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Morphological Diversity of Isolated Mycobacterial Cultures in the Republic of Kazakhstan: Implications for Diagnostics and Treatment

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Abstract: The study of mycobacterial morphology in the Republic of Kazakhstan is relevant due to the increasing burden of tuberculosis and other mycobacterial diseases. The aim of this study was to comprehensively describe the morphological characteristics of different mycobacterial species identified in Kazakhstan, which will help to improve diagnostic methods and develop new approaches to treatment. Comparative and cross-cultural analyses were conducted by synthesizing data from multiple international and local studies to identify species-specific variations in growth rates, colony morphology, pigmentation, and temperature preferences. A systematic literature review and meta-analysis were conducted to synthesize various findings and examine theoretical viewpoints on the influence of environmental variables on mycobacterial morphology. The results demonstrate that slow-growing species, such as *Mycobacterium tuberculosis*, need 3-8 weeks for detectable colony development at 37°C, whereas fast-growing species, such as *Mycobacterium abscessus*, establish colonies within 2-5 days at comparable temperatures. Moreover, environmental adaptation is apparent, as *Mycobacterium marinum* flourishes at 30°C, in contrast to infections acclimated to humans. These results possess considerable diagnostic and therapeutic implications. Identifying temperature-dependent growth patterns might enhance the adjustment of culture conditions for a precise diagnosis, thereby reducing delays in the detection of slow-growing infections. Moreover, understanding colony shape and color changes improves species distinction, potentially minimizing misdiagnosis. The study offers a systematic approach that might enhance treatment tactics by taking into account species-specific growth traits when choosing antimicrobial medicines, especially in the fight against drug-resistant mycobacteria. The practical significance of the study is to provide an important database for the development of new antimicrobial agents targeting specific morphological and physiological characteristics of mycobacteria. This is particularly important in light of the growing problem of drug resistance among mycobacteria, which requires new approaches to therapy.

Keywords: Tuberculosis, Species Specificity, Mycolic Acid, Pathogenicity, Pigmentation

Introduction

The examination of mycobacterial morphology in Kazakhstan is essential due to the increasing prevalence of tuberculosis and nontuberculous mycobacteria, especially among immunocompromised populations.

The country's distinctive environment probably contains unique mycobacterial species, which is essential for local epidemiology and management measures. Comprehending these illnesses within veterinary contexts is crucial due to concerns regarding treatment resistance and zoonotic threats. The variety of mycobacterial species complicates diagnosis and treatment, underscoring the necessity for studying the morphological and genetic traits of local strains to formulate tailored vaccines and antimicrobials. A thorough examination of the current literature is essential to adequately address these concerns.

Globally, tuberculosis continues to pose a considerable public health issue, with around 10 million new cases documented each year (World Health Organization, 2022). In addition to tuberculosis caused by *M. tuberculosis*, infections from nontuberculous mycobacteria are on the rise, resulting in severe infections that frequently exhibit resistance to conventional therapies. Kazakhstan has significant public health challenges due to the elevated incidence of multidrug-resistant tuberculosis and an increase in nontuberculous mycobacterial infections (Kalizhanova et al., 2024). The country's varied climate, animal agriculture, and historical contamination contribute to the maintenance of several local mycobacterial strains, necessitating the examination of their morphology for enhanced diagnostic techniques and management strategies.

Kazakhstan encounters distinct obstacles in the diagnosis of mycobacterial illnesses. Conventional diagnostic techniques frequently do not differentiate between tuberculosis and nontuberculous species, resulting in misdiagnoses. Restricted availability of sophisticated molecular diagnostics in rural regions impedes early detection and control initiatives (Adekenov et al., 1991b). Moreover, zoonotic strains such as *M. bovis* provide concerns through cattle reservoirs, heightening human transmission through unpasteurized dairy products. These characteristics underscore the necessity for customized surveillance and management methods for mycobacterial infections in Kazakhstan.

Following the information of Kirimbayeva et al. (2023), *Mycobacterium* (*M.*) is the only genus in the family *Mycobacteriaceae*. In recent years, the number of species in the genus *Mycobacterium* has experienced changes, and nowadays, more than 170 diverse species are known. Three to ten new *Mycobacterium* species are added to the world of science each year. In the time interval between 2003 and 2012, over 60 different species of *Mycobacterium* have been identified. In animals, the genus *Mycobacterium* is classified into three key categories:

1. The *M. tuberculosis* complex, which includes the species responsible for the development of tuberculosis in mammals (*M. tuberculosis*, *M. africanum*, *M. canettii*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. mungi*, *M. orygis* and in 2013 the species *M. suricattae* was added)
2. The *M. avium* complex is the group that causes tuberculosis in birds
3. Nontuberculous mycobacteria comprise over 160 species that, in contrast to the previously mentioned groups, are not considered obligate pathogens

Turgenbayev et al. (2021) have shown that the majority of mycobacteria exist in the environment as saprophytes, symbionts, and commensals. Nonetheless, certain organisms may function as opportunistic infections for both animals and people, transmitted by direct contact or aerosols. Pathogenic organisms often exhibit preferred hosts but are capable of infecting other hosts, resulting in chronic illnesses in domestic animals. Daniyarov et al. (2021) indicated a reduction in poultry illnesses in industrialized nations attributable to enhanced production methods. Simultaneously, lesser-researched nontuberculous mycobacteria are being increasingly detected in both domestic and wild animals, but their health implications remain ambiguous, particularly in the absence of overt clinical manifestations.

