

Mycobacterium tuberculosis complex in wastewater: An emerging One Health concern at the human-animal-environment interface

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ABSTRACT

Members of the *Mycobacterium tuberculosis* complex (MTBC), which comprise both human and animal-adapted species, continue to be a major global health concern. There is growing evidence that MTBC can be found in wastewater systems outside of clinical settings, indicating ongoing shedding from infected people, animals, and sources connected to healthcare. The role of wastewater as a possible reservoir and indicator of TB dynamics at the community level is called into doubt by its environmental presence. A pertinent viewpoint for comprehending MTBC circulation across human, animal, and environmental interfaces is offered by the One Health framework. Wastewater is a useful matrix for evaluating disease transmission outside of conventional surveillance systems because it serves as a confluence point for biological elements from many origins. However, concerns regarding viability, persistence, and its link to real transmission risk hamper the interpretation of MTBC detection in wastewater. The current understanding of MTBC occurrence in wastewater, including sources of contamination, environmental survival, detection techniques, and epidemiological significance, is compiled in this review. Improved detection has been made possible by molecular techniques, especially PCR-based technologies, however there are still issues with identifying viable organisms and measuring infection load. MTBC in wastewater is a new environmental issue that could have an impact on ecosystem integrity and public health monitoring. A One Health approach that incorporates wastewater monitoring may improve early detection skills and offer further insights into the dynamics of tuberculosis in human, animal, and environmental systems.

Introduction

Members of the *Mycobacterium tuberculosis* complex (MTBC) continue to produce tuberculosis (TB), a serious infectious disease that continues to provide a challenge to public health systems around the world (Hartady *et al.*, 2026). TB is one of the major causes of death from a single infectious agent despite long-standing control efforts, especially in low- and middle-income nations with frequently inadequate diagnostic capabilities, treatment coverage, and surveillance systems (Gulumbe *et al.*, 2025). A combination of the pathogen's biological traits and socio-environmental factors that promote continued exposure and infection are what cause MTBC to spread over time (Forrellad *et al.*, 2013).

Mycobacterium tuberculosis, the main human pathogen, and animal-adapted species including *M. bovis*, *M. caprae*, and *M. africanum* are among the closely related mycobacterial species that make up the MTBC (Delogu *et al.*, 2013). Although the host range, epidemiological patterns, and ecological adaption of these species vary, they have a high degree of genetic similarity (Behruzniya *et al.*, 2025). Because MTBC can infect a variety of host animals, it poses a serious threat to veterinary health, human medicine, and wildlife conservation (Szacawa *et al.*, 2025). The intricacy of tuberculosis epidemiology and the requirement for integrated control tactics are highlighted by this interspecies distribution (Warner *et al.*, 2025).

The environmental aspect of MTBC, especially its presence in wastewater systems, has received more attention in recent years (Mtetwa *et al.*, 2022a). Biological elements from institutional, animal, and human sources come together in wastewater (Mtetwa *et al.*, 2022b). Fecal shedding, respiratory secretions, hospital effluents, slaughterhouse waste, and agricultural runoff are some of the ways that MTBC bacilli might get into these systems (Zhang *et al.*, 2022). Wastewater may serve as an environmental matrix that reflects disease load and pathogen circulation patterns at the community level, despite not being usually thought of as a key transmission channel (Wijayanti *et al.*, 2025).

Following the successful implementation of wastewater-based epidemiology for viral infections, the idea of using wastewater as a monitoring tool has gained traction in environmental microbiology and public health (Dos Santos *et al.*, 2024). Wastewater analysis provides a non-invasive method for tracking population-level shedding in the context of tuberculosis and may offer early warning signs of shifts in the disease's prevalence (de-la-Rosa-Martinez *et al.*, 2026). However, due to ambiguities around viability, durability, and association with real infection rates, the interpretation of MTBC signals in wastewater is still difficult (Aw and Rose, 2012).

Because it highlights the connections between environmental, animal, and human health, the One Health paradigm offers a pertinent conceptual basis for tackling these issues (Danasekaran, 2024). Through zoonotic transmission, shared environmental exposure, and overlapping ecological niches, tuberculosis serves as an example of this interconnectivity (Maity *et al.*, 2026). By incorporating wastewater surveillance into a One Health strategy, more thorough disease monitoring strategies may be supported and the understanding of MTBC circulation across various compartments may be improved (Martínez-Lirola *et al.*, 2023).

With a focus on sources of contamination, environmental persistence, detection techniques, and epidemiological consequences, this study attempts to summarize the state of the art regarding the presence and behavior of MTBC in wastewater systems. Additionally, it addresses the applicability of wastewater-based surveillance within an integrated One Health framework and emphasizes its potential function as a supplementary tool in tuberculosis control.

Overview of MTBC

The MTBC is a group of closely related pathogenic mycobacteria responsible for tuberculosis in humans and a range of animal species, characterized by high genetic similarity but distinct host adaptation and epidemiological roles. Table 1 summarizes the major members of the MTBC,

including species adapted to humans, domestic animals, and wildlife.

