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First Insights into Whole Genome Sequencing of the *Mycobacterium tuberculosis* Complex: Molecular Diversity and Drug Susceptibility Patterns in Senegal

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Abstract

Background: *We conducted a bibliographic analysis of the Mycobacterium tuberculosis complex (MTBC) in sub-Saharan Africa, which included the analysis of 8,139 genomic sequences from 34 of the 49 sub-Saharan African countries. Notably, only one complete sequence from Senegal was identified, which had been generated in the United States. Our primary objective was to utilize whole genome sequencing (WGS) to detect resistance in anti-tuberculous strains of the MTBC in Senegal. This work seeks to advance the understanding of the molecular diversity and distribution of tuberculosis in Senegal.* ***Methods:*** *A prospective study was conducted in Senegal involving patients diagnosed with tuberculosis (TB). Genomic DNA was extracted from positive culture isolates and subjected to whole genome sequencing (WGS) using Illumina MiSeq technology. This approach enabled the characterization of Mycobacterium tuberculosis complex (MTBC) species and lineages, as well as the identification of mutations associated with treatment resistance. The findings confirmed the significant presence of MTBC and diverse lineages in Senegal.* ***Results:*** *Our results showed that all strains isolated from patients were Mycobacterium tuberculosis sensu stricto, with a predominance of Euro-American sub-lineages. Through bioinformatics analysis, we predicted resistance to 20 anti-tuberculosis (first and second line) drugs for each sample. In silico antibiogram analysis identified strains resistant to isoniazid and other multi-drug-resistant strains, with resistance associated mutations detected in the katG and progenies.* ***Conclusion:*** *This study significantly contributed to the molecular surveillance of Mycobacterium tuberculosis complex in Senegal. The species identification of M. tuberculosis isolates is crucial for the rapid diagnosis of tuberculosis in patients, thereby facilitating improved clinical management and treatment outcomes.*

Keywords: *Mycobacterium tuberculosis* complex, Lineage 4, molecular distribution, drug susceptibility test, Senegal

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INTRODUCTION

The *Mycobacterium tuberculosis* complex (MTBC) consists of 13 closely related species, commonly referred to as ecotypes [1]. These ecotypes exhibit variable distribution across different mammalian hosts, without strict specificity to humans or any mammalian species [2].

In Africa, tuberculosis (TB) is one of the top five public health priorities. Pathovars of the *Mycobacterium tuberculosis* complex (*M. tuberculosis*) has been identified in patients in West Africa, as described by studies involving the molecular mapping of strains over the past two decades [3].

Senegal, a West African country with a population of 18,126,390 in 2023 [4], is projected to reach 21,734,274 by 2030 [5]. It shares borders with six other countries: The Gambia or Republic of The Gambia Mali, Mauritania, Guinea-Bissau, Guinea-Conakry and Cabo Verde. Among the pathogens of public health importance in Senegal the *M. tuberculosis* complex (MTBC) is the causative agent of tuberculosis (TB). Tuberculosis remains the leading cause of death attributable from a single infectious agent, surpassing HIV/AIDS [6].

According to the 2022 WHO report, the incidence of tuberculosis in Senegal was 113 cases per 100,000 inhabitants, with an incidence of multidrug-resistant and extensively drug-resistant (MDR/XDR) TB at 1.6 cases per 100,000 inhabitants. This incidence has risen slightly since 2017. Mortality due to this infection was reported as 16 deaths per 100,000 inhabitants among HIV-negative people and 1.3 deaths per 100,000 inhabitants among people living with HIV (PLHIV) [6].

The Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formations (IRESSEF) and the National Program of Tuberculosis (PNT) have combined their efforts to combat tuberculosis in Senegal (Figure 1). Their collaborative efforts focus on the molecular characterization of *Mycobacterium tuberculosis* complex strains isolated during the 2023 drug resistance survey. This collaboration has resulted in the first whole genome sequencing (WGS) analysis of MTBC in Senegal, making a significant milestone in the study of tuberculosis and mycobacterial infections. These advancements are expected to enhance patients' care and treatment outcomes.

