facility conditions, ventilation performance, and other unmeasured factors may explain the observed pattern. The ventilation gap of roughly 17-fold between the measured maximum and the recommended standard nonetheless points to a concrete, quantifiable area for infection-control improvement.

(b) Ventilation and occupancy in transport and congregate settings.

Deol et al. (2022) reported that estimated potential-transmission rates reached up to nine times the median on 12 of 43 minibus taxi journeys, a finding driven mainly by low ventilation (44 to 443 L/s), whereas risk on trains, where occupancy reached 132, was driven mainly by crowding. Ventilation and occupancy are thus shown to jointly shape potential airborne exposure, and the two drivers are seen to differ by setting: taxis were found to be ventilation-limited, while trains were found to be occupancy-limited. Since *M. tuberculosis* was not directly detected in the study, its contribution lies in identifying where exposure conditions are most unfavorable, rather than in confirming transmission. The elevated risk observed in the clinic during high-occupancy periods, despite good ventilation, further illustrates that neither factor alone is sufficient.

(c) Household physical conditions.

Marni et al. (2024) reported an odds ratio of 2.667 for the association between inadequate overall household conditions and TB incidence, with MTB detected in 4 of 15 households and 10 of 15 households (66.7%) failing the healthy-home criteria. The household emerges as a plausible exposure domain, supported by the concurrent detection of MTB in roughly a quarter of sampled homes and the predominance of substandard conditions. However, the small sample and wide confidence interval warrant cautious interpretation, and the association does not establish that any single household factor, ventilation, humidity, temperature, lighting, or occupancy density, independently caused infection.

(d) Prison-cell surfaces.

Verma et al. (2022) detected *M. tuberculosis* DNA in 16% of swabs (55 of 340) from cells occupied by people with active TB, with surface detection associated with occupants' sputum bacterial load and antibiotic-resistance markers identified in some samples. Surface contamination therefore marks potentially contaminated locations and may help target investigations of shared spaces, and its correlation with sputum load provides a plausible exposure gradient. Nevertheless, positive surface findings do not prove surface-mediated transmission and must

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be weighed against the dominant evidence for airborne spread, particularly since viable bacteria were not confirmed in any sample.

(e) Overall interpretation across factors.

Table 4 links potential TB exposure to mechanical rather than natural ventilation in healthcare facilities (OR = 4.77), to facility location in the Western Cape rather than Gauteng (OR = 4.82), and to low ventilation and high occupancy in minibus taxis, where estimated potential-transmission rates reached up to nine times the median. It also links potential exposure to inadequate overall household conditions (OR = 2.667) and to *M. tuberculosis* DNA on 16% of swabs from prison cells occupied by people with active TB. Yet the interpretation column consistently states that none of these associations establishes causation, direct detection, or confirmed transmission. The appropriate conclusion is that these ventilation, occupancy, household, and surface findings mark settings deserving further assessment, while their actual contribution to TB infection and disease remains to be demonstrated through evidence that links them directly to confirmed human infection.

Beyond potential exposure, the persistence and viability of the detected material remain central to interpretation. Verma et al. (2022) detected *M. tuberculosis* DNA on prison surfaces, but viable bacteria were not confirmed in the environmental samples. Similarly, Matuka et al. (2021), Middlekoop et al. (2022), and Bunyasi et al. (2022) reported molecular detection of airborne bacterial material without establishing that the detected DNA represented viable infectious organisms. These findings demonstrate the usefulness of environmental molecular surveillance while also identifying an important limitation in interpreting positive results.

The review by Martinez et al. (2019) examined evidence concerning the detection, survival, and infectious potential of *M. tuberculosis* in environmental settings. The review described reports of detection in soil, water, air, dust, and contaminated materials, as well as experimental findings indicating that the bacterium could remain viable in soil for months or longer. However, the authors emphasized that the contribution of environmental reservoirs to human TB transmission remains insufficiently established. Experimental evidence of survival supports the possibility of persistence under particular conditions but falls short of demonstrating that environmental reservoirs account for a measurable proportion of human TB cases. The wastewater review by Mtetwa et al. (2022) also identified uncertainty concerning bacterial survival and infectiousness in wastewater. Although MTBC DNA was reported in untreated and treated wastewater, wastewater exposure was left unconfirmed as a route of infection. Similarly, Matthews et al. 's soil investigation identified MTBC DNA, but culture was unavailable owing to regulatory restrictions on sample transport. The viability of the detected organisms therefore remained undetermined.