Research by Akhmetova et al. (2021) demonstrates that the *M. tuberculosis* complex had a completely clonal origin, with no indication of genetic recombination across strains. This clonal structure is rare among living creatures and is often regarded as a secondary characteristic. Unlike other species, which possess genomes derived from several ancestral origins, the *M. tuberculosis* complex has minimal homoplasy and stable molecular markers, hence affirming its clonality. In these types of systems, homoplasy can only happen when genes mix or when different mutations happen at the same locus. On the other hand, Tarabayeva and Abilbayeva (2022) say that the *M. tuberculosis* complex has very little genetic diversity, which means that the chance of homoplasy due to repeated mutations is very low. This is only the case when the locus is subject to directional selection (for example, genes related to resistance or genes related to antibiotic resistance), when the frequency of mutations in the locus is higher than the total frequency of nucleotide mutations (transposition of TR2323), or when the mutational process creates similar alleles in different strains (variable number tandem repeat loci).

This study offers significant insights into the underrepresented mycobacterial flora of Kazakhstan, although the region has a substantial incidence of tuberculosis. It enhances current understanding by providing a localized examination of strain distribution, host interactions, and morphological adaptations. The prevalence of Beijing genotype strains exhibiting substantial multidrug-resistant tuberculosis underscores regional strain development and resistance trends. The study highlights environmental and ecological variables influencing local mycobacterial diversity, answering a significant gap in worldwide research and supporting focused public health measures for the region.

Therefore, the aim of this article is to comprehensively analyze and describe the morphological characteristics of different *Mycobacterium* species isolated in the territory of the Republic of Kazakhstan. This study seeks to systematize existing data obtained from laboratory analysis of mycobacterial cultures in order to identify specific morphological features that may contribute to the improvement of methods of diagnosis and treatment of diseases caused by these pathogens.

Materials and Methods

A comparative study was performed to synthesize morphological data from the existing research across diverse geographical locations. This method enabled a structured assessment of species-specific morphological characteristics, encompassing growth rates, colony formations, and pigmentation patterns. This included not only examining the results of studies conducted in Kazakhstan but also analyzing data from international studies. A cross-cultural investigation was conducted to evaluate the variability in mycobacterial morphology under different environmental settings. The research integrated published results from Kazakhstan and global studies to ascertain any regional changes. It was established how environmental factors influence the morphological and physiological features of microorganisms, revealing important aspects of their survival and spread.

The investigation examined many environmental factors that affect mycobacterial morphology. This encompassed temperature ranges particular to many species, including *M. marinum*, which flourishes at roughly 30°C, and *M. tuberculosis*, which favors 37°C. Geographical variations were analyzed to assess their influence on species distribution in conjunction with zoonotic variables that promote the transfer of mycobacteria from animals to people. This study employs a systematic literature review to analyze existing research on mycobacterial morphology, which is known globally and in Kazakhstan in particular. No primary data collection or laboratory experiments, including polymerase chain reaction methods, were conducted. Instead, data were meticulously extracted from peer-reviewed studies that documented mycobacterial strains isolated in Kazakhstan and their morphological characteristics. Inclusion criteria for the review encompassed studies published in peer-reviewed journals from 2000 to 2023 that focused on *Mycobacterium* morphology, pigmentation, and environmental adaptations, featuring clearly defined methodologies and morphological assessments, detailed descriptions of growth patterns, colony morphology, and pigmentation characteristics, and the use of validated microbiological or molecular techniques for species identification. Studies that didn't give clear methodological descriptions, studies that only looked at clinical symptoms or treatment effectiveness without morphological analysis, reports that only looked at one case study or anecdotal findings, and studies that didn't have data sources that could be verified were excluded.

In addition to the aforementioned methods, meta-analysis was applied to evaluate and synthesize data from different studies. Meta-analysis facilitated the pooling of results from multiple studies, leading to more generalized and reliable conclusions regarding the morphological and physiological features of mycobacteria. The study employed publications by Tarlykov et al. (2020) to comprehend contemporary techniques for growing mycobacteria and their morphological analyses, establishing a basis for current categorization methodologies. Pai et al. (2022) elucidated the cell wall architecture, cellular morphology, and colony development attributes of mycobacteria, therefore augmenting the understanding of their morphological and physiological traits. Daniyarov et al. (2023) provided insights into mycobacterial survival processes across various environmental circumstances, enhancing comprehension of their adaptability. Additional information was obtained from electronic resources, including the World Health Organization (2022), the Ministry of Agriculture of Kazakhstan (2023), and its veterinary control committee's portal, which provided up-to-date data on mycobacterial taxonomy, genetics, and recent microbiological findings, facilitating the analysis of the situation in Kazakhstan.

Statistical analyses employing chi-square tests were conducted to determine the disparities in genotype prevalence and medication resistance patterns between Kazakhstani and global mycobacterial strains. The genotypic distribution of et al. (2023) and Tarlykov et al. (2020) were employed for the study in Kazakhstan, whilst worldwide prevalence statistics were sourced from publications by the World Health Organization (2022); Dedrick et al. (2023). The Beijing genotype was much more common in Kazakhstani people than in people from other countries, as shown by the chi-square test result of $\chi^2 = 45.67$ and $p<0.0001$. In contrast, the LAM genotype was seen as underrepresented in Kazakhstan ($\chi^2 = 22.91$, $p<0.0001$). The correlation between morphological characteristics and treatment resistance was investigated by analyzing the incidence of multidrug-resistant tuberculosis in both Kazakhstani and global strains. The research employed data from Daniyarov et al. (2023) and World Health Organization reports to analyze multidrug-resistant tuberculosis frequencies in Beijing genotype strains. The chi-square test revealed a substantially elevated frequency of multidrug-resistant tuberculosis in Kazakhstani Beijing strains ($\chi^2 = 18.23$, $p = 0.0003$). A statistical assessment of total multidrug-resistant tuberculosis prevalence across all strains indicated that Kazakhstan exhibited a considerably elevated rate compared to global trends ($\chi^2 = 15.84$, $p = 0.0007$). The statistical studies validated notable geographical variations in mycobacterial strain distribution and patterns of treatment resistance. The research utilized theme and narrative synthesis methods to amalgamate results from many sources to discern significant patterns in mycobacterial morphology. A comparative framework analysis was employed to cross-reference data from worldwide and regional research, emphasizing similarities and differences in morphological traits. The method used a mix of statistical comparisons and qualitative synthesis to allow for a thorough and well-organized study of the different types of mycobacteria. *M. tuberculosis* was assessed by examining the prevalence of the Beijing and Latin American-Mediterranean (LAM) genotypes in Kazakhstan and globally. Statistics from Auganova