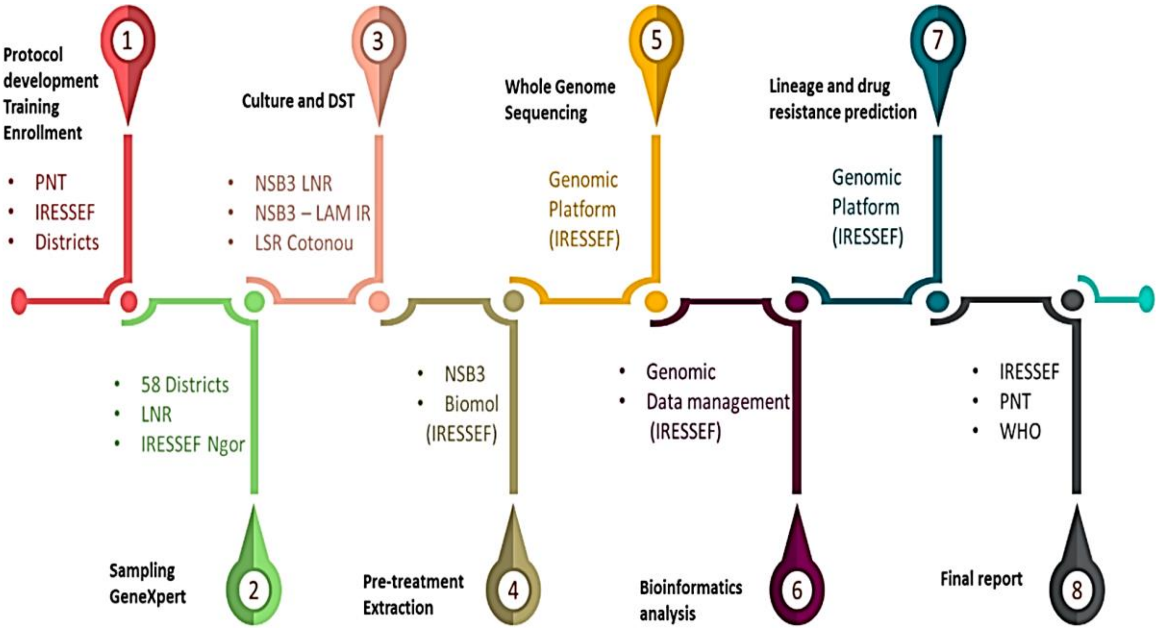

Figure 1. IRESSEF interventions during pharmaco-resistance survey in 2023 in Senegal.

M. bovis is a frequent cause extrapulmonary tuberculosis, which is difficult to diagnose due to a lack of tools for the specific identification of the bacterium by the standard diagnostic tests used in Senegal. Current laboratory procedures used to diagnose tuberculosis in Senegal are unable to differentiate between *M. tuberculosis* and *M. bovis*. This shows that zoonotic tuberculosis is underdiagnosed, which is an obstacle to tracing the zoonotic source of these pathovars. The lack of identification and characterization in humans is since there is no algorithm that takes into account screening for *M. tuberculosis* complex in infected animals, food matrices, such as unpasteurized dairy products or undercooked meat, and environmental sources to understand the sources of transmission of the disease. In addition, atypical mycobacteria, led by *M. avium*, are increasingly found in animals or in foods of animal origin and, therefore, represent a potential risk of infection for humans [7].

Mycobacteria in Senegal are characterized mainly by spoligotyping, which shows the predominance of the species *M. tuberculosis sensu stricto* and *M. africanum*. As far as lineages are concerned, Senegal mainly has Haarlem, T, Beijing, LAM and L5 [8–9]. This technique, however, does not provide sufficient information on the genome and mutations that may lead to resistance to anti-tuberculosis drugs. No whole genome studies have been carried out Senegal to understand the genomic diversity of *M. tuberculosis* strains and the variability of pathovars.