Another consideration is that the *M. tuberculosis* complex includes multiple related organisms. The detection of *M. bovis* DNA in soil, for example, provides evidence relevant to environmental animal tuberculosis but should not be treated as equivalent to detecting *M. tuberculosis* in a human-associated environment. Organism identification and the specificity of the detection method must therefore be considered when interpreting environmental findings. The reviewed evidence highlights the need to distinguish four related but different findings: detection of bacterial genetic material, confirmation of viable bacteria, demonstration of infectious potential, and evidence of actual transmission. These findings require different levels of evidence. Future studies combining molecular detection with suitable culture-based or other viability assessments, where technically feasible, could help clarify whether environmental detections represent viable organisms. Investigations linking environmental results with epidemiological evidence would also help determine the actual contribution of environmental occurrence to TB infection and disease.

IV. Implications for Tuberculosis Prevention, Environmental Surveillance, and Infection Control

The reviewed literature suggests that environmental investigation can complement established TB prevention and infection-control measures by identifying settings that warrant further assessment. Environmental sampling, indoor air monitoring, and the evaluation of ventilation and occupancy can provide additional information about possible exposure conditions in healthcare facilities, schools, households, prisons, and public transportation. In healthcare settings, Matuka et al. (2021) identified airborne *M. tuberculosis* DNA in public healthcare facilities and reported ventilation-related differences in detection. These findings support the importance of evaluating ventilation performance and existing infection prevention and control measures. Bunyasi et al. (2022) also demonstrated the feasibility of detecting airborne bacterial DNA in school classrooms and public health clinics. Environmental monitoring could therefore be considered as an additional investigative tool in settings where people gather, particularly when assessing possible exposure conditions.

The findings of Deol et al. (2022) emphasize the importance of considering ventilation and occupancy together. Their results suggest that crowded public transportation and periods of high occupancy deserve attention when evaluating potential airborne exposure. In households, the findings of Marni et al. (2024) support further assessment of physical conditions associated with TB incidence. However, because these studies do not

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independently establish the causal effect of individual environmental factors, prevention measures should be based on the broader evidence and established TB control practices rather than on environmental detection alone. Environmental surface sampling, as demonstrated by Verma et al. (2022), may also help identify locations associated with people who have active TB. Such information could support environmental investigations and the assessment of shared spaces. Nevertheless, positive surface samples should not automatically be interpreted as proof that contaminated surfaces are a major route of TB transmission. The findings must be evaluated alongside evidence concerning airborne transmission, bacterial viability, and the circumstances of potential exposure.

Wastewater surveillance and soil investigation provide additional opportunities for environmental research. The findings summarized by Mtetwa et al. (2022) indicate that MTBC genetic material can be detected in wastewater systems, while the investigation by Matthews et al. highlights the potential for environmental sampling to investigate mycobacteria in natural settings. These approaches may contribute to broader environmental surveillance, but their value for predicting human TB risk requires further validation. Overall, environmental surveillance should be viewed as a complementary tool rather than a replacement for patient-based diagnosis, contact investigation, or established infection-control measures. The reviewed studies support further development of standardized environmental sampling protocols, improved methods for evaluating bacterial viability, and stronger integration of environmental results with epidemiological information. These improvements would help determine when environmental detection provides actionable information for TB prevention and when additional investigation is necessary before drawing conclusions about transmission risk.

V. Limitations of the Reviewed Literature

The reviewed literature has several limitations that affect the interpretation and comparison of its findings. First, the studies investigated different environmental sources, including air, surfaces, soil, and wastewater. They also used different sampling procedures, analytical methods, and sample sizes. This methodological variability limits direct comparisons of reported detection percentages and precludes treating the findings as measurements of a single, consistent environmental occurrence rate. Second, six of the seven primary studies relied on molecular detection methods (PCR, qPCR, ddPCR, GeneXpert, or sequencing). Although PCR-based techniques and related assays can identify bacterial genetic material, they cannot independently establish the presence of viable or infectious bacteria. Studies lacking confirmation of viability therefore provide limited evidence about whether the detected organisms could cause infection under the conditions investigated.