Taxonomy and species diversity

A collection of genetically similar mycobacterial species that cause tuberculosis in humans and a variety of animal hosts make up the MTBC (Mvubu and Jacoby, 2023). These organisms differ in host preference, pathogenicity, geographic distribution, and epidemiological relevance despite having a high degree of genetic similarity (Santos-Pereira *et al.*, 2021). Clarifying transmission mechanisms and evaluating possible dangers at the human–animal–environment interface require an understanding of the diversity of MTBC (Rajbhandari *et al.*, 2022).

The most common member of the complex and the main cause of tuberculosis in humans globally is *Mycobacterium tuberculosis* (Mohammadnabi *et al.*, 2024). The great majority of tuberculosis infections are caused by this species, which is highly suited to human hosts (Alsayed and Gunosewoyo, 2023). Because *M. tuberculosis* is so common, it is the main cause of MTBC shedding into municipal wastewater through human waste and effluents related to healthcare (Coleman *et al.*, 2022).

Bovine tuberculosis is caused by *Mycobacterium bovis*, which has a wide host range that includes humans, animals, and cattle (Borham *et al.*, 2022). From the standpoint of One Health, it is among the most significant MTBC members due to its zoonotic potential (Khairullah *et al.*, 2024). The interdependence of human and animal health is highlighted by the possibility of transmission through direct animal contact, contaminated animal products, or environmental exposure (Vayr *et al.*, 2018).

Goats are the primary host of *Mycobacterium caprae*, although it has also been isolated from humans, livestock, and wildlife (Rodríguez *et al.*, 2011). Despite being less common than *M. bovis*, this species contributes to the epidemiology of animal tuberculosis in a number of areas and shows how MTBC members can transcend species boundaries (Mahrous *et al.*, 2025).

In West Africa, *Mycobacterium africanum* is a major cause of tuberculosis in humans (de Jong *et al.*, 2010). Although it has a smaller geographic range and different epidemiological traits than *M. tuberculosis*, it nevertheless contributes significantly to the prevalence of tuberculosis in the area (Sharma *et al.*, 2016).

Other members of the MTBC include *Mycobacterium microti*, *My-*

cobacterium pinnipedii, and *Mycobacterium orygis*, which are linked to rodents, marine mammals, and a variety of domestic and wild animals, respectively (Szacawa *et al.*, 2025). The complex's numerous host-adapted species demonstrate the biological complexity of MTBC and highlight the necessity of integrated surveillance strategies that take into account environmental, animal, and human reservoirs (Nederlof *et al.*, 2026).

Epidemiology and public health importance

Both human and animal populations continue to suffer greatly from tuberculosis, which is still a major global health concern (LoBue *et al.*, 2010). *Mycobacterium tuberculosis* is the primary cause of tuberculosis in humans, although animal-adapted species like *M. bovis* and *M. caprae* are major contributors to tuberculosis in cattle and wildlife (Mohammed *et al.*, 2025). Members of the MTBC are found in a variety of geographical areas (Bayraktar *et al.*, 2011). The potential of MTBC to endure in interrelated human, animal, and environmental systems is shown in its ubiquitous prevalence (Kirika *et al.*, 2026).

MTBC is linked to a significant illness burden (Danchuk *et al.*, 2025). Tuberculosis is still one of the most common infectious disease-related causes of death in humans, especially in low- and middle-income nations where socioeconomic and healthcare inequalities make transmission easier (Cioboata *et al.*, 2025). Cattle production and wildlife protection are still impacted by bovine tuberculosis, which causes financial losses through reduced output, surveillance expenses, travel restrictions, and eradication efforts (Bonilla-Aldana *et al.*, 2024). By sustaining transmission cycles outside of domestic animal populations, the existence of wildlife reservoirs makes disease management efforts even more difficult (Lekko *et al.*, 2020).

Beyond its immediate therapeutic impact, MTBC is important for public health (Yang *et al.*, 2023). Vulnerable groups are further at risk from zoonotic transmission of animal-adapted MTBC species, especially those who have frequent contact with livestock or animal products (Nuraini *et al.*, 2024). The possible importance of environmental contamination in pathogen transmission is further highlighted by growing evidence of MTBC detection in environmental matrices, including wastewater (Mtetwa *et al.*, 2026). These elements highlight the necessity of integrated surveillance and control methods that, under a One Health paradigm,

Table 1. Diversity, host range, and epidemiological significance of MTBC members.