The species and lineage characterization of *M. tuberculosis* isolates provides more information that will benefit TB strain-infected patients and improve their care management. Techniques, such as real time PCR (qPCR) and linear probe tests, which are based on partial amplification of MTBC DNA, are commonly used to detect *M. tuberculosis* complex directly in clinical samples. However, these methods are limited in their ability to identify the different species of MTBC [10].

The objective of this study was to identify the lineages and sublineages of *Mycobacterium tuberculosis sensu stricto* isolates using whole genome sequencing (WGS) and to determine their resistance profiles by in silico antibiogram analysis.

MATERIALS AND METHODS

Sample Collection

A total of 1,147 samples were included in this study, comprising 1,070 bacteriologically confirmed new cases of pulmonary TB and 77 retreatment cases. These samples were collected during the national survey on tuberculosis drug resistance in Senegal under the supervision of the National Tuberculosis Program (PNT) team. The inclusion criteria were adults or children diagnosed as TB positive by GeneXpert and patients naïve to or on anti-tuberculosis treatment. All patients who declined participation, either personally (for adult patients) or through their parents or legal guardians (for pediatric patients), were excluded from this study.

The sample size for this study was determined using statistical calculations to ensure reliable and representative findings. The calculation assumed of a 1% prevalence of rifampicin-resistant tuberculosis (RR-TB) among new bacteriologically confirmed pulmonary tuberculosis (TPBC) cases. To achieve statistically significant results, a 95% confidence interval was applied, indicating that there is a 95% probability that the true prevalence lies within the calculated range. Additionally, a precision level of 0.6% was incorporated, ensuring that the margin of error in the prevalence estimate remains minimal. To account for potential challenges during data collection, such as incomplete or unusable samples, a sample loss rate of 10% was factored into the calculation. Exhaustive sampling was employed to maximize coverage and capture a comprehensive representation of the target population. These considerations ensured that the study's sample size was sufficiently robust to detect meaningful patterns in RR-TB prevalence while maintaining statistical rigor.

GeneXpert Detection of *M. tuberculosis* Complex

Samples that were initially tested using only microscopy were subsequently confirmed with GeneXpert. For each sample, 1 mL of sputum was mixed with 2 mL of GeneXpert reagent in a 2:1

ratio of reagent to sample (Cepheid, Sunnyvale, CA, USA). The mixture was incubated at room temperature for 15 minutes, following the protocol previously described [11]. After incubation, the prepared mixture was transferred into a GeneXpert cartridge and processed using the GeneXpert system, adhering to the manufacturer's instructions (Cepheid). This ensured accurate confirmation of *M. tuberculosis* presence in the samples.

Culture and Inactivation

Positive GeneXpert samples were further confirmed by culturing on solid media supplemented with glycerol and pyruvate. The resulting bacterial colonies were transferred into sterile tubes containing nuclease-free water and sterile glass beads. A 200 µL aliquot of the resuspended colonies was then subjected to heat inactivation at 95°C for 30 minutes to ensure the deactivation of viable bacteria while preserving DNA for subsequent analysis.

DNA Extraction

The inactivated colonies were processed for DNA extraction using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). For each sample, 180 µL of ATL buffer and 20 µL of proteinase K were added to 200 µL of the resuspended bacterial colonies. The mixture was incubated at 56°C for 15 minutes to facilitate cell lysis and protein digestion then washed according to manufacturer's instructions (Qiagen, Hilden, Germany), resulting in elution of purified DNA in 200 µL of AE buffer. This extracted DNA was then ready for downstream analyses.

QC and Genome Sequencing

The extracted DNA samples underwent quality control assessments using the Qubit Flex Fluorometer to measure nucleic acid concentration and ensure suitability for downstream applications. Library preparation was performed using the DNA Prep kit, strictly adhering to the manufacturer's protocol to achieve optimal results. The prepared libraries were subsequently loaded onto an Illumina MiSeq sequencing platform for high-throughput sequencing. The raw sequence data, in the form of FastQ files, were then extracted and utilized for comprehensive bioinformatics analysis, enabling species identification, lineage classification, and resistance profiling.