This study is theoretical, so no direct sample collection occurred. Nonetheless, the reviewed research examined mycobacterial strains from various environmental sources, such as soil, water, and clinical specimens. The environmental factors affecting morphological adaptation, including temperature, humidity, and host interactions, were evaluated based on prior studies.

Results and Discussion

Mycobacterial Representatives Isolated on the Territory of the Republic of Kazakhstan and their Impact on the Main Hosts

Species belonging to the *M. tuberculosis* complex continue to be the leading cause of tuberculosis worldwide, particularly in low- and middle-income countries (Panivska & Shevchuk, 2024). Despite significant advances in the diagnosis and therapy of the disease, *M. tuberculosis* remains the primary causative agent of tuberculosis in humans and may rarely affect animals. The pathogen is globally distributed, and the incidence in animals is declining along with the decline in human tuberculosis cases (Olifer, 2024). Animals susceptible to *M. tuberculosis* include donkeys, horses, cattle, carnivores, and pigs. *M. bovis*, on the other hand, is primarily a threat to cattle but can also affect goats, camels, horses, pigs, dogs, and cats. Although *M. bovis* causes only a few cases of tuberculosis in humans, it has a significant economic impact on domestic and wild animals in many countries, especially in those where there is little data on the spread of infection in the population (Tarabaeva & Abilbaeva, 2024).

M. microti was originally identified as the causative agent of tuberculosis in voles. This species has some differences from M. tuberculosis and is sometimes considered a subspecies of the latter. The disease among wild rodents in the Republic of Kazakhstan was first observed in 1931 during a study of cyclical fluctuations in vole populations. Voles, wood mice, and other mouse species show high susceptibility to *M. microti* infection, while small mammals such as guinea pigs, rabbits, mice, and rats show resistance to infection, even when high doses are administered (Belov et al., 2005). Sporadic cases have been reported in large mammals (cattle, llamas, cats) and humans (Ministry of Agriculture of the Republic of Kazakhstan, 2023). M. caprae, isolated in 2003 in Almaty from the lymph nodes and lungs of goats, was recognized as a new species within the M. tuberculosis complex in the following years, 2004 and 2005. This pathogen has become known as a significant causative agent of tuberculosis in cattle in Kazakhstan and has also been found in deer and wild boar. Even in 2023, cases have been confirmed in cattle, pigs, and even humans, demonstrating its wide host range (Auganova et al., 2023).

In conformity with the statements of Baid and Sengupta (2022), bacteria have the ability to change their shape, with many external cues contributing to their shape plasticity, although the key molecular mechanisms of this process are still poorly understood. Bacteria are capable of adopting different morphologies guided by multiple factors. Under conditions of limited access to nutrients, for example, bacteria can change their surface area to volume ratio to enhance nutrient uptake and diffusion. Mobility also plays a significant role in determining the shape of bacteria: flagella may be located at one end of the cell to enable locomotion, while proteins forming an actin ‘tail’ may be used to move around the host. Adhesion to surfaces by specialized adhesive proteins and structures, the symmetrical distribution of chromosomes prior to division, which facilitates accurate cell division, and the presence of complex secretory systems also contribute to morphological variation in bacteria. In reviewing the study by Auganova et al. (2023), similar trends were also noticed. Among mycobacteria that do not cause tuberculosis, many are opportunistically pathogenic and can cause a variety of infections in animals, including lymphadenitis, lung lesions, skin, soft tissues, tendons, joints, and bones (Pai et al., 2022). Mycobacteria are also known to cause morbidity and mortality in fish, both marine and freshwater, including fish raised for food and ornamental aquarium fish (Meulenbroek et al., 2020; Shumka & Apostolou, 2018). One of the most common agents of mycobacteriosis in fish is *M. marinum*, which causes severe and intractable disease.

Avian tuberculosis, a threat to the poultry industry and poultry lovers, is mainly caused by *M. avium* (Basybekov et al., 2018; Yerzhan et al., 2024). Sensitivity to the disease varies according to the bird species; poultry, sparrows, pheasants, and partridges are particularly susceptible, while domestic pigeons show greater resistance. Infected birds and contaminated water and soil are key sources of the spread of infection, with mycobacteria able to remain viable in the external environment for several months (Pangaliyev et al., 2024; Shumka et al., 2023). Similar observations of susceptibility to infections caused by *M. avium* have been reported in other animals, such as pigs, which can have a negative impact on the economy (Committee for

Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan, 2023). Wild birds, pigs, and certain mammalian species now represent the main natural reservoirs of avian tuberculosis (Bogoyavlenskiy et al., 2012).