MTBC species	Primary host(s)	Epidemiological characteristics	Public health and One Health relevance	References
<i>Mycobacterium tuberculosis</i>	Humans	Principal cause of human tuberculosis worldwide; highly adapted to human hosts and responsible for most TB cases globally	Major contributor to the global TB burden and an important source of MTBC shedding into wastewater through human excreta and healthcare-related effluents	Alsayed and Gunosewoyo (2023); Mohammadnabi <i>et al.</i> (2024); Coleman <i>et al.</i> (2022)
<i>Mycobacterium bovis</i>	Cattle, wildlife, humans	Broad host range with capacity for interspecies transmission; causative agent of bovine tuberculosis	Major zoonotic MTBC species; transmission may occur through direct animal contact, contaminated animal products, or environmental exposure, highlighting human–animal–environment interactions	Borham <i>et al.</i> (2022); Vayr <i>et al.</i> (2018); Khairullah <i>et al.</i> (2024)
<i>Mycobacterium caprae</i>	Goats, cattle, wildlife, humans	Primarily associated with goats but capable of infecting multiple domestic and wild animal species as well as humans	Contributes to animal tuberculosis epidemiology and illustrates cross-species transmission within livestock–wildlife–human interfaces	Rodríguez <i>et al.</i> (2011); Mahrous <i>et al.</i> (2025)
<i>Mycobacterium africanum</i>	Humans (predominantly in West Africa)	Human-adapted MTBC member with restricted geographic distribution and distinct epidemiological characteristics	Important contributor to regional tuberculosis burden in West Africa and demonstrates geographic diversity within MTBC	de Jong <i>et al.</i> (2010); Sharma <i>et al.</i> (2016)
<i>Mycobacterium microti</i>	Rodents, occasionally other mammals and humans	Primarily maintained in rodent populations with sporadic spillover infections	Highlights the role of wildlife reservoirs in MTBC maintenance and transmission ecology	Szacawa <i>et al.</i> (2025)
<i>Mycobacterium pinnipedii</i>	Seals, sea lions, and other marine mammals	Host-adapted to aquatic mammals and associated with tuberculosis in marine wildlife	Demonstrates the ecological diversity of MTBC and the importance of environmental and wildlife surveillance	Szacawa <i>et al.</i> (2025)
<i>Mycobacterium orygis</i>	Cattle, wildlife, humans	Emerging MTBC species reported in domestic animals, wildlife, and humans across several regions	Increasingly recognized as a zoonotic pathogen at the human–animal interface, supporting the need for integrated One Health surveillance	Szacawa <i>et al.</i> (2025); Kirika <i>et al.</i> (2026)

address the interrelated aspects of environmental, animal, and human health (Katale *et al.*, 2019).

Occurrence of MTBC in wastewater

The occurrence of MTBC in wastewater reflects the environmental dissemination of bacilli originating from human, animal, and healthcare sources through multiple contamination pathways.

Sources of contamination

MTBC enters wastewater systems via a number of interrelated institutional, animal, and human routes (Ferreira *et al.*, 2024). The main source is domestic wastewater, where infected people excrete bacilli through respiratory secretions that are swallowed and then expelled in feces or other bodily fluids (Mtetwa *et al.*, 2022b). The baseline environmental load of MTBC in municipal sewage systems is influenced by this ongoing shedding (Zhang *et al.*, 2022).

Due to the large concentration of tuberculosis patients and the release of untreated or partially treated clinical waste, healthcare facilities—including hospitals and clinics—represent another significant source (Gopalaswamy *et al.*, 2021). Hospital effluents, which frequently mix with municipal wastewater streams, can be directly exposed to MTBC through sputum disposal, laboratory washings, and contaminated liquid waste (Click, 2015).

Particularly in regions where bovine tuberculosis is widespread, livestock-related operations in the veterinary field contribute to environmental contamination through dung, urine, and waste from affected animals (Allen *et al.*, 2021). When appropriate biosafety precautions are not taken, processing waste and slaughterhouse effluents may increase the spread of MTBC (Pereira *et al.*, 2023).

Furthermore, a possible but controlled source of contamination is laboratory trash from research and diagnostic centers that handle MTBC cultures (Bartolomeu-Gonçalves *et al.*, 2024). Viable organisms may unintentionally leak into wastewater systems as a result of improper disposal or insufficient inactivation techniques (Falkinham, 2022). The major human, veterinary, and institutional pathways contributing to MTBC contamination in wastewater are summarized in Figure 1.

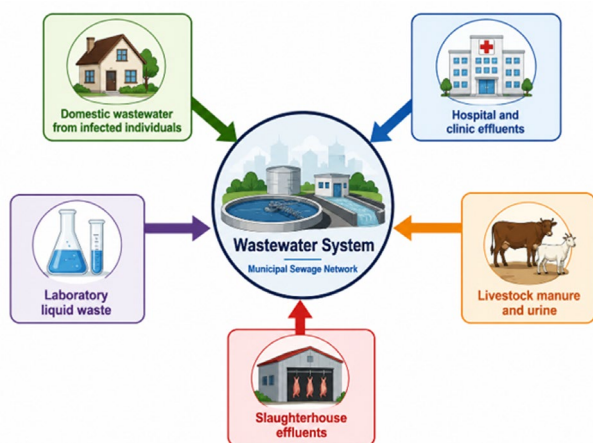


Figure 1. Main contamination pathways of MTBC entering wastewater.

Detection of MTBC in wastewater

Both molecular and microbiological methods, each with unique advantages and disadvantages, are used to detect MTBC in wastewater (Mtetwa *et al.*, 2022a). These methods are crucial for evaluating environmental pollution and assisting epidemiological investigations based on wastewater (Chen *et al.*, 2023).

