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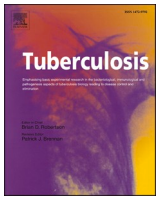

Identification of non-tuberculous mycobacteria in slaughtered cattle from Chennai, India

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A B S T R A C T

Non-tuberculous mycobacteria (NTM) are emerging pathogens in human and veterinary medicine, with a globally increasing incidence. In India, sporadic studies have identified an upward trend in NTM infections, but accurate prevalence estimates are lacking due to the absence of nationwide surveillance. Non-tuberculous mycobacteria have been reported in clinically healthy cattle and wildlife globally, complicating tuberculosis (TB) diagnostics and surveillance. This study aimed to characterize NTM species isolated from tissue samples of slaughtered cattle in Chennai using culture and targeted *hsp65* gene sequencing. A total of 118 presumed NTM samples from 115 animals were processed, and 49 isolates were confirmed as NTMs by PCR. Sequencing identified 18 different species, with *Mycobacterium intracellulare* (9/49) being the most frequent, followed by *Mycobacterium* sp. *strain 79_MI18_10584* (6/49) and *Mycobacterium elephantis* (6/49). Several identified species, including *M. intracellulare, M. fortuitum* (5/49)*, M. kansasii* (4/49)*, and M. avium*, have caused infections in humans as well. NTMs in cattle lymph nodes without visible lesions suggest their asymptomatic persistence, albeit there being a possibility of transient colonization. Non-tuberculous mycobacteria complicate bovine tuberculosis (bTB) diagnostics by inducing cross-reactive immune responses and forming granulomatous lesions resembling those caused by *Mycobacterium tuberculosis* complex (MTBC). This study highlights the presence and diversity of NTMs in Indian cattle and emphasizes the need for better surveillance, improved molecular characterization, and better understanding of their epidemiological and immunological roles in both veterinary and public health contexts.

1. Introduction

Non-tuberculous mycobacteria (NTM) are a diverse group of mycobacteria distinct from the *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium leprae*. While MTBC primarily causes tuberculosis (TB) in humans and animals, NTMs are increasingly being recognized as emerging pathogens in both human and veterinary medicine. Traditionally considered opportunistic, some NTM species can cause chronic granulomatous infections resembling TB but with distinct transmission dynamics and epidemiology [1].

The incidence of NTM infections in humans has increased worldwide, especially in North America and Europe, in recent decades. In the United States, the prevalence of NTM-positive cultures ranges between 1.4 and 6.6 per 100,000 individuals, while in the United Kingdom, the incidence of NTM-positive cultures rose from 4 per 100,000 to 6.1 per 100,000 between 2007 and 2012. In Canada, the prevalence of NTM pulmonary disease (NTM-PD) increased from 29.3 per 100,000 individuals in 1998–2002 to 41.3 per 100,000 individuals in 2006–2010. This increase may be attributed to the increasing incidence of chronic lung disease, changes in host immunity due to an aging and immunocompromised population, declining herd immunity due to the reduced TB burden in high-income countries, and environmental and climatic changes driven by expanding human-made infrastructure. Additionally, improved diagnostics, heightened clinical awareness, and expanded surveillance may also contribute to this trend [2].

India reflects this global trend, although in this case, an increasing burden of NTM infections identified through sporadic studies, rather than systematic surveillance. A comprehensive review of 56 studies spanning 1981 to 2020 reported that NTM isolation rates nearly doubled, increasing from 0.9 % (2001–2010) to 1.6 % (2011–2020). In patients suspected of having TB, 1.1 % (395 of 34,829 individuals) were diagnosed with NTM pulmonary disease (NTM-PD), highlighting the need for greater differentiation between TB and NTM in clinical settings. The species most frequently isolated in India include *M. avium complex* (MAC)*, M. chelonae, M. fortuitum,* and *M. abscessus*, with infections reported in both pulmonary and extrapulmonary cases [3]. While studies

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have confirmed an upward trend, accurate prevalence estimates remain elusive due to the absence of nationwide data collection and mandatory reporting.