Phylogenetic and Phylogeographic Analysis

To establish a phylogenetic relationship among the *Mycobacterium* types, present in our study samples, a comprehensive pipeline consisting of two main scripts written in linux/bash language was developed (Figure 2). This pipeline included two primary steps: generating consensus sequences via assembly by mapping and performing multiple sequence alignments to calculate genetic distances bectween the study samples, the reference genome, and genomes from various *Mycobacterium tuberculosis* complex (MTBC) species (*M. tuberculosis sensu stricto*, *M. canettii*, *M. bovis*, *M. microti*, *M. africanum*, and *M. caprae*). The respective genome identifiers were obtained from NCBI: NC_015848.1, AM408590.1, LR882497.1, FR878060.1, and CP016401.1. The assembly step is characterized by the alignment of raw sequence reads on the H37Rv reference genome using the Burrows Wheeler Aligner (BWA) tool (https://github.com/lh3/bwa). Single nucleotide polymorphism (SNP) variants were then called to identify locations with a base difference between H37Rv and the already aligned reads and generate the corresponding genome of the sample studied. The tools used are samtools (https://www.htslib.org/doc/samtools.html) and bcftools (https://samtools.github.io/bcftools/bcftools.html). Then, genomes of other variants of the complex are downloaded from NCBI to identify similarities with the strains obtained. Finally, after a multiple alignment with MAFFT (https://github.com/GSLBiotech/mafft) and a genetic distance calculation with MEGA (https://www.megasoftware.net/) to identify the degree of similarity between the samples and the MTBC. Finally, a phylogenetic tree was constructed using the Iqtree2 tool (https://github.com/iqtree/iqtree2).

Using metadata that includes both the sampling site and the lineage identification of each sample, a map was distribution of lineages circulating in the regions of Senegal where samples were collected.