Third, the evidence connecting environmental occurrence with confirmed human TB infection or disease was limited. Five primary studies identified environmental DNA, and Deol et al. (2022) examined factors associated with potential exposure, such as ventilation and occupancy, without direct detection. However, these findings do not necessarily establish a direct causal relationship between environmental contamination and TB transmission. Even studies reporting associations between household conditions and TB incidence require cautious interpretation because other factors can contribute to the observed relationship. Fourth, the geographic distribution of the literature was uneven. Five of the seven primary studies were conducted in South Africa, including the soil investigation at Kruger National Park; the remaining two were conducted in Indonesia (Marni et al., 2024) and Brazil (Verma et al., 2022). Other studies examined Brazil, Indonesia, and a natural environment in South Africa, while review articles covered multiple countries or broader global evidence. This distribution limits the extent to which the findings can represent all geographical regions, environmental conditions, and populations.

Fifth, one primary study (Matthews et al., 2025) investigated *M. bovis* rather than *M. tuberculosis*, and the three review articles (Martinez et al., 2019; Mtetwa et al., 2022; Zhang et al., 2022) addressed MTBC generally. Although these studies are relevant to understanding the environmental occurrence of members of the complex, their findings cannot automatically be generalized to *M. tuberculosis* or human TB transmission. The organism identified in each study must remain clear when synthesizing the evidence. Finally, the reviewed material includes both original research and review articles. Review articles may summarize evidence from studies that overlap with the original investigations included in the same review. Consequently, their findings should not be counted as independent environmental observations, and the ten publications should not be interpreted as ten equivalent original experiments. In addition, the evidence summarized in the study table does not provide a complete, independently verified account of every study published on the topic. The results therefore describe the reviewed publications rather than the total global evidence base. Despite these limitations, the reviewed literature provides useful evidence concerning the environmental settings in which mycobacterial material has been detected, the methods used to investigate its occurrence, and the environmental conditions relevant to potential exposure. The principal research gaps concern confirmation of bacterial viability, standardization of detection methods, broader geographic representation, and stronger evidence connecting environmental findings with confirmed TB infection and disease.

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CONCLUSION

This review aims to explore the presence of *Mycobacterium tuberculosis* and *M. tuberculosis* complex organisms within the environment, how they are identified and potentially how they may relate to TB exposure, infection and disease. Ten publications were included in this review with 7 primary studies and 3 review articles. It has been found that Mycobacterial genetic material can be found in many environments including healthcare facilities, schools, homes, prison cells, public transport, surfaces, soil and waste water. This provides additional information on where Mycobacterial material may be present.

Whilst there appears to be a link between environmental factors such as poor ventilation and overcrowding increasing risk of TB exposure, there is insufficient evidence to conclude that environmental contamination will lead to causing or contributing to TB in humans. Six of the seven primary studies used molecular identification methods such as PCR-based techniques to identify the presence of genetic material from the bacteria; however, a positive molecular result does not necessarily mean the bacteria were viable. Differences in sampling methods, identification methods, study setting and geographical areas make it difficult to compare results. Studies relating to MTBC or other species within the complex such as *Mycobacterium bovis* need to be considered separately to studies looking specifically at *M. tuberculosis*.

This review has found that while environmental surveillance has potential to be used as an adjunct tool for TB surveillance and infection prevention, it is unclear whether it can be used to determine risk of transmission. Standardised sampling methods should be used in future studies alongside viability methods. The link between the identification of bacteria in the environment and epidemiological evidence for infection in humans should also be explored. More research is needed in a variety of geographical areas and environments to improve evidence. Until these issues are resolved, it cannot be assumed that the identification of bacteria in the environment is evidence that it is infectious. Environmental surveillance should not be considered as an alternative to current methods of diagnosing TB, identifying contacts and preventing infections.