Non-tuberculous mycobacterial infections have also been reported globally in animals, with studies confirming their presence in clinically healthy cattle and wildlife and highlighting their potential impact on bovine health and disease surveillance. In Switzerland, NTMs were detected in 20 % of 108 slaughtered cattle, with 22 isolates belonging to *M. avium* subsp. *hominissuis* (63 %), *M. kansasii* (18 %), *M. persicum, M. europaeum, and M. lymphaticum* [4]. Similarly, in northern China, analysis of 163 livestock samples, including hilar lymph nodes from 55 cows, liver tissue from 87 sheep, throat swabs from 20 dairy cows, and a lung sample from one reindeer, identified 39 NTM isolates. The most frequently detected species include *M. senuense, M. nonchromogenicum, M. kumamotonense, M. gordonae, M. algericum, M. arupense, and M. intracellulare*, reflecting significant species diversity in livestock populations [5].

Moreover, NTMs have been increasingly detected in wildlife, further complicating their epidemiology. In Spain, *M. avium, M. lentiflavum,* and *M. nonchromogenicum* have been frequently isolated from wild boars (*Sus scrofa*) and other wildlife species, suggesting a potential role wildlife plays in NTM transmission to cattle [6]. Similarly, a study in Argentina detected NTMs in endangered wild species such as giant anteaters, peccaries, tapirs, and pampas deer, with *M. hominissuis, M. intracellulare, M. terrae, and M. fortuitum* being the most prevalent [7]. These findings emphasize the importance of wildlife-livestock interactions in NTM transmission, reinforcing the need for enhanced surveillance of both domestic and wild animal populations.

Despite the increasing recognition of NTM infections in cattle worldwide, there are limited data on the prevalence and species distribution of NTMs in cattle populations in India. A recent study from a slaughterhouse in Kolkata found that 94 % of Mycobacterium isolates from tuberculosis-like lesions (32 out of 34) were NTMs, whereas only two belonged to MTBC. Species-level identification revealed that *M. fortuitum* was the most prevalent, followed by *M. abscessus, M. chelonae, M. parascrofulaceum, and M. novocastrense*. The high proportion of NTMs in affected cattle suggests their potential role in lesion formation and their impact on bovine tuberculosis (bTB) diagnostics [8].

Beyond their presence and eventual clinical importance in cattle, NTMs complicate TB surveillance by interfering with diagnostic tests. In Spain, a study of 373 isolates from skin test-positive cattle in TB-free herds identified 32 different NTM species, with *M. hominissuis* (69 % of MAC isolates), *M. nonchromogenicum* (27.6 %), and *M. bourgelatii* (5.6 %) being the most common species. Additionally, 53 isolates displayed mixed genotypes, suggesting the potential presence of previously uncharacterised NTM species. These findings underscore the diagnostic interference of NTMs in tuberculin skin tests (TST), leading to false-positive results, unnecessary culling, and economic losses [9].

Understanding the epidemiology and species diversity of NTMs in cattle is crucial to develop effective diagnostic, therapeutic, and preventive measures to control their spread. Given the limitations of traditional culture and biochemical methods, sequencing of the *hsp65* gene has emerged as a highly reliable method for identifying NTM species because of its high interspecies variability. The *hsp65* gene encodes the 65-kDa heat shock protein, which plays a crucial role in bacterial stress response. Its hypervariable regions allow for clear discrimination between closely related NTM species, making it particularly useful in epidemiological studies and routine diagnostics [10]. This study aimed to characterize NTM species isolated from tissue samples of clinically healthy slaughtered cattle in Chennai using culture and targeted gene sequencing using the *hsp65* gene.

2. Materials and methods

2.1. Study site and sample collection

This study was conducted at the Greater Chennai Corporation slaughterhouse, Perambur, Chennai, with the necessary permits from the City Health Officer, Department of Public Health, Tamil Nadu, India. As this study was conducted on samples obtained from routinely slaughtered cattle, no animal ethics approval was necessary in accordance with committee for control and supervision of experiments on animals (CCSEA) guidelines. The study design, sample collection methods, and animal details were previously described [11]. In total, 118 presumed NTM samples from 115 animals were included in this study. Presumed NTMs, in the context of this study are defined as culture smear-positive by acid fast staining and MPT64 immunochromatographic test (ICT)-negative isolates. Tissue samples, including bronchial and mediastinal lymph nodes, as well as tissues with or without gross visible tuberculosis-like lesions, were collected from slaughtered cattle and further processed at ICMR- National Institute for Research in Tuberculosis.