In turn, Nakane et al. (2022) add that infections caused by nontuberculous mycobacteria can be associated with about 200 different species, which are mainly found in the natural environment and among animals. Despite the decline in the incidence of tuberculosis, the trend for infections caused by nontuberculous mycobacteria is exactly the opposite, with the incidence showing a steady and significant increase at the global level since 1965. This indicates the great danger of such diseases and that the situation with nontuberculous mycobacteria requires global intervention from the state control of Kazakhstan. American researchers such as Di Capua et al. (2022) and Pavlik et al. (2022) have developed a unique theory describing the stages of evolution of *M. tuberculosis* based on its genetic variability and the sequential phases of development from the moment of division to the formation of mature cells and their subsequent transformation under the influence of a variety of environmental conditions. This theory provides a holistic understanding of the genetic adaptability of *M. tuberculosis*. Genetic adaptability indicates the capacity of bacteria to develop by mutations, horizontal gene transfer, or regulatory modifications, enabling their survival under selection pressures like antibiotics or host immunological responses (Zhumanov et al., 2015; Ospanov et al., 2024). Mutations in the katG gene of *M. tuberculosis* produces resistance to isoniazid, a principal first-line tuberculosis medication, illustrating genetic adaptation to antibiotic therapy. Various morphological forms of the tuberculosis pathogen, including cocciform, bulbous, granular, virus-like, filterable, L-forms, and others, have been found to represent different stages of the biological cycle of *M. tuberculosis*, the identification of which is often beyond the capabilities of standard microbiological analysis methods. These findings are extremely important in the study of mycobacterial structure and allow the addition of previously open questions.

Basic Morphological Characteristics of Mycobacteria and a Detailed Description of the Structure of M. tuberculosis

The comprehensive analysis and comparison of morphological characteristics of different *Mycobacterium* species isolated in Kazakhstan highlight the efforts of Tarlykov et al. (2020) aimed at a thorough understanding of the diversity within this genus. This approach not only contributes to the development of scientific knowledge in the field of microbiology but also plays a key role in the accurate diagnosis, effective treatment, and reliable management of diseases caused by mycobacteria. Based on data from their work, the following details the unique and common features of each species, highlighting their specific morphological traits, adaptations, and behavior in different environments. These characteristics not only aid in the identification and classification of mycobacteria but also provide valuable information on their ecological niches, transmission routes, and mechanisms of interaction with the host.

The classification of *Mycobacterium* species is essential for comprehending their pathogenic capabilities, host preferences, and environmental adaptations. *Mycobacterium* species differ in their main and secondary hosts, with some displaying extensive host ranges and others showing strict host specificity. Moreover, variations in growth rate, color, and colony architecture provide significant insights into their ecological niches and pathogenic potential. Table 1 shows a detailed comparison of important mycobacterial species, focusing on how they interact with hosts, how they cause illness, and what their physical traits are.

The data given reveal specific patterns in host range, illness presentation, and physical traits among *Mycobacterium* species. Pathogenic species, like *M. tuberculosis* and *M. bovis*, mostly infect people and pets and tend to have slow growth and colonies that aren't colored. This is often linked to the progression of chronic diseases. Conversely, environmental and opportunistic species, such as *M. marinum* and *M. senegalense*, exhibit accelerated growth rates and pigmented colonies, indicating tolerance to varied circumstances and ephemeral infections. The existence of secondary hosts in certain species suggests their capacity for zoonotic transmission, which has significant ramifications for disease management measures. Comprehending these attributes is crucial for enhancing diagnostic methodologies, surveillance initiatives, and focused treatment strategies.

Pigmentation and growth rates are critical diagnostic indicators for distinguishing *Mycobacterium* species in laboratory environments. The categorization of growth rates into slow-growing (≥7 days) and fast-growing (<7 days) species facilitates the preliminary identification of pathogenic mycobacteria. Slow-growing species, such as *M. tuberculosis* and *M. bovis*, are generally implicated in chronic infections and need extended incubation on a culture medium, hence rendering quick molecular diagnostics essential for early diagnosis. In contrast, rapidly proliferating species like *M. senegalense* are frequently linked to environmental infections and opportunistic diseases, facilitating expedited culture-based diagnosis. Pigmentation patterns offer supplementary diagnostic insights (Turmagambetova et al., 2017). Photochromogenic species, such as *M. marinum*, exhibit pigmentation in response to light exposure, but scotochromogenic species, like certain strains of the *M. avium* complex, generate pigment in both illuminated and dark environments. Non-pigmented (achromogenic) mycobacteria, such as *M. tuberculosis* and *M. bovis*, are frequently associated with human and animal tuberculosis, underscoring the significance of pigmentation studies in distinguishing between environmental and harmful species. The integration of these visual characteristics with genetic and biochemical assays markedly improves the precision of species identification and informs treatment choices in clinical and veterinary microbiology.

M. tuberculosis are aerobic bacilli that range from 2 to 4 µm in length and about 0.2-0.5 µm in width. Bacilli may be slightly curved, which gives them a characteristic appearance under microscopy (Adekenov et al., 1991a). A feature of the cell wall is its high lipid content, including mycolic acids, which give the bacterium acid resistance. This structure serves as a barrier not only against phagocytosis but also provides resistance to antibiotics and disinfectants to a large extent. When cultured on specific nutrient media such as Lowenstein-Jensen medium, M. tuberculosis forms characteristic dry, rough, and wrinkled colonies. These colonies usually appear after 3-8 weeks, reflecting the slow growth rate of the bacterium. The colonies may vary in color from pale yellow to dark brown, depending on the culture conditions. M. tuberculosis is highly genetically variable, which favors the development of antibiotic resistance. The development of multidrug-resistant and extensively resistant strains is a serious problem in the treatment of tuberculosis, requiring continuous monitoring and adaptation of therapeutic regimens (Skak et al., 2017; Tabriz et al., 2021). The bacterium is airborne and can persist in the environment for long periods of time (Danilenko et al., 2021). The efficiency of transmission and infection depends on multiple factors, including the concentration of bacteria in the air, the duration and frequency of contact, and the state of the potential host's immune system. M. tuberculosis is able to survive and multiply within macrophages, making it an effective intracellular pathogen. The bacterium induces a complex immune response that can lead to the formation of granulomas, the characteristic structures of macrophages (Auganova et al., 2023; Montayeva et al., 2015).