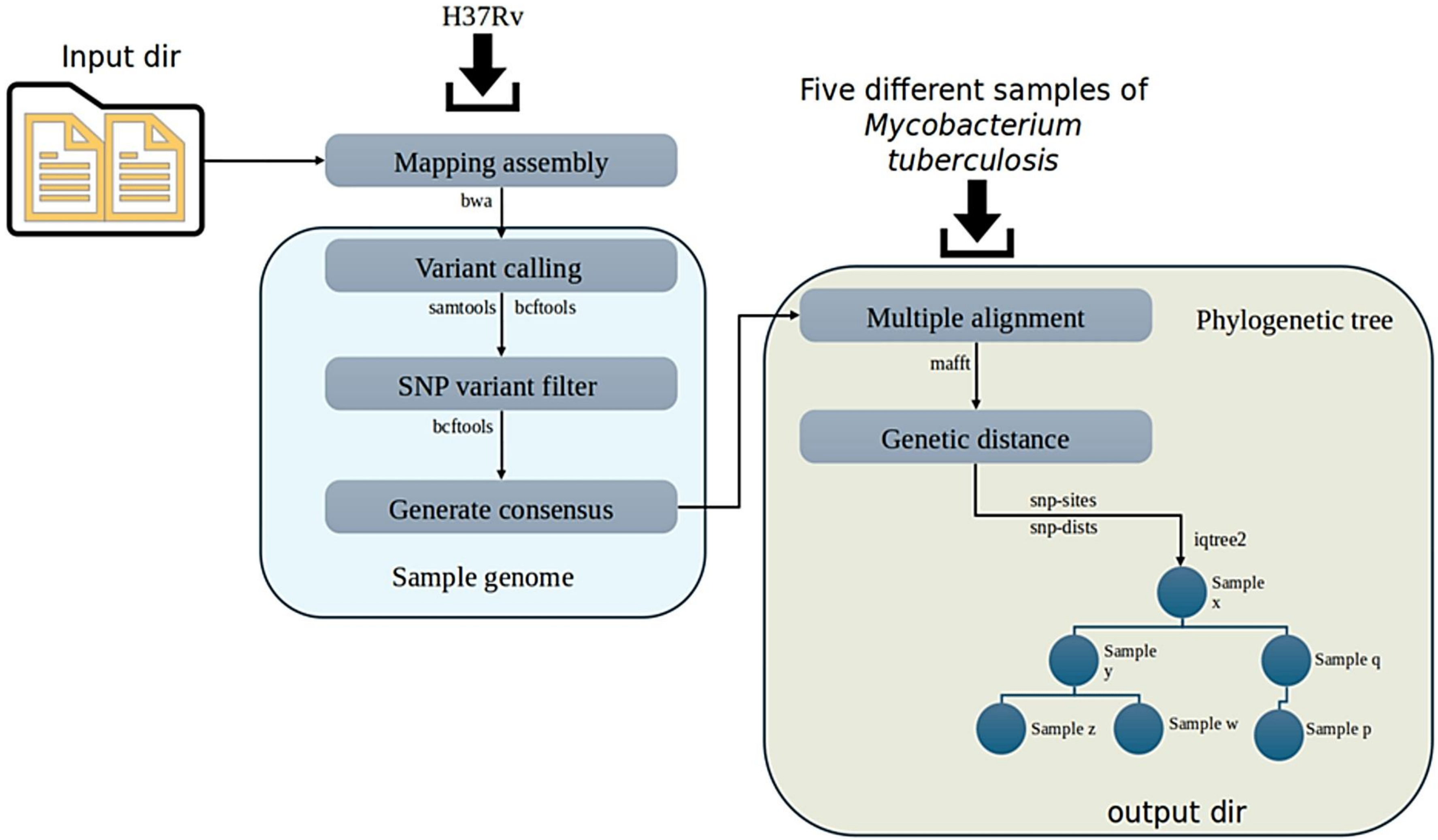

Figure 2. Pipeline for phylogenetic analysis architecture.

RESULTS

Overall data A total of 27 isolates were successfully sequenced in this study. The geographic origins of these isolates are as follows: 5 from Dakar, 6 from Thiès, 4 from Diourbel, 2 from Ziguinchor, 4 from Saint Louis, 3 from Louga, 1 from Fatick, and 1 from Sédhiou. All sequenced samples were identified as *Mycobacterium tuberculosis sensus stricto* isolates. This diverse geographic representation provides a comprehensive overview of the distribution and characteristics of MTBC strains across Senegal, contributing valuable data for understanding the regional epidemiology of tuberculosis in the country.

Species, Lineages and Sublineages Identification

As shown in Figure 2, the 27 sequenced and analyzed isolates were classified as *M. tuberculosis sensu stricto*. These isolates were predominantly grouped within lineage 4 (Euro-American), with several distinct sublineages identified. The distribution of these sublineages was as follows: L4 (2/27; 7.4%), L4.1.2.1 (8/27; 29.6%), L4.1.2.2 (1/27; 3.7%), L4.3.3 (3/27; 11.1%), L4.3.4 (1/27; 3.7%), L4.3.4.2 (1/27; 3.7%), L4.6.2.2 (6/27; 22.2%), and L4.8 (5/27; 18.5%). These sublineages represented most of the strains isolated in our study, providing valuable insight into the molecular diversity of *M. tuberculosis* within the region.