2.2. Sample processing and culture

Samples were processed using the saline dilution method, homogenized, decontaminated with 4 % NaOH, and neutralized using phosphate-buffered saline (pH 7.4). Cultures were set up in MGIT 960, Lowenstein-Jensen (LJ) slopes, LJ with sodium pyruvate (LJ-SP), and Selective Kirchner's medium (SK). Ziehl-Neelsen (ZN) staining was performed on smears from processed deposits [11].

2.3. DNA extraction and hsp65 gene sequencing for NTM identification

DNA was extracted from pure NTM isolates using the CTAB-NaCl method. The *hsp65* gene was amplified by PCR using the forward primer (5′-ACCAACGATGGTGTGTCCAT-3′) and reverse primer (5′-CTTGTCGAACCGCATACCCT-3′) [12]. Simultaneously, amplification was performed using the primers for the MPT64 gene: forward (5′-TCCGCTGCCAGTCGTCTTCC-3′) and reverse (5′-GTCCTTCGCG AGTCTAGGCCA-3′). This was performed to determine whether the isolates belonged to MTBC. The PCR products were resolved on a 1.5 % agarose gel, purified using a Qiagen PCR Purification Kit (Qiagen, Germany), and sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). Species identification was conducted using NCBI-BLASTn (https://blast.ncbi.nlm.nih.gov), and species confirmation was achieved through phylogenetic analysis with appropriate reference NTM strains (Dai et al., 2011). The study protocol is illustrated in Fig. 1.

3. Results

3.1. Culture and PCR

Of the 118 presumptive NTM cultures, 48 were obtained from bronchial lymph nodes, 39 from mediastinal, 16 from cranial, 9 from liver, 3 from spleen, 2 from lung, and 2 from prescapular lymph nodes, while growth was observed on LJ, LJ-SP, or MGIT media. All cultures exhibiting growth were confirmed as acid-fast positive using the Ziehl-Neelsen staining method. The DNA isolated from all 118 samples was subjected to PCR targeting the *hsp65* and MPT64 genes. A characteristic 441 bp amplicon for *the hsp65* gene was detected in 49 samples (Supplementary Table S1), while no amplification was observed for the MPT64 gene, confirming the absence of any MTBC in these samples. To monitor potential contamination, PCR-grade water was included as a no-template negative control during each batch of DNA extraction and PCR amplification, and no amplification was observed in these controls.