The traditional method for determining viable MTBC is still culture-based procedures (Campelo *et al.*, 2021). Usually, samples are de-

contaminated before being injected onto liquid culture systems (like MGIT) or selective medium like Köwenstein-Jensen (Kassaza *et al.*, 2014). The sluggish growth rate of MTBC, overrun by non-target microorganisms, and low bacterial numbers in environmental samples limit the use of culture in wastewater, even if it offers conclusive proof of living organisms (Mtetwa *et al.*, 2022b).

Because of their high sensitivity and specificity, polymerase chain reaction (PCR) and quantitative PCR (qPCR) are frequently utilized for quick detection (Lv *et al.*, 2017). These techniques enable detection even in cases where viable organisms cannot be recovered by focusing on conserved genomic sequences like IS6110 or other MTBC-specific markers (Coros *et al.*, 2008). Although it is unable to differentiate between live and dead cells, qPCR also enables the calculation of bacterial load, which makes it helpful for comparative environmental surveillance (Dorn-In *et al.*, 2019).

By describing the whole microbial community in wastewater samples, metagenomic sequencing provides a more thorough method (Li *et al.*, 2023). This technique offers insights into co-occurring diseases and microbial ecology while enabling MTBC detection without the need for prior target selection (Severova *et al.*, 2025). However, its expensive cost, difficult data analysis, and decreased sensitivity for low-abundance species like MTBC continue to limit its application (Okumu *et al.*, 2017).

Survival and persistence of MTBC in aquatic environments

The survival and persistence of MTBC in aquatic environments are influenced by its intrinsic resistance to environmental stressors and the physicochemical characteristics of wastewater systems.

Environmental factors affecting survival

MTBC's survivability and possible environmental stability are determined by a number of physicochemical and biological parameters that affect its persistence in aquatic habitats, including wastewater (Pereira *et al.*, 2020).

MTBC viability is significantly impacted by temperature (Zheng *et al.*, 2023). While higher temperatures hasten cellular damage and reduce persistence, lower temperatures typically increase bacterial survival by lowering metabolic stress (Huang *et al.*, 2019). Seasonal and process-related temperature variations can consequently have a major impact on MTBC lifetime in wastewater systems (Shadi *et al.*, 2025).

Since MTBC can withstand neutral to slightly alkaline surroundings better, pH levels also have an impact on survival (Reichlen *et al.*, 2017). Because of its lipid-rich cell wall, MTBC is more resilient than many non-mycobacterial organisms, yet extreme acidic or strongly alkaline environments can compromise cellular integrity and lower viability (Ghazaei, 2018).

Organic matter has both inhibitory and protective properties (Fieweger *et al.*, 2019). By encouraging aggregation or association with particulate matter, organic-rich environments may protect MTBC from environmental stressors and disinfectants (Pereira *et al.*, 2023). Over time, MTBC survival may be indirectly decreased by intense microbial competition in such matrices (Ghodbane *et al.*, 2014).

One significant inactivating element is ultraviolet (UV) radiation, especially in surface waters that are exposed to sunlight (Mamahlodi, 2019). Although MTBC may be partially protected when embedded in sediments or biofilms, UV exposure damages DNA and decreases infectivity, reducing the efficacy of natural photoinactivation (Assefa and Girmay, 2024).

Potential for environmental persistence

Because of its hydrophobic, lipid-rich cell wall structure, MTBC is remarkably resilient in environmental matrices (Zhang *et al.*, 2022). Compared to many non-mycobacterial infections, this trait increases

resistance to desiccation, chemical disinfectants, and different physical stresses, allowing for longer life in wastewater conditions (Pereira *et al.*, 2020).

The ability of MTBC to bind to organic aggregates and particulate matter is a major element in environmental persistence (Cortacans and Cardona, 2025). In wastewater systems' sediments and sludge fractions, where microbial inactivation mechanisms are less effective, this interaction promotes retention and partial protection from unfavorable conditions (Prus and Styszko, 2025).

There have also been reports of MTBC participating in or remaining in biofilm communities (Liu *et al.*, 2024). Extracellular polymeric materials function as a protective matrix within biofilms, limiting exposure to environmental changes and disinfectants (Chakraborty and Kumar, 2019). During routine wastewater treatment procedures, this microenvironment may increase bacterial survival and make eradication more difficult (Amha *et al.*, 2017).

The removal of MTBC is still difficult despite standard treatment techniques such sedimentation, biological treatment, and disinfection (Mtetwa *et al.*, 2022b). Treatment effectiveness may be lowered by the organism's inherent resistance mechanisms and limited disinfectant penetration into particle and biofilm-associated niches (Nguyen, 2016). As a result, possibly viable cells or leftover MTBC genetic material may endure through wastewater treatment phases, underscoring the necessity of focused and effective control measures (Nguyen *et al.*, 2025). The key environmental mechanisms supporting MTBC persistence in wastewater are summarized in Figure 2.

One health implications of mtbc in wastewater

The detection of MTBC in wastewater highlights the interconnected

risks across human, animal, and environmental systems, reinforcing the relevance of a One Health approach. Table 2 summarizes the One Health implications of MTBC presence in wastewater systems, highlighting exposure pathways and associated risks across human, animal, and environmental domains.