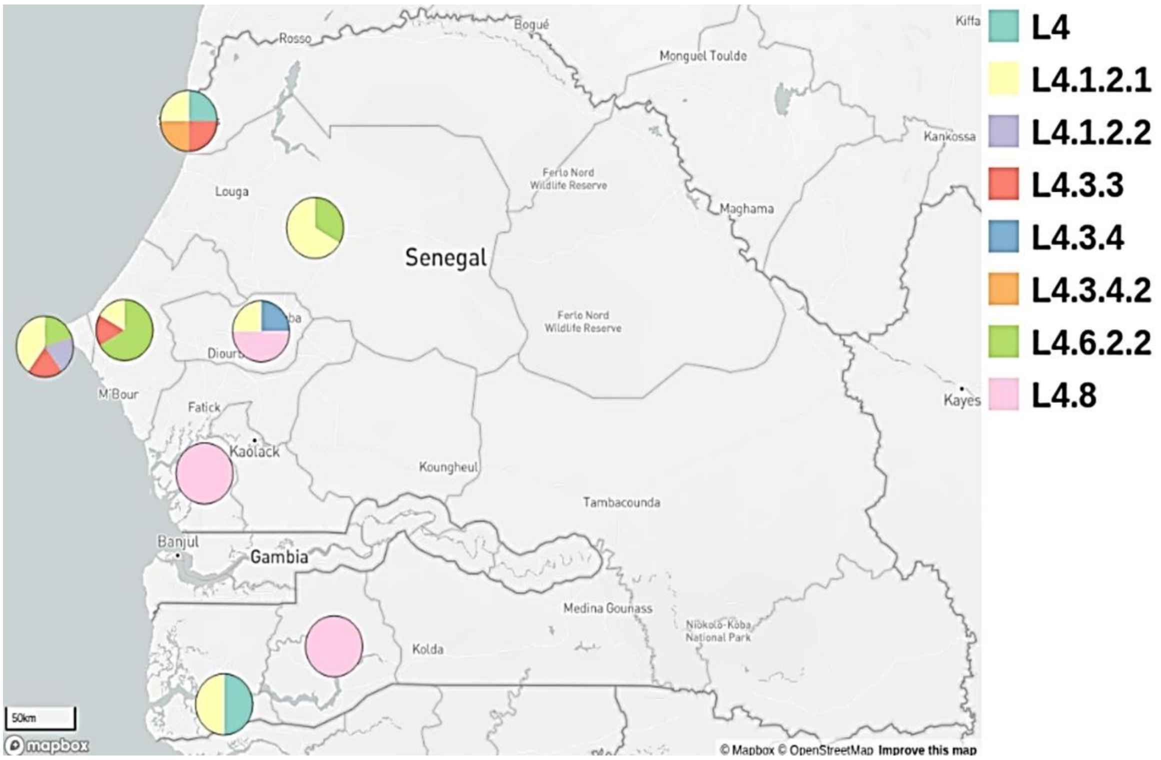

Figure 3. Preliminary distribution of *M. tuberculosis* lineages in samples collected during 2023 pharmacoresistance survey.

Geography of Sublineages

Our preliminary results indicate a high degree of diversity in the geographical distribution of *M. tuberculosis sensu stricto* sub-lineages across the eight regions where samples were collected. The identified lineages and sublineages include L4, found in Saint Louis (1/2) and Ziguinchor (1/2); L4.1.2.1, with isolates from Dakar (2/8), Thiès (1/8), Diourbel (1/8), Louga (2/8), Saint Louis (1/8), and Ziguinchor (1/8); L4.1.2.2 from Dakar (1/1); L4.3.3, represented in Dakar (1/3), Thiès (1/3), and Saint Louis (1/3); L4.3.4 from Diourbel (1/1); L4.3.4.2 from Saint Louis (1/1); L4.6.2.2, found in Dakar (1/6), Thiès (4/6), and Saint Louis (1/6); and L4.8, with isolates from Diourbel (2/4), Fatick (1/4), and Sedhiou (1/4) (Figure 3). Additionally, there was one L4.8 isolate of unknown origin. These findings highlight the complexity and geographical variation of *M. tuberculosis* lineages in Senegal, which could have significant implications for understanding the epidemiology of tuberculosis in the region.