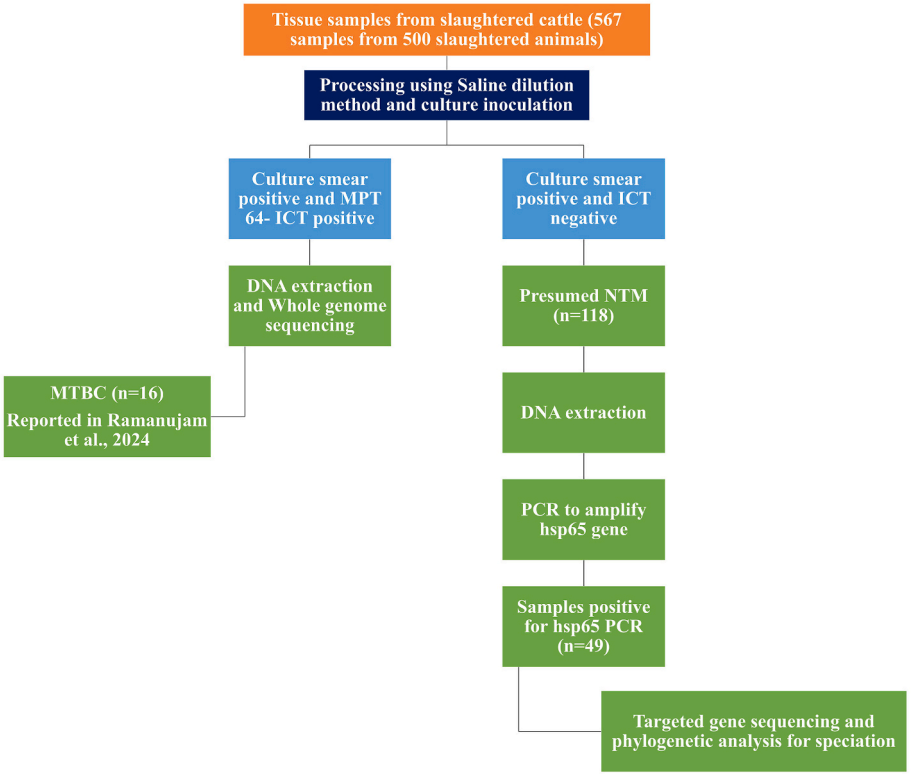

Fig. 1. Schematic overview of the study workflow outlining the sequential steps employed for the isolation, presumptive identification, molecular confirmation, and speciation of non-tuberculous mycobacteria (NTM) from bovine tissue samples.

3.2. Targeted gene sequencing and phylogenetic analyses

Targeted gene sequencing was performed for all 49 samples that showed *hsp65* amplification. Sequencing was successful for all amplicons with an average read length of 426 bp (Supplementary Table S2). Species identification was conducted using NCBI BLAST, with assignments based on high percentage identity and query cover (>95 %) and a low E-value [8]. To further validate species identification, a phylogenetic tree was constructed using RAxML with 1000 bootstrap replicates following multiple sequence alignment with ClustalW. All 49 study sequences were included in the phylogenetic analysis to enhance resolution and confirm species delineation through clustering with corresponding reference strains. The tree was rooted at the midpoint and included representative reference sequences retrieved from NCBI GenBank to ensure accurate clustering and comparative analysis [13] (Supplementary Table S3).

The 49 NTM isolates were identified as representing 18 different species (Supplementary Table S4). *Mycobacterium intracellulare* was the most frequently isolated species (9 isolates), followed by *Mycobacterium* sp. *strain 79_MI18_10584* (6 isolates) and *Mycobacterium elephantis* (6 isolates). *Mycobacterium fortuitum* (5 isolates) and *Mycobacterium kansasii* (4 isolates) were also among the predominant species. Several species, including *Mycobacterium nonchromogenicus*, *Mycobacterium malmesburyense*, *Mycobacterium scrofulaceum MS3*, *Mycobacter kumamotonensis*, and *Mycobacterium asiaticum*, were detected in two samples. Additionally, *Mycobacterium avium*, *Mycobacterium* sp. *strain HDG*, *Mycobacterium phlei*, *Mycobacterium alsense*, *and Mycobacterium* sp. *FI-06192*, *Mycobacterium simiae*, *Mycobacterium saskatchewanense*, and *Mycobacterium gordonae* were each detected in a single sample (Table 1). The identified strains clustered well with their respective reference strains in the phylogenetic tree (Fig. 2).

Table 1
Non-tuberculous mycobacteria species identified in slaughtered cattle in Chennai along with their sample IDs and counts.

S. no	Species	Count	Samples
1	*Mycobacterium intracellulare*	9	KL010, KL045, KL126, KL215, KL216, KL218, KL378, KL399, KL476
2	*Mycobacterium* sp. *strain 79_MI18_10584*	6	KL124, KL162, KL194, KL380, KL393, KL414
3	*Mycobacterium elephantis*	6	KL003, KL005, KL006, KL028, KL029, KL065
4	*Mycobacterium fortuitum*	5	KL035, KL102, KL351, KL464, KL481
5	*Mycobacterium kansasii*	4	KL267, KL376, KL480, KL488
6	*Mycobacterium nonchromogenicus*	3	KL349, KL356, KL377
7	*Mycobacterium malmesburyense*	2	KL020, KL026
8	*Mycobacterium scrofulaceum MS3*	2	KL023, KL123
9	*Mycobacterium kumamotonensis*	2	KL085, KL325
10	*Mycobacterium asiaticum*	2	KL313, KL466
11	*Mycobacterium avium*	1	KL286
12	*Mycobacterium* sp. *strain HDG*	1	KL270
13	*Mycobacterium phlei*	1	KL008
14	*Mycobacterium alsense*	1	KL032
15	Mycobacterium sp. FI-06192	1	KL210
16	*Mycobacterium simiae*	1	KL211
17	*Mycobacterium saskatchewanense*	1	KL475
18	*Mycobacterium gordonae*	1	KL477