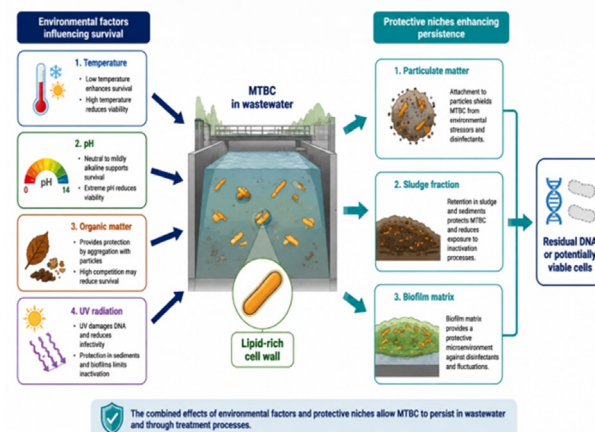


Figure 2. Environmental persistence mechanisms of MTBC in wastewater systems.

Human health risks

Although the precise transmission risk is still unknown, human exposure to MTBC in wastewater environments is seen as a developing occupational and environmental concern (Rahmatullah *et al.*, 2025). Wastewater treatment plant employees are among the main groups at risk because they may come into close contact with untreated or partially treated sewage while doing everyday tasks including aeration, mainte-

Table 2. One Health implications of MTBC in wastewater systems across human, animal, and environmental interfaces.

One Health domain	Exposure pathways and sources	Key risks and implications	References
Human health	Occupational exposure among wastewater treatment plant workers during sludge handling, maintenance activities, and aeration processes	Increased likelihood of contact with untreated or partially treated wastewater containing MTBC; potential exposure through inhalation of aerosols and accidental ingestion of contaminated droplets	Poopedi <i>et al.</i> (2023); Jones-López <i>et al.</i> (2013)
	Aerosol generation from aeration tanks, high-pressure flows, and splashing during wastewater treatment operations	Potential inhalation exposure, particularly in enclosed environments with inadequate ventilation; possible occupational health concern for wastewater workers	Coleman <i>et al.</i> (2022); de Sousa <i>et al.</i> (2020)
	Contamination of surface water and groundwater due to inadequate wastewater treatment or uncontrolled effluent discharge	Indirect community exposure through domestic, agricultural, and recreational water use; potential dissemination of MTBC genetic material and possibly viable organisms into downstream water systems	Nishmitha <i>et al.</i> (2025); Zhang <i>et al.</i> (2022)
Animal health	Exposure through contaminated irrigation water, soil, and feed resources affected by wastewater reuse or runoff	Livestock exposure, particularly in cattle and small ruminants, through grazing and water consumption; increased risk in environments supporting MTBC persistence	Santos <i>et al.</i> (2015); Pickup <i>et al.</i> (2006); Reil <i>et al.</i> (2026)
	Wildlife interaction with contaminated aquatic and terrestrial habitats influenced by anthropogenic wastewater discharge	Wildlife may act as recipients and maintenance hosts of MTBC, contributing to pathogen persistence within ecosystems	Lekko <i>et al.</i> (2020); Ferreira <i>et al.</i> (2024)
	Cross-species transmission involving animal-adapted MTBC members, particularly <i>Mycobacterium bovis</i>	Maintenance of infection cycles among livestock, wildlife, and occasionally humans; environmental contamination may facilitate indirect transmission and pathogen circulation across species	Mohammed <i>et al.</i> (2025); Malone and Gordon (2017); Santos <i>et al.</i> (2015); Yang <i>et al.</i> (2023)
Environmental health	Discharge of inadequately treated wastewater into rivers, lakes, reservoirs, and irrigation channels	Introduction of MTBC genetic material and potentially viable organisms into aquatic ecosystems; deterioration of microbiological water quality	Mtewa <i>et al.</i> (2023a); Löffler <i>et al.</i> (2023)
	Association of MTBC with sediments, suspended particles, and aquatic transport systems	Enhanced environmental persistence and downstream dissemination beyond the original contamination source	Zhang <i>et al.</i> (2022); Primm <i>et al.</i> (2004)
	Agricultural reuse of contaminated water and ecological interactions within soil–water systems	Increased exposure of livestock, wildlife, and environmental microbiota; incorporation of MTBC into broader ecological cycles	Matthews <i>et al.</i> (2025); Cavalcante-Silva <i>et al.</i> (2023)
	Hydrological transport and ecosystem connectivity between aquatic and terrestrial environments	Redistribution of MTBC across interconnected ecosystems, potentially sustaining environmental contamination and reinforcing One Health transmission pathways	Guégan <i>et al.</i> (2025); Szacawa <i>et al.</i> (2025)

nance, and sludge handling (Poopedi *et al.*, 2023). By inhaling or inadvertently consuming contaminated aerosols and droplets, these actions may increase the risk of exposure (Jones-López *et al.*, 2013).