Table 1: Characteristics of Mycobacteria: Host Range, Disease Associations, and Morphological Traits

Types of Mycobacteria	Main host	Secondary hosts	Disease	Growth Rate	Pigmentation	Optimal growth temperature	Colony morphology
M. tuberculosis	Humans, captive primates	Dogs, cattle, canaries	Tuberculosis (Global)	Slow	Unpigmented	37°C	Rough, dry, wrinkled
M. bovis	Cattle	Deer, badgers, cats, humans, other mammals	Tuberculosis	Slow	Unpigmented	37°C	Rough, similar to M. tuberculosis
M. microti	Voles	Occasionally other mammals	Tuberculosis	Slow	Unpigmented	37°C	Rough, yellow-brown
M. caprae	Goats	Cattle	Tuberculosis	Slow	Unpigmented	37°C	Rough, dry
M. avium complex	Numerous bird species (excluding parrots)	Pigs, cattle	Tuberculosis	Slow	Pigmented/Unpigmented	37°C	Smooth, often pigmented
M. marinum	Fish	Humans, aquatic mammals, amphibians	Tuberculosis	Slow	Pigmented	30-33°C	Yellow pigmented
M. lepraemurium	Rodents (rats, mice)	Cats	Leprosy in rodents, leprosy in cats	Slow	Unpigmented	37°C	Rod-shaped
M. avium subsp. *paratuberculosis*	Cattle, sheep, goats, deer	Other ruminants	Paratuberculosis	Slow	Unpigmented	37°C	Dense, lumpy
Unspecified fast-growing mycobacteria	Cattle	None	Skin tuberculosis, sensitization due to tuberculinization	Fast	Pigmented/Unpigmented	28-37°C	Smooth, pigmented
M. senegalense	Cattle	None	Chronic lymphadenitis	Fast	Pigmented	28-37°C	Yellow pigmented

Source: Compiled by the authors based on Tarlykov et al. (2020) and Committee for Veterinary Control and Supervision of the Ministry of Agriculture of the Republic of Kazakhstan (2023)

Liang and Rubinstein (2023) note that mycolic acids are a characteristic component of all mycobacteria, providing them with the unique property of resistance to acids and alcohols. Other types of bacteria, like Corynebacterium, Gordonia, Nocardia, Rhodococcus, and Tsukamurella, also make these acids. However, mycobacteria are different because they make mycolic acids with very long chains (70 to 100 carbon atoms), which break down at high temperatures into saturated acid methyl esters with chain lengths of C23 to C27. The vast majority of Mycobacterium species are characterized by the synthesis of mycolic acids enriched with additional oxygen-containing groups (methoxy-, keto-, epoxy-, and carboxy groups), which makes the presence of oxygenated forms of mycolates a significant feature for assigning a bacterium to the genus Mycobacterium, although it is not a strictly obligatory condition. At the same time, the specificity of the esters released during pyrolysis serves as a key to distinguishing the genus Mycobacterium from other genera containing mycolic acids. In a study of mycobacteria from Kazakhstan, it can be seen that it is M. tuberculosis, which is not the most frequent pathogen in animals, that has the highest level of mycolic acids, suggesting the importance of mycobacterial morphology in host selection. The categorization of mycobacterial colony shape is affected by several interconnected elements, including growth rate, environmental adaptations, and host association. Fig. 1 illustrates the contribution of these features to the morphological variation seen among different Mycobacterium species.

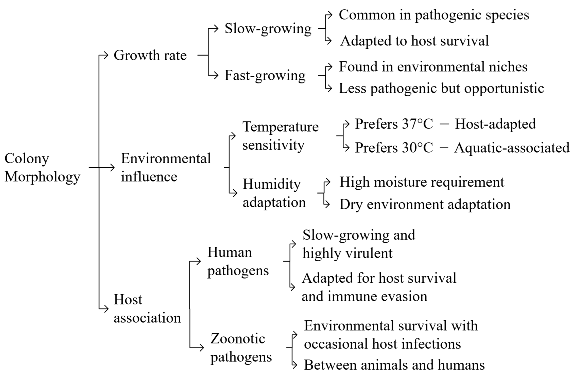

Fig. 1: Mycobacterial colony morphology classification

Figure 1 depicts the structure of mycobacterial colony morphology, categorizing species according to their growth rate (slow or fast-growing), environmental adaptability (temperature and humidity), and host relationship (human or zoonotic infections). Slow-growing species, such as *M. tuberculosis*, are often host-adapted infections that depend on extended survival strategies, whereas fast-growing species, like *M. abscessus*, are frequently environmental opportunists. Moreover, environmental variables, including temperature preferences, affect species distribution, with *M. marinum* flourishing in aquatic habitats at around 30°C and *M. tuberculosis* preferring the human body temperature of 37°C. This categorization facilitates comprehension of species adaptation techniques and their ramifications for diagnostics and disease control.

Variations in growth rates, temperature preferences, and colony morphologies substantially affect diagnostic processes, impacting both the speed and precision of identification. Slow-growing species such as *M. tuberculosis* and *M. leprae* need extended incubation periods (about 2-3 weeks) prior to apparent colony development, hence delaying diagnosis and the commencement of therapy. Conversely, rapidly proliferating species such as *M. abscessus* can be identified within a few days, facilitating prompt action. Temperature preferences are essential in diagnostic culturing. For example, *M. marinum*, which flourishes at around 30°C, may be overlooked if incubated at the typical 37°C conditions suited for *M. tuberculosis*. Furthermore, changes in colony morphology, exemplified by the rough, non-pigmented colonies of *M. tuberculosis* compared to the smooth, pigmented colonies of *M. marinum*, act as first phenotypic indicators but may occasionally result in misdiagnosis without molecular validation. These points make it clear that temperature-controlled incubation, longer culture times for organisms that grow slowly, and extra molecular techniques are all needed to make sure that the right species is identified in environmental and clinical diagnostics.