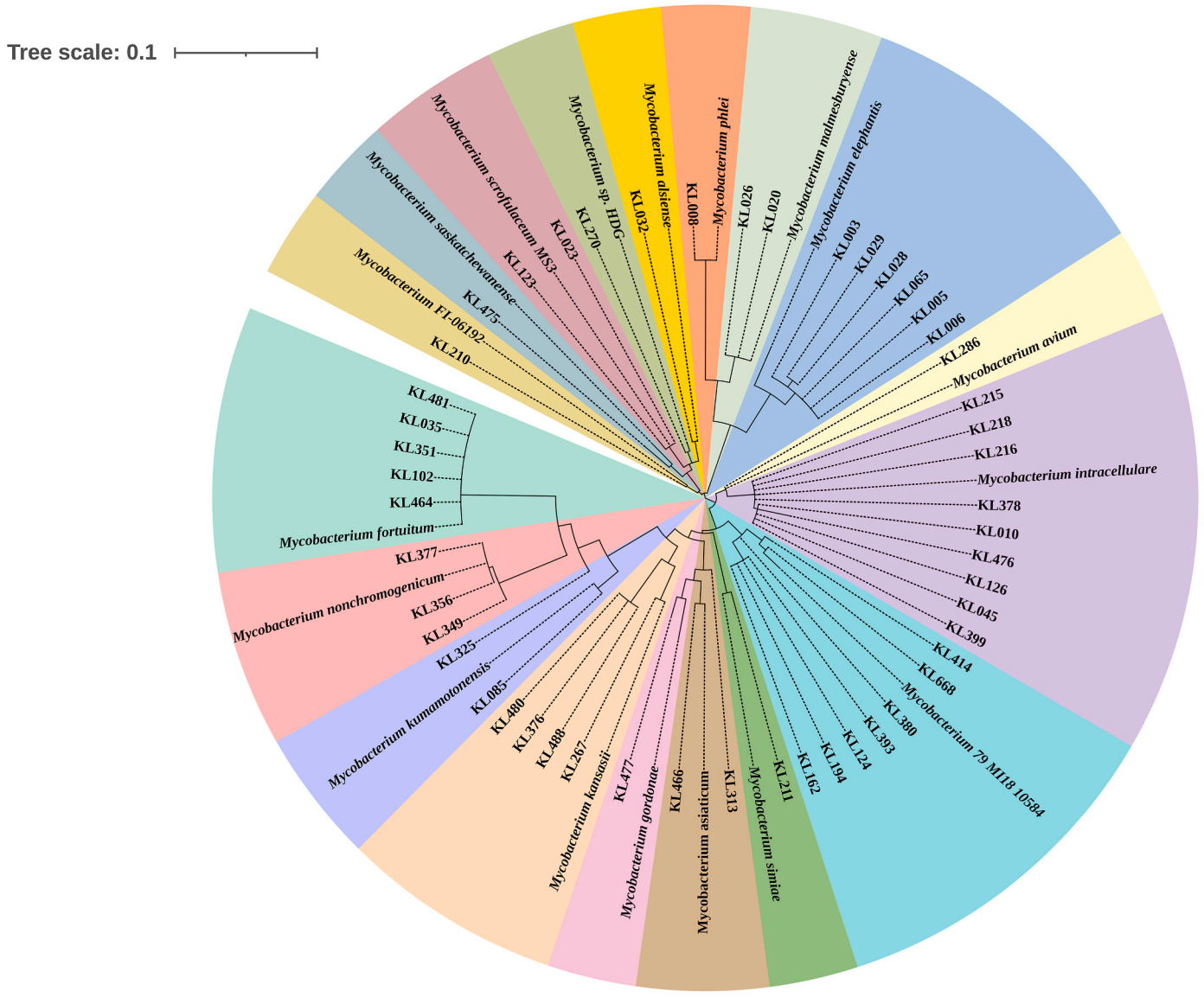

Fig. 2. Maximum likelihood phylogenetic tree based on partial hsp65 gene sequences, depicting the relationship between NTM isolates from this study and corresponding reference strains. The tree was constructed using the RAxML algorithm with 1000 bootstrap replicates and rooted at midpoint.

4. Discussion

This study characterized NTM in slaughtered cattle from Chennai, India, and identified 49 isolates representing 18 species using *hsp65*-targeted gene sequencing. *Mycobacterium intracellulare* was the most frequent species, followed by *M. kansasii*, *M. avium*, and *M. fortuitum*, all of which have pathogenic and zoonotic potentials [14,15]. The presence of *M. kansasii* and *M. avium*, both known causative agents of pulmonary NTM infections in humans, highlights the importance of characterizing NTM species in cattle, given their potential implications for public health and zoonotic risks [16].

In this study, a majority of NTM species were isolated from lymph nodes without TB-like lesions, a trend also observed by Gcebe et al., in South African cattle [1]. Their study identified *M. intracellulare, M. yongonense*, and other TB members, as well as *M. sherrisii, M. porcinum*, and *M. virginiense*, from specimens without visible lesions, suggesting that NTM colonization may occur without overt pathology. Ghielmetti et al., identified NTMs in wild boars without visible lesions, where *M. avium, M. nonchromogenicum, M. scrofulaceum, M. fortuitum*, and *M. phlei* (all of which were also found in our study) were recovered from 75 lymph node samples (n = 101) of wild boars despite the absence of detectable tuberculosis-like pathology [17]. Similarly, in a study of slaughtered pigs in Uganda, the authors reported that 31 % of lymph nodes without visible lesions harbored NTMs, with *M. avium* being the most frequently isolated species. These findings indicate that NTMs may persist asymptomatically in lymphoid tissues across multiple host species [18].