Contamination of surface and groundwater resources is another possible route, especially in environments where wastewater treatment is insufficient or effluent discharge is not well controlled (Nishmitha *et al.*, 2025). Indirect exposure concerns to nearby communities may increase if MTBC genetic material and potentially viable organisms infiltrate downstream water systems used for residential, commercial, or recreational purposes (Zhang *et al.*, 2022).

Furthermore, contaminated particles may spread through the air due to aerosolization processes in wastewater systems, such as those produced during high-pressure flow, aeration tanks, or splashing in treatment units (Coleman *et al.*, 2022). Theoretically, one way to be exposed to such aerosols is through inhalation, particularly in small spaces with inadequate ventilation (de Sousa *et al.*, 2020).

Animal health risks

In places where effluent management is insufficient, animals may be exposed to MTBC from wastewater through environmental contamination of water sources, soil, or feed ingredients (Santos *et al.*, 2015). When runoff from contaminated sites enters grazing areas or wastewater is utilized for irrigation, livestock—especially cattle and small ruminants—may be exposed (Pickup *et al.*, 2006). Opportunities for indirect contact with infectious material are created by such exposure, particularly in environments that encourage pathogen persistence (Reil *et al.*, 2026).

Interactions between wildlife populations and contaminated water bodies or habitats impacted by anthropogenic wastewater discharge may also have an impact (Lekko *et al.*, 2020). Because they may serve as both recipients and maintenance hosts of MTBC within local ecosystems, species that share ecological interfaces with agricultural systems or human settlements are especially vulnerable (Ferreira *et al.*, 2024).

The possibility of cross-species transmission is a crucial epidemiological factor (Mohammed *et al.*, 2025). Members of the MTBC that have adapted to animals, like *M. bovis*, can spread from domestic animals to wildlife and occasionally even into human populations (Malone and Gordon, 2017). By sustaining the presence of pathogens outside of host organisms, environmental contamination may promote indirect transmission and enable infection cycles across several species (Santos *et al.*, 2015). The significance of environmental channels in maintaining MTBC circulation within One Health systems is shown by this interrelated dynamic (Yang *et al.*, 2023).

Environmental health concerns

Because MTBC contaminates aquatic environments, its presence in wastewater systems has wider effects on environmental health (Löffler *et al.*, 2023). Inadequately treated effluent discharge can change the microbiological quality of rivers, lakes, and irrigation channels by introducing MTBC genetic material and possibly live organisms (Mtetwa *et al.*, 2023a).

After being discharged into the environment, MTBC may attach itself to suspended particles and sediments, enabling extended persistence past the initial site of contamination (Zhang *et al.*, 2022). This may make it easier for the organism to spread throughout downstream habitats, particularly in water networks that are connected to urbanized or agricultural areas (Primm *et al.*, 2004).

Hydrological mobility and ecological interactions also have an impact on environmental spread, allowing pollutants to be redistributed into soil and surface water systems (Cavalcante-Silva *et al.*, 2023). The use of tainted water for irrigation in agricultural contexts may increase the exposure of livestock, wildlife, and ambient microbiota to MTBC, integrating the disease into larger ecological cycles (Matthews *et al.*, 2025).

The recorded finding of MTBC in aquatic habitats highlights its signif-

icance as an environmental pollutant, even if environmental transmission of tuberculosis is not yet firmly established as a primary route of infection (Guégan *et al.*, 2025). This emphasizes how crucial wastewater management is to preserving environmental integrity and halting ecological spread under a One Health paradigm (Szacawa *et al.*, 2025).

Wastewater-based surveillance as a tool for tuberculosis monitoring

Wastewater-based surveillance offers a complementary epidemiological approach for monitoring MTBC circulation at the population level through environmental sampling.

Principles of wastewater surveillance

An epidemiological technique called wastewater-based surveillance uses the methodical collecting and examination of sewage to get data on a population's health (Punch *et al.*, 2025). This approach is predicated on the idea that biological markers—such as viruses, genetic material, or metabolites—that diseased people excrete into wastewater systems can be found at the community level (Metwa, 2025).

Wastewater surveillance in the context of MTBC depends on the identification of bacterial DNA or, occasionally, living organisms derived from human and animal sources (Mtetwa *et al.*, 2023a). Wastewater provides a composite signal that captures ongoing transmission patterns without requiring individual-level sampling since it aggregates inputs from huge populations (Oliveira *et al.*, 2025).

For illnesses like tuberculosis, where a significant percentage of infections may remain asymptomatic or undetected, this strategy is very beneficial (Mohammadnabi *et al.*, 2024). Wastewater surveillance can supplement traditional clinical reporting and improve early warning capabilities for public health monitoring by recording population-level shedding trends (Nguyen *et al.*, 2025).

The stability of target biomarkers in environmental circumstances, analytical sensitivity, and sampling method are some of the variables that affect this system's efficacy (Zhuang *et al.*, 2024). Within a One Health framework, wastewater surveillance provides a non-invasive and affordable method of monitoring disease trends when combined with epidemiological data (Dos Santos *et al.*, 2024). A simplified workflow of wastewater-based surveillance for MTBC monitoring is presented in Figure 3.