In the context of *M. tuberculosis*, as indicated in the study by Orujyan et al. (2022) and Hodges et al. (2023), a variety of morphological forms can be observed, which sometimes appear in response to stressors or environmental changes. These include spherical, elliptical, filamentous, spore-like, and L-cell wall-resistant forms. This indicates that *M. tuberculosis* cells are capable of a much wider range of morphological adaptations than is generally assumed. In particular, the bacteria can acquire an elongated L-shape, possibly playing a role in their survival and resistance to some forms of treatment. This capacity for morphological diversity not only highlights the complexity of *M. tuberculosis* biology but also suggests possible mechanisms by which these microorganisms may adapt to different environments and evade the host immune system. Also, morphological plasticity might make it easier for bacteria to hide from antibiotics, which would make getting rid of them harder, and more research is needed to come up with new ways to treat them. Thus, understanding the morphological adaptation of *M. tuberculosis* offers new perspectives for tuberculosis control, highlighting the need to develop innovative approaches to the diagnosis and therapy of this disease. Morphological plasticity refers to the capacity of bacteria to modify their form, size, or structural composition in reaction to environmental factors, facilitating survival and persistence. *M. tuberculosis* may develop a concealed, cell-wall-deficient state under stressors like nutritional scarcity or antibiotic exposure, enabling it to avoid immunological recognition and pharmacological intervention.

Comparative Characteristics of Mycobacteria of the Tuberculosis and Non-Tuberculosis Complex Isolated on the Territory of the Republic of Kazakhstan

M. bovis is closely related to *M. tuberculosis*, having a similar bacilliform morphology. However, under laboratory conditions, *M. bovis* can show slightly more active growth in the presence of pyruvate. The cell walls of these bacteria are also rich in complex lipids, which gives them acid resistance. Colonies of *M. bovis* are generally rough and rigid, although they may exhibit slight variations in morphology depending on culturing conditions.

Since the genome of a clonal organism evolves as a compact genetic network, the history of all clonal organisms traces back to a single ancestor, a single cell that existed at a particular place and time. As Nishiuchi (2021) indicates, bovine tuberculosis in cattle reported worldwide probably originated from a single source that then spread across the planet. There are now three identified *M. bovis* clonal complexes, each with its own deletions and spoligotyping patterns. Two of them are only found in Africa, while the third is found all over the world. The “African 1” and “African 2” complexes are characterized by unique genetic markers reflecting their geographical affiliation and adaptation to local conditions. “European 1”, in turn, shows a wide distribution, indicating its ability to adapt to a variety of environments and hosts on different continents. These clonal complexes illustrate the complexity and diversity of *M. bovis* evolutionary pathways and emphasize the importance of genetic monitoring in understanding the spread and control of bovine tuberculosis internationally.

Based on the data obtained in the above study, it can be established that the isolated *M. bovis* structures belong to the European 1 type, which helps to establish the characteristics of this species and to adjust the control methods. *M. microti* differs from *M. tuberculosis* in that it has smaller bacilli and may exhibit a more fragile bacillary form. *M. microti* colonies are often wetter and may acquire a yellow-brown pigmentation, which sets them apart from the classic dry and rough appearance of *M. tuberculosis* colonies. This emphasizes the variability of colony morphology within the tuberculosis complex. *M. caprae*, under the microscope, exhibits a classic bacilliform shape, similar to *M. tuberculosis* and *M. bovis*. However, *M. caprae* can exhibit unique features of colony morphology when cultured, where colonies sometimes appear more compact and smoother, making them distinguishable from the typically rough colonies of *M. tuberculosis*. *M. avium* complex mycobacteria are characterized by thin bacilliform forms that can vary in size but are usually slightly larger compared to other mycobacteria such as *M. tuberculosis*. The cell wall of these microorganisms contains unique lipids and glycolipids that provide them with acid resistance and the ability to evade the host immune response.

In vitro, *M. avium* complex can exhibit considerable diversity in colony morphology. They can be either smooth and shiny or rough or even wrinkled. Pigmentation due to carotenoid production varies from pale yellow to intense orange, especially when exposed to light, which is a hallmark of these microorganisms. Pigmentation may serve as a defense against ultraviolet radiation and other harmful environmental influences (Rubins et al., 1992). According to the classification system proposed by Hedin et al. (2023), nontuberculous mycobacteria are divided into groups based on their growth rate into slow-growing mycobacteria (categories I, II, III), which take more than 7 days to develop, and fast-growing mycobacteria (category IV), which take less than 7 days to grow. In the slow-growing mycobacteria group, the classification of each subtype is based on their ability to pigment. Subtype I is characterized by the ability to synthesize yellow pigment only when exposed to sunlight, i.e., it is photochromogenic. Subtype II is able to produce pigment regardless of light, i.e. scotochromogenic, while subtype III either produces minimal or no pigment, making it achromogenic. Category IV, or fast-growing mycobacteria, is not associated with pigmentation characteristics. Thus, it can be said that mycobacteria isolated on the territory of the Republic of Kazakhstan, falling under many classifications, show quite a wide species diversity, which must be taken into account when controlling the development of diseases caused by these bacteria.