Also, 118 presumptive NTM isolates were identified based on their growth on LJ, LJ-SP, or MGIT media, along with AFB positivity and negative MPT64 ICT results. However, PCR amplification of *hsp65* confirmed that only 49 of these isolates were mycobacteria. This discrepancy highlights the limited specificity of AFB staining, as acid-fastness is not exclusive to mycobacteria and can also be exhibited by other organisms. Acid-fastness is primarily attributed to the high lipid content, particularly mycolic acids, in the cell walls of certain bacteria, enabling them to retain specific stains even after acid-alcohol decolorization. Acid-fast organisms beyond Mycobacterium include *Nocardia* spp. such as *N. brasiliensis, N. cyriacigeorgica, N. farcinica*, and *N. nova* [19]. Additionally, some protozoan parasites, such as *Cryptosporidium parvum*, exhibit acid-fast properties. The presence of these organisms could explain the AFB-positive and *hsp65* PCR-negative results observed in this study. However, characterisation and speciation of these isolates

is beyond the scope of this current study.

Similar findings have been reported previously. A study in Nigeria identified 12 acid-fast bacterial species other than *Mycobacterium* in 97 culture-positive, AFB-positive specimens from patients with tuberculosis-like symptoms, in addition to 81 MTBC species and 4 NTM species. The authors of this study also speculate that these could be due to N*ocardia* species or *Rhodococcus equi*, considering their similar appearance in acid fast staining [20]. Similarly, while isolating NTM from African buffaloes in South Africa, Carke et al., reported 112 culture positive samples, out of which 40 samples did not produce any bands when amplified with primers for *hsp65/rpoB/esat6* primers [21]. Also in the study conducted in India that reports NTMs isolated from cattle, from 97 culture positive samples, only 34 samples were positive for PCR using the primers for *hsp65* gene [8].