Susceptibility Profile of M. Tuberculosis Complex During TB-Pharmacoresistance Survey in Senegal

According to the World Health Organization (WHO), mono-resistant tuberculosis is defined as resistance to a single first-line anti-tuberculosis drug [12]; multidrug resistant (MDR) tuberculosis is defined as resistance to at least isoniazid and rifampicin; extensive drug resistance tuberculosis (XDR-TB) is defined as additional resistance to any fluoroquinolone and at least one additional group A drug, Bedaquiline and Linezolid.

Pre-MDR resistanc includes any resistance in the form of mono-resistance to either rifampicin or isoniazid [6]; pre-XDR tuberculosis is defined as tuberculosis fulfilling the definition for MDR or rifampicin-resistant tuberculosis also resistant to any fluoroquinolone [13]. For other types of anti-tuberculosis resistance (“other”), this includes any resistance in the form of mono-resistance, poly-resistance, without MDR, pre-MDR, XDR and pre-XDR [6].

In this study, the resistance profile of the 27 *Mycobacterium tuberculosis* complex (MTBC) isolates was carefully analyzed, revealing that most of the samples (23 out of 27, or 85.18%) were fully susceptible to first-line anti-tuberculosis drugs, indicating no resistance to the commonly used drugs, such as isoniazid and rifampicin. Among the remaining samples, 2 (7.4%) were classified as multidrug-resistant (MDR) isolates, meaning they were resistant to both isoniazid and rifampicin, which are the primary drugs used in tuberculosis treatment.

Additionally, 1 (3.7%) sample was identified as a pre-MDR isolate, characterized by resistance to either rifampicin or isoniazid but not both (Figure 4). When examining the overall prevalence of drug resistance within the sequenced samples, 4 of the 27 (14.81%) isolates displayed resistance, with MDR strains accounting for most resistant isolates. Notably, within the phylogenetic tree, the distribution of MDR isolates was particularly prominent, as 2 out of the 4 drug-resistant samples (50%) exhibited multidrug resistance.

This high proportion of MDR strains highlights the critical need for monitoring and addressing drug resistance in tuberculosis management in the region. The data also suggests that while the overall resistance rate is relatively low, the presence of MDR and pre-MDR isolates underscores the importance of continued surveillance to track emerging drug-resistant strains, which may complicate treatment regimens and require more specialized therapeutic approaches.

Geography of Drug Resistance

The susceptibility profiles of the *Mycobacterium tuberculosis* complex (*M. tuberculosis*) isolates were analyzed across eight regions included in this survey. Drug-resistant isolates were detected in 4 out of the 8 regions (50%), specifically in Dakar, Thiès, Louga, and Saint Louis. The proportions of drug-resistant profiles varied across these regions, suggesting potential regional differences in resistance patterns.

In contrast, antibiotic-susceptible isolates were reported in the remaining 4 regions (50%), namely Diourbel, Fatick, Ziguinchor, and Sédhiou, where no resistance to first-line anti-tuberculosis drugs was detected. This distribution of drug-resistant and susceptible isolates highlights the variability in tuberculosis resistance patterns within different geographical areas, emphasizing the need for region-specific surveillance and tailored treatment strategies to effectively address drug resistance in these regions.