M. marinum stands out among mycobacteria by its unique ability to adapt to cooler conditions, with an optimal growth temperature of about 30-33°C (Doroshkevich et al., 2017). This species is capable of forming yellow-pigmented colonies, which is an important diagnostic feature. The pigmentation that occurs in response to exposure to light emphasizes the unique adaptive mechanisms of M. marinum, allowing it to exist in both aquatic environments and host tissues (Liu et al., 2024; Irgashev et al., 2021). M. lepraemurium, mainly found in mice, shows morphological similarities to M. tuberculosis, although it is characterized by a slow growth rate and the ability to form dense colonies. This feature of colony morphology, where they appear smoother and more clustered, may play a role in their survival and spread within the host.

M. avium subsp. Paratuberculosis is the causative agent of Johne’s disease in ruminants and is characterized by the formation of dense, poorly separated colonies, reflecting its ability to consolidate in intestinal tissues. Its thin bacilli and complex cell wall composition ensure its acid tolerance and ability for long-term survival in the environment. As noted by Sparks et al. (2023) and Komine et al. (2023), M. paratuberculosis bacilli and those belonging to the M. avium-M. Intracellular complex exhibited elongation and a change in surface texture from rough to smooth when Kirin 40 was added to nutrient media, in contrast to their condition without Kirin 40. The researchers suggest that the surface roughness of the bacilli is related to the presence of glycopeptidolipids on their surface. Although there is a marked correlation between colony morphology and glycopeptidolipid production, the relationship between colony morphology and virulence remains an open question. Putting Kirin 10 or 20 on Löwenstein-Jensen agar changed the texture of the colonies from rough to smooth, which is similar to what Tween 80 does. However, using Kirin 10, Mitar-C200, or Vieran-400 gave the colonies a rougher texture. However, it was found that concentrations of these three surfactants above 0.02% (wt/vol) did not favor bacilli growth. No such studies have been conducted in a review study of mycobacteria in Kazakhstan; these data will be able to add to the information base on the morphological characteristics of these subjects on different nutrient media.

Fast-growing mycobacteria, including M. senegalense, differ from slow-growing species in their ability to form colonies in a much shorter period. These species, which exhibit diversity in colony morphology and bacillus shape, are adapted to a wide range of environmental conditions. According to Zhou et al. (2022), M. senegalense, producing yellow-pigmented colonies, exhibits unique adaptations related to cell wall composition that ensure its survival in a variety of environments. These examples highlight the remarkable diversity of mycobacteria in nature and their unique morphological and physiological adaptations to different environments. From pigmentation and colony shape to temperature preferences and cell wall structure, each mycobacterial species presents a unique set of adaptations that allow them to successfully colonize a wide variety of ecological niches and hosts (Daniyarov et al., 2023).

Studying the internal features of mycobacteria, Dedrick et al. (2023) concluded that they are capable of producing carotenoids that give their colonies a color ranging from yellow to red. Carotenoid synthesis in scotochromogenic mycobacteria is independent of the presence of light (Petrenko et al., 2022; Kizatova et al., 2017). Exposure of these microorganisms to light is not a prerequisite for pigmentation, although prolonged illumination may enhance colony color. In contrast, photochromogenic mycobacteria require the presence of light and oxygen to initiate carotenogenesis. There are also mycobacteria that do not exhibit the ability to pigment under all lighting conditions. Nevertheless, non-photochromogenic strains may show pigmentation varying from visible to bright yellow or from pinkish to coral coloration. Most of these observations are also confirmed in the above study, indicating the mutual objectivity of the data obtained.

The statistical analysis of *M. tuberculosis* strains in Kazakhstan compared to global trends revealed notable disparities in genotypic distribution and treatment resistance frequency. The findings, as shown in Table 2, indicate a greater incidence of the Beijing genotype and multidrug-resistant tuberculosis in Kazakhstan, reflecting regional adaptations and resistance patterns that might influence tuberculosis control initiatives (Auganova et al., 2023; Tarlykov et al., 2020; Dedrick et al., 2023; World Health Organization, 2022).

Table 2: Comparison of M. tuberculosis Genotypic Distribution and Drug Resistance Between Kazakhstan and Global Data

Comparison	Kazakhstan (%)	Global (%)	Chi-square (χ^2)	p-value	Significance
Beijing genotype prevalence	60% (324/540)	35%	45.67	<0.0001	Highly Significant
LAM genotype prevalence	12.9% (70/540)	30%	22.91	<0.0001	Highly Significant
Multidrug-resistant tuberculosis in Beijing genotype	69.1% (224/324)	50%	18.23	0.0003	Significant
Overall multidrug-resistant tuberculosis prevalence	40.7% (132/324)	30%	15.84	0.0007	Significant

The results demonstrate that M. tuberculosis strains in Kazakhstan show a much greater prevalence of the Beijing genotype and a stronger correlation with multidrug resistance relative to worldwide patterns. The majority prevalence of the Beijing genotype (60%) indicates that this lineage has become the most common strain in Kazakhstan, raising concerns due to its recognized correlation with elevated transmission rates and antibiotic resistance. The reduced incidence of the LAM genotype (12.9%) relative to worldwide statistics (30%) reinforces the notion of regional strain selection pressures potentially shaped by previous epidemiological patterns or changes in treatment efficacy. The elevated multiple drug-resistant incidence in Beijing strains (69.1%) underscores the necessity to address drug resistance mechanisms, considering that the Beijing lineage is recognized for its capacity to acquire resistance through genetic alterations and efflux pump mechanisms. The heightened overall multidrug-resistant tuberculosis prevalence (40.7%) in Kazakhstan, in contrast to the global average (30%), highlights the necessity for focused intervention strategies, including improved surveillance, accelerated molecular diagnostics, and customized treatment protocols to address the increasing multidrug-resistant tuberculosis burden in the region.