The phylogenetic tree constructed in this study using partial *hsp65* gene sequences (Fig. 2) revealed species-dependent sequence variability among the NTM isolates. Isolates identified as *M. fortuitum* formed a compact, well-supported cluster, indicating minimal intraspecies variation and close similarity to reference strains available in NCBI. In contrast, isolates classified as *Mycobacterium* sp. *strain 79_MI18_10584*, exhibited a higher degree of sequence diversity and formed distinct subclusters. These isolates grouped exclusively with *Mycobacterium* sp. *strain 79_MI18_10584* in the phylogenetic tree, with no branching towards other known Mycobacterium species, suggesting that this was the closest matching reference available. The observed sequence heterogeneity may reflect natural intra-species diversity or unresolved taxonomic complexity within this group. Further molecular characterization using additional genetic markers or whole genome sequencing would help clarify the phylogenetic relationships and confirm whether these represent true intraspecific variants or emerging subspecies.

The findings of this study align with those of previous reports on NTM isolation in animals across various regions. *Mycobacterium intracellulare*, the most frequently identified species in our study, has been reported in cattle from Uganda, Ghana, and Mexico. It has also been isolated from African buffalo (*Syncerus caffer*) in South Africa, wild boar (*Sus scrofa*) in Spain, and tapirs (*Tapirus terrestris*) in Argentina [7, 21–24] The strain *Mycobacterium* sp. *strain 79_MI18_10584* has been previously reported in cattle that were positive for the tuberculin skin test, but negative for MTBC from officially bTB free herds in Spain [9].

Similarly, *M. fortuitum* has frequently been reported in both domestic and wild animals. It has been identified in cattle from Ghana and Mexico as well as in roe deer (*Capreolus capreolus*) in Europe. Its detection has also been reported in a collared peccary (*Pecari tajacu*) in Argentina. *Mycobacterium kansasii*, a well-documented human pathogen, has been recovered from cattle in Mexico and roe deer in Europe [7,23–25].

Other NTMs identified in our study have been previously reported across different hosts, emphasizing their broad adaptability. *Mycobacterium scrofulaceum*, detected in cattle in Mexico, has also been recovered from wild boar in Spain. *Mycobacterium kumamotonense*, found in cattle in China, has been detected in badgers (*Meles meles*) in European collared peccary in Argentina. Similarly, *M. elephantis*, a species of emerging clinical interest, has been reported in wild boars, whereas *M. asiaticum* has been found in African buffalo in South Africa. The detection of *M. nonchromogenicum* in cattle, wild boars, and sheep highlights its ability to thrive in both livestock and free-ranging wildlife. Meanwhile, *M. gordonae*, often considered an environmental mycobacterium, has been isolated from African buffalo in South Africa and giant anteaters (*Myrmecophaga tridactyla*) in Argentina [5,7,21–23,25].

Reports of NTM isolation in cattle in India remain extremely scarce, with most of the available data coming from human infections. However, several species identified in our study, including *M. intracellulare, M. fortuitum, M. kansasii, M. gordonae, M. scrofulaceum,* and *M. simiae*, have been reported in Indian patients. *Mycobacterium intracellulare* has also been documented in New Delhi and Pondicherry. Similarly, *M. fortuitum* is one of the most commonly reported NTMs in New Delhi, Rajasthan, Wardha, and Bangalore. *Mycobacterium kansasii* has been detected in New Delhi, Bangalore, and Pondicherry, whereas *M. gordonae* and *M. scrofulaceum* have been reported in New Delhi, Wardha, and Bangalore. Although *M. simiae* was detected in lower numbers in our study, it has also been reported in Bangalore and Rajasthan. The repeated detection of these species in both cattle and humans highlights their potential zoonotic significance, although direct transmission remains unconfirmed [26–31]. Although rare *M. elephantis* has been isolated from humans in Canada, Iran, and Belgium, with cases involving lymphocutaneous spread and respiratory specimens [32,33].