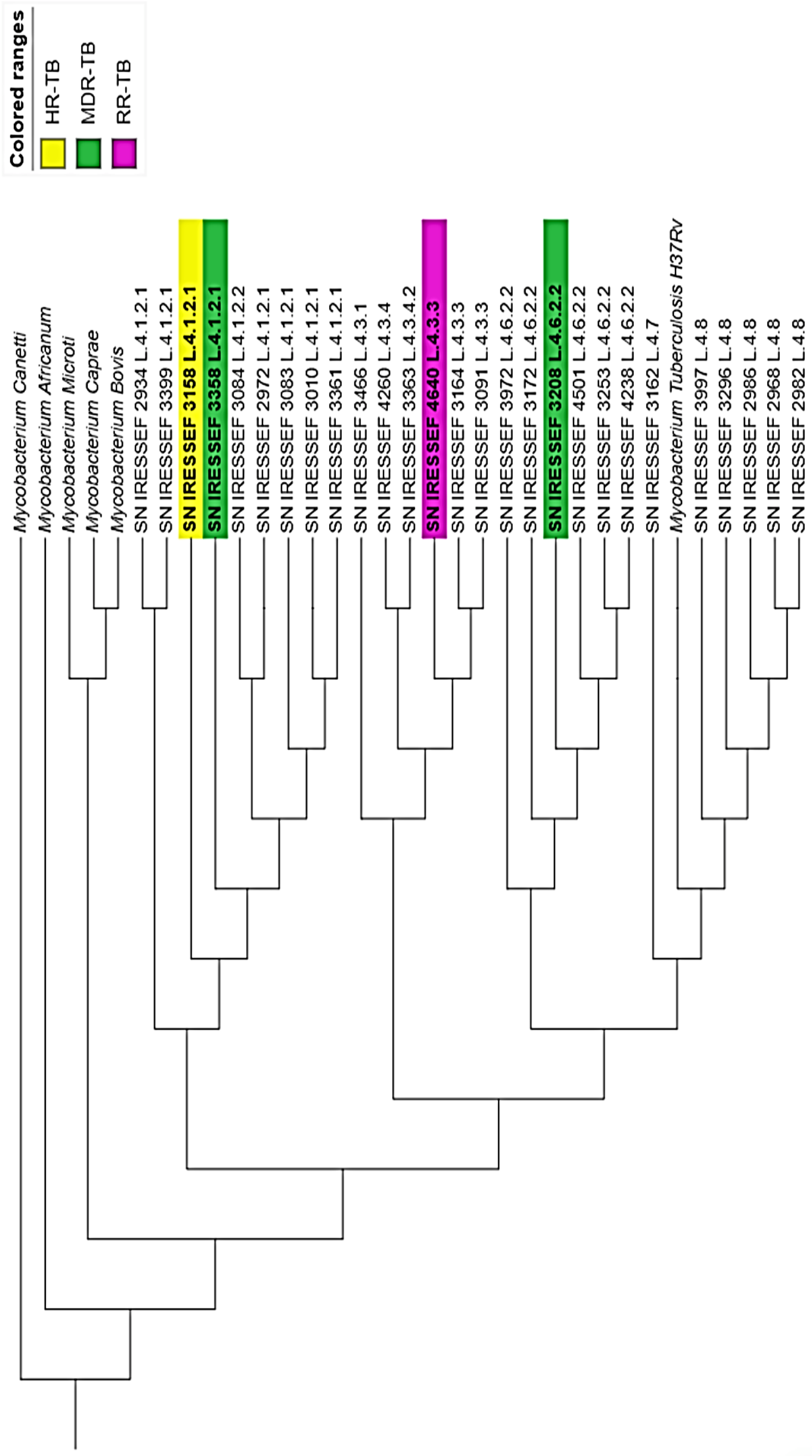

Figure 4. Phylogenetic distribution of strains including the resistance during pharmacoresistance survey in Senegal in 2023. 271.

Association Sublineages Drug Resistance and Mutations

In this study, eight distinct sublineages of *M. tuberculosis* were identified. When examining the association between drug resistance and sublineage, the overall results, as shown in Figure 4, revealed that the highest proportion of drug-resistant isolates was found in the L4.1.2.1 sublineage, which included 1 MDR isolate (3.7%) and 1 HR isolate (3.7%). Additionally, the susceptibility profiles of *M. tuberculosis* complex isolates from sublineages L4.1.2.1, L4.6.2.2, and L4.3.3 were reported, with 4 out of the 27 isolates (14.8%) showing drug resistance. These included 2 MDR isolates (2/4), 1 HR isolate (1/4), and 1 RR isolate (1/1) (Figure 5). These findings indicate a notable presence of drug-resistant strains within specific sublineages, highlighting the importance of continued surveillance to track resistance patterns across different genetic lineages of *M. tuberculosis* and to inform targeted treatment strategies.