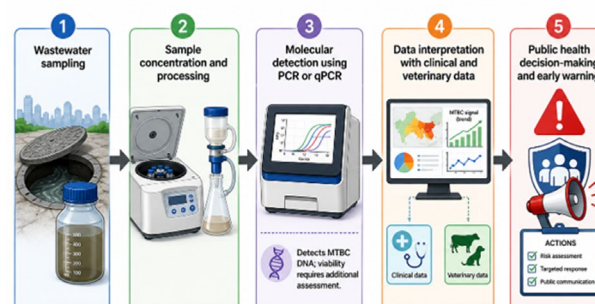


Figure 3. Wastewater-based surveillance workflow for tuberculosis monitoring.

Current evidence and applications

According to recent research, MTBC genetic material has been found in municipal wastewater in a variety of geographic locations, suggesting that wastewater can reveal underlying tuberculosis transmission within communities (Mtetwa *et al.*, 2022a). The majority of findings use molecular methods that target MTBC-specific genetic markers, such as PCR and qPCR, which have verified environmental detection even in the absence of cultured isolates (Parsons *et al.*, 2011). These results imply that waste-

water could be used as an additional matrix for population-level tuberculosis biomarker monitoring (Alli et al., 2011).

Wastewater surveillance has demonstrated potential in real-world applications as an indirect measure of the TB burden at the community level, especially in cities with centralized sewage systems (Oliveira et al., 2025). It has been suggested that variations in MTBC detection signals over time can serve as stand-ins for variations in treatment coverage, infection prevalence, or case detection effectiveness (Greaves and Rodriguez, 2026). Compared to wastewater monitoring for viral pathogens, the method is still in its early stages of development, but it offers important insight into cases of tuberculosis that are hidden or underreported (Wang et al., 2026).

One important new use is the idea of early detection (Zeng et al., 2025). Wastewater analysis may provide an early warning of increased transmission activity before clinical case numbers increase by detecting pathogen signals released by both identified and undiagnosed patients (Mtetwa et al., 2022b). Conventional surveillance may underestimate the disease burden in environments with low diagnostic capacity or delayed reporting systems (Hartady et al., 2026).

However, methodological heterogeneity, a lack of standardization, and confusion about the relationship between detected DNA levels and true infection prevalence continue to hinder the interpretation of available data (Kim et al., 2023). Notwithstanding these drawbacks, research to date indicates that wastewater-based monitoring is a viable supplementary method for tuberculosis surveillance in a One Health context (Smolinski et al., 2025).

Challenges and limitations

Data reliability and comparability are impacted by a number of methodological and interpretive issues that limit the use of wastewater-based surveillance for MTBC (Zhang et al., 2022).

Analytical sensitivity is one of the main constraints (Hemeg et al., 2023). MTBC is usually found in wastewater at low concentrations, frequently below what traditional culture techniques can detect (Mtetwa et al., 2023b). Even while molecular methods like PCR and qPCR increase sensitivity, they can still be unable to reliably identify low-abundance targets or differentiate between viable and non-viable organisms, which could result in an overestimation of epidemiological significance (Chen et al., 2025).

The absence of consistent sampling and processing procedures is another major problem (Alli et al., 2011). Significant variation in detection results can be caused by differences in storage conditions, concentration techniques, DNA extraction processes, and sampling tactics (grab vs. composite sampling) (De Almeida et al., 2013). This methodological heterogeneity lowers the repeatability of results across various surveillance systems and restricts comparability among investigations (Severova et al., 2025).

Another significant difficulty is data interpretation (Maciejak-Jas-trzębska et al., 2025). The prevalence or intensity of infection are not directly correlated with the identification of MTBC genetic material in wastewater (Biyang et al., 2024). Quantitative evaluations are complicated by variables such environmental nucleic acid degradation, dilution effects in sewage networks, and shedding heterogeneity among infected persons (Kerubo et al., 2022). Therefore, careful calibration and integration with clinical surveillance data are necessary to convert molecular signals into significant epidemiological markers (Alli et al., 2011).

Prevention and control strategies

The prevention and control of MTBC in wastewater systems require integrated measures that address pathogen sources, environmental dissemination, and treatment effectiveness within a One Health framework.

Wastewater treatment approaches

Physical, biological, and chemical treatment methods are used to remove MTBC from wastewater; each method has a distinct role in reducing pathogens (Huang et al., 2022).

Through biodegradation and adsorption to biomass flocs, biological treatment technologies including membrane bioreactors and activated sludge are crucial in lowering microbial burdens (Yang et al., 2025). Although MTBC cells are largely extracted from the liquid phase when they are integrated into sludge particles, their lipid-rich cell wall can increase survivability in these matrices, preventing total inactivation (Jacobo-Delgado et al., 2023).

After secondary treatment, disinfection procedures are essential for lowering the number of pathogenic bacteria that remain (Zhang et al., 2022). Ozonation, UV irradiation, and chlorination are common methods (Mtetwa et al., 2022b). Due to their capacity to damage nucleic acids and cellular structures, UV and ozone are often more efficient against mycobacteria (Tyagi et al., 2015). However, MTBC has comparatively stronger resistance than many intestinal bacteria, necessitating ideal exposure conditions to achieve significant inactivation (Payaslıoğlu et al., 2025).