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RECOMMENDATION

Based on the findings and limitations identified in this systematic review, further research is recommended to strengthen the understanding of the environmental occurrence of *Mycobacterium tuberculosis* and its possible association with tuberculosis (TB) exposure, infection, and disease. Although the reviewed literature demonstrates that mycobacterial genetic material can be detected in different environmental settings, the available evidence remains insufficient to establish the extent to which environmental contamination contributes directly to human TB transmission. Therefore, future investigations should focus on improving environmental detection methods, determining bacterial viability, and establishing stronger links between environmental findings and epidemiological evidence.

Future studies should adopt standardized environmental sampling and detection procedures to improve the reliability and comparability of findings. Differences in sampling locations, sample sizes, collection techniques, laboratory procedures, and detection methods make it difficult to compare results across studies. Researchers should establish consistent protocols for collecting and processing environmental samples from air, surfaces, soil, and wastewater, while clearly reporting sampling conditions, analytical procedures, detection limits, and results. Standardized methods would allow more meaningful comparisons between environmental settings and help determine whether differences in reported detection rates reflect actual environmental variation or differences in study design and analytical sensitivity.

Researchers should also prioritize the assessment of bacterial viability in environmental samples. Molecular methods, including polymerase chain reaction (PCR) and related techniques, are useful for detecting bacterial genetic material; however, a positive molecular result does not necessarily confirm that viable or infectious bacteria are present. Whenever feasible and appropriate, future studies should combine molecular detection with validated viability assessments and culture-based methods under suitable biosafety conditions. These approaches could provide stronger evidence regarding the persistence of *M. tuberculosis* in environmental settings and help distinguish the detection of residual genetic material from the presence of potentially infectious bacteria.

Furthermore, future investigations should examine the relationship between environmental detection and human TB infection or disease more directly. Environmental findings should be interpreted alongside relevant epidemiological information, such as the presence of individuals with active TB, exposure histories, and available clinical or public health data. Longitudinal studies and investigations that integrate environmental sampling with epidemiological surveillance may help clarify whether environmental detection corresponds with periods or locations of increased exposure. Such research is necessary because the presence of bacterial genetic material in an environmental sample does not, by itself, establish that transmission occurred through that environment or that the detected material caused infection or disease.

Additional research should be conducted in geographically diverse locations and environmental settings to address the uneven distribution of the existing literature. Since several of the reviewed investigations focused on healthcare facilities and other indoor environments in South Africa, studies in underrepresented regions and different environmental conditions are needed to determine the broader applicability of the findings. Research should also clearly distinguish *M. tuberculosis* from other members of the *M. tuberculosis* complex, particularly *Mycobacterium bovis*. Although evidence involving other members of the complex may contribute to understanding environmental mycobacteria, findings should not automatically be generalized to *M. tuberculosis* or human TB transmission without appropriate supporting evidence.

Environmental surveillance should also be evaluated as a complementary approach to existing TB prevention and infection-control strategies. Monitoring selected environments may help identify locations that warrant further investigation, particularly where poor ventilation, overcrowding, or close contact may increase the potential for airborne exposure. However, environmental surveillance should not replace established TB diagnostic procedures, contact investigation, appropriate ventilation, or other evidence-based infection-control measures. Further research should assess the practical value, reliability, and limitations of environmental monitoring to determine when its findings can meaningfully support public health decisions and the allocation of prevention resources.

Finally, future systematic reviews should incorporate newly available primary research while maintaining transparent and rigorous review procedures. Reviewers should carefully verify the eligibility, methodological quality, and findings of each included publication. Original research studies should be distinguished from review articles to avoid counting overlapping evidence as independent observations. Clear reporting of inclusion and exclusion criteria, data extraction procedures, and study limitations would improve the transparency and reproducibility of future reviews. Where sufficient comparable evidence becomes available, quantitative synthesis may also be considered; however, studies with substantially different sampling methods, environmental sources, or outcomes should not be combined without appropriate methodological justification.

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Overall, further research is needed to determine whether environmental detection of *M. tuberculosis* represents viable infectious material and to clarify the extent of its association with human TB exposure, infection, and disease. Improving sampling consistency, evaluating bacterial viability, strengthening epidemiological links, and expanding research across diverse settings will help address the current limitations in the evidence. These efforts may ultimately establish a clearer and more reliable role for environmental surveillance in supporting TB prevention, infection control, and public health decision-making.

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