Despite these overlaps, several NTMs frequently reported in Indian patients were absent in our cattle isolates such as *M. abscessus* and *M. chelonae*, *M. peregrinum, M. mucogenicum, M. moriokanese, M. goodii,* and *M. wolinskyi* [16,26,27,30]. While these differences may be influenced by host-specific factors, environmental distribution, or diagnostic methodologies, their absence in this study underscores the potential ecological distinctions between human- and bovine-associated NTMs in India.

While *hsp65* gene sequencing served as the primary method for species-level identification in this study, the use of a single genetic marker has inherent limitations, particularly in differentiating closely related mycobacterial taxa. Although *hsp65* is widely recognized for its discriminatory power in NTM speciation, incorporation of additional targets such as *rpoB* or *16S rRNA* genes could further enhance taxonomic resolution, especially in cases where species boundaries remain ambiguous.

Of the 118 presumptive NTM isolates selected based on culture growth and acid-fast positivity, only 49 yielded successful amplification of the *hsp65* gene and were confirmed to belong to the genus *Mycobacterium*. The remaining 69 cultures could not be confirmed as mycobacteria and may represent acid-fast organisms from other genera, degraded nucleic acids, or limitations in primer specificity. Although comprehensive molecular analysis of these isolates was beyond the scope of the present study, this observation underscores the need for a more integrative approach in future investigations to resolve ambiguities in species identification.

The role of NTMs in complicating the diagnosis of bTB has been increasingly recognized, particularly because of their ability to induce cross-reactive immune responses and form granulomatous lesions that resemble those caused by MTBC. The presence of NTMs in cattle not only mimics bTB pathology but also interferes with widely used diagnostic tests, including tuberculin skin tests (TST) and interferon-gamma release assays (IGRAs). These challenges contribute to false-positive test results, leading to misclassification of animals, unnecessary culling, and economic losses [1].

A study by Gómez-Buendía et al., demonstrated the widespread occurrence of NTMs in skin test-positive cattle from officially tuberculosis-free herds in Spain, in which 32 different NTM species were identified. Among these, MAC, *M. kansasii*, and *M. nonchromogenicum* were frequently detected, also highlighting the potential for environmental exposure to NTMs to prime the immune system and interfere with bTB diagnostics [9]. These findings are consistent with earlier studies showing that cattle exposed to NTMs may develop cross-reactive immune responses even in the absence of *M. bovis* infection, further complicating surveillance efforts (Michel et al., 2011). One of the key mechanisms by which NTMs interfere with bTB diagnosis is their antigenic similarity to *M. bovis*. For example, *M. kansasii*, shares T-cell epitopes with *M. bovis*, particularly the ESAT-6 and CFP-10 antigens, which are integral components of IGRA-based diagnostics [34]. This antigenic overlap leads to false-positive IGRA results, making it difficult to differentiate between true bTB infections and NTM-related immune responses.

5. Conclusion

This study provides important insights into the diversity of NTMs in slaughtered cattle from Chennai, identifying 18 species, including

Mycobacterium intracellulare, M. fortuitum, M. kansasii, and *M. avium*. Notably, several NTMs were detected in lymph node samples without tuberculosis-like lesions, similar to the findings of previous studies, highlighting their presence in bovine tissues. While their clinical significance in cattle remains unclear, many of these species are known to cause disease in humans, raising questions about their broader epidemiological role. These findings emphasize the need for continued surveillance, improved molecular characterization, and better understanding of NTMs in both veterinary and public health contexts.

CRediT authorship contribution statement

Harini Ramanujam: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Manohar Nesakumar:** Methodology, Formal analysis. **Kannan Thiruvengadam:** Formal analysis. **Rajaraman Kannan:** Methodology. **Sivaraman Palanisamy:** Methodology. **Sivakumar Shanmugam:** Methodology, Formal analysis, Data curation. **Kannan Palaniyandi:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability statement

The partial *hsp65* gene sequences of the non-tuberculous mycobacteria (NTM) isolates characterized in this study have been deposited in the NCBI GenBank database under accession numbers PV915643 to PV915691.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2025.102673.

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