To increase removal efficiency, advanced treatment technologies are being used more and more (Omoteso et al., 2025). While sophisticated oxidation methods produce reactive species that accelerate microbial destruction, membrane filtration technologies (such as ultrafiltration and reverse osmosis) offer a physical barrier that can hold microbial cells (Shastri et al., 2018). These technologies are especially useful in high-risk environments where pathogen concentrations may be raised, like hospital wastewater treatment (Sharma et al., 2026).

One Health-based control measures

An integrated One Health approach that connects the fields of environmental, animal, and human health is necessary to control MTBC in wastewater ecosystems (Martínez-Lirola et al., 2023). In order to decrease pathogen shedding, restrict environmental diffusion, and improve surveillance systems across interconnected interfaces, this strategy places a strong emphasis on coordinated action (Zhang et al., 2022).

Cross-sectoral cooperation between environmental management organizations, veterinary services, and public health authorities is a crucial element (Lajaunie et al., 2025). In terms of human health, this involves reducing bacterial shedding into sewage systems by early detection and efficient treatment of tuberculosis patients (Mtetwa et al., 2022b). Environmental contamination from animal sources is decreased in animal health by controlling bovine and zoonotic tuberculosis by testing, culling of affected livestock, and biosecurity measures (Desu et al., 2025).

To identify and monitor MTBC circulation across various compartments, integrated monitoring systems are necessary (Díaz-Fernández et al., 2026). A more thorough understanding of transmission patterns is made possible by the integration of wastewater surveillance with clinical and veterinary data (Szacawa et al., 2025). Integration of data from many sources can enhance early warning systems' ability to respond to outbreaks or shifts in the incidence of diseases (Elbehiry et al., 2025).

Policy and regulatory frameworks are essential for ensuring successful implementation from a governance standpoint (Cole et al., 2020). To reduce environmental discharge, standards for wastewater treatment, biosafety in medical and laboratory settings, and appropriate disposal of infectious material are required (Mtetwa et al., 2022b). Enforcing regulations more strictly improves compliance and promotes long-term disease control, especially in high-burden areas (Sazali et al., 2023).

Research gaps and future perspectives

There is still little and inconsistent information about the presence and behavior of MTBC in wastewater, despite increased interest in the

environmental aspect of tuberculosis (Nguyen *et al.*, 2025). It is difficult to generalize results at regional or global dimensions because the majority of research that are currently accessible are limited to certain geographic areas and rely on small datasets (Guterres *et al.*, 2026). This emphasizes the necessity of larger surveillance networks and coordinated data gathering in a variety of environmental contexts (Nederlof *et al.*, 2026).

Current detection techniques still have significant methodological limitations. Assessing actual infectious risk is challenging because, despite their sensitivity, molecular tests do not reliably distinguish between living and non-viable organisms (Song *et al.*, 2025). Therefore, improving environmental interpretation requires the development of more sophisticated methods that can combine viability assessment with high sensitivity (Rabello and de-Paris, 2026).

The inadequate incorporation of wastewater surveillance into regular tuberculosis control initiatives represents another significant gap (Mtetwa *et al.*, 2021). While wastewater-based surveillance has been extensively studied for viral infections, its use for tuberculosis is still in its infancy and mostly experimental (Chukwu *et al.*, 2024). Enhancing early detection skills and offering a more complete picture of disease dynamics could be achieved by fortifying the connection between environmental data and clinical surveillance (Yayan *et al.*, 2024).

Standardizing collection procedures, validating quantitative models connecting wastewater signals to disease burden, and evaluating MTBC survivability in actual environmental settings should be the top priorities for future study (Mtetwa *et al.*, 2022b). Furthermore, in order to adequately characterize the function of wastewater in MTBC ecology within a One Health paradigm, interdisciplinary research incorporating microbiology, environmental engineering, epidemiology, and veterinary science are necessary (Marfil *et al.*, 2026).

Conclusion

Emerging data suggests that MTBC is present in wastewater systems as part of environmental contamination pathways, and it continues to be a complex collection of pathogens with serious consequences for human and animal health. The finding of MTBC in sewage illustrates the potential function of wastewater as a secondary reservoir for pathogen surveillance at the community level and reflects continuous shedding from infected populations.

By collecting aggregated signals from both symptomatic and asymptomatic persons as well as contributions from animal and health-care sources, wastewater-based data offer an alternative viewpoint to traditional tuberculosis surveillance. The persistence and detectability of MTBC highlight the importance of environmental matrices in comprehending disease transmission, even though its infectious potential in wastewater environments is still unclear.

Particularly in environments with low diagnostic coverage, the incorporation of wastewater surveillance into tuberculosis monitoring frameworks presents encouraging prospects for enhancing epidemiological understanding. These results highlight the significance of a One Health strategy that links environmental, animal, and human health systems to enhance tuberculosis diagnosis, prevention, and control at many levels.

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Conflict of interest

The authors declare that there is no conflict of interest.

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