Previous studies have established those structural alterations in the bacterial cell wall significantly contribute to the development of antimicrobial resistance in mycobacteria, highlighting the link between morphological features and drug resistance. The increased lipid content of the mycobacterial cell envelope, especially the presence of mycolic acids, has been associated with enhanced resistance to many antibiotics, including isoniazid and rifampicin (Sachan et al., 2023). These long-chain fatty acids form a highly impermeable barrier, diminishing medication penetration and limiting antibacterial activity. In drug-resistant strains, heightened cross-linking of arabinogalactan-peptidoglycan complexes further fortifies this defensive mechanism (Dulberger et al., 2020). Modifications in cell wall structure not only improve survival but also lead to phenotypic variations, such as rougher colony morphology and heightened biofilm formation, which have been noted in multidrug-resistant and extensively drug-resistant strains (Torrens & Cava, 2024). Moreover, research demonstrates that morphological plasticity, namely the capacity of mycobacteria to shift between planktonic and biofilm-associated growth phases, profoundly influences drug resistance (Sharma et al., 2023). Biofilm development, often linked to rough colony morphotypes, provides protection by restricting antibiotic diffusion and promoting bacterial persistence (Lyubchyk et al., 2015). Additionally, cell elongation and filamentation, prevalent in stress-induced mycobacterial populations, have been associated with enhanced survival under antibiotic pressure (Zhao et al., 2023). These structural changes may confer a survival advantage, especially in intracellular habitats where antibiotic exposure is intermittent and suboptimal.

A supplementary mechanism connecting morphology and resistance is the existence of lipid inclusions and internal lipid bodies in drug-tolerant mycobacterial populations. Resistant strains of *M. tuberculosis* frequently demonstrate the accumulation of neutral lipids, which is posited to function as an energy store during metabolic dormancy, facilitating survival under antibiotic stress (Goossens et al., 2020). This dormancy-associated phenotype is notably important in latent tuberculosis infections since modified metabolic activity and morphological alterations diminish sensitivity to conventional anti-tubercular treatment.

The analysis conducted by scientists from Kazakhstan not only highlights the global importance of mycobacteriological research but also highlights the critical need for the development and implementation of comprehensive diagnostic approaches. These approaches should take into account the wide morphological, physiological, and genetic diversity of mycobacteria in order to provide effective solutions to public health problems at the global level. In this context, it is important to emphasize that improving the diagnosis and treatment of mycobacterial infections requires international cooperation and knowledge sharing to accelerate progress against diseases caused by these resistant and adaptive microorganisms.

Variations in colony texture and pigmentation might affect the identification procedure, necessitating differential staining methods and adjustments to culture conditions to enhance species recognition. Some mycobacteria can also form biofilms and filamentous growth, which may make them more resistant to antibiotics. This means that specific treatment plans need to be made to target these resistant types. Understanding the differences in growth rates between mycobacterial species makes it easier to find the best treatment length, especially for infections that grow slowly (Dushayeva et al., 2013).

This research enhances the comprehension of mycobacterial variety in Kazakhstan by emphasizing local adaptations, although it mostly confirms known characteristics of mycobacteria rather than presenting new ideas. The focus on regional strain distribution and morphological characteristics establishes a core framework that can guide regional diagnostic techniques and treatment options. However, the limitation of this study is that it does not propose novel molecular markers or diagnostic tools, which restricts its immediate practical applicability.

Conclusion

The integration of regional and international studies showed that comparison of *Mycobacterium* species indeed reveals an extensive range of growth rates, temperature preferences, colony morphology, and differences in pigmentation, indicating considerable ecological diversity and adaptability in this genus. For example, slow-growing species such as *M. tuberculosis* and *M. bovis* require considerable time to form colonies on nutrient media, making rapid diagnosis difficult. In contrast, fast-growing species, such as *M. abscessus*, form colonies in a few days, requiring other approaches to diagnosis and treatment. Temperature preferences of *Mycobacterium* species also vary. The analyzed studies suggest thatadapts to cooler aquatic environments, whereas *M. tuberculosis* thrives at 37°C. This diversity emphasizes the adaptation of mycobacteria to different hosts and habitats.

Colony morphology also provides important information for species identification. Colonies may be smooth, rough, or pigmented, depending on the species. For example, colonies of the *M. avium* complex can change color when exposed to light, which is not the case for many other species. These morphological features of colonies, combined with their growth characteristics, provide the primary data for laboratory identification, but more accurate diagnosis often requires the use of molecular techniques such as deoxyribonucleic acid sequencing. This is particularly relevant in cases where there is close similarity between species, which can make differentiation based solely on phenotypic characters difficult.

The study's results underscore the necessity for temperature-modulated incubation methods, prolonged culture periods for slow-growing mycobacteria, and the use of molecular methodologies to improve diagnostic precision, especially in areas with a variety of environmental mycobacterial species. Future studies should concentrate on refining temperature-specific culture methodologies, creating quick molecular diagnostics for slow-growing species, and exploring the environmental adaptation of zoonotic mycobacteria to enhance detection and disease management measures.

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Author’s Contributions

Assiya Borsynbayeva: Conceptualized the study and designed the methodology.

Kairat Turgenbayev: Conducted the comprehensive literature review and performed the comparative and cross-cultural analyses.

Gulzhauhar Sarsenova: Synthesized the findings and developed the theoretical framework.

Zhuldyz Tlegenova: Supervised the research process and provided critical revisions to the manuscript.

Salika Berdiakhmetkyzy: Coordinated the project, managed manuscript preparation, and facilitated the publication process.

Ethics

This article is entirely original. The corresponding author confirms that all of the other authors have read and approved the manuscript, and no ethical issue is involved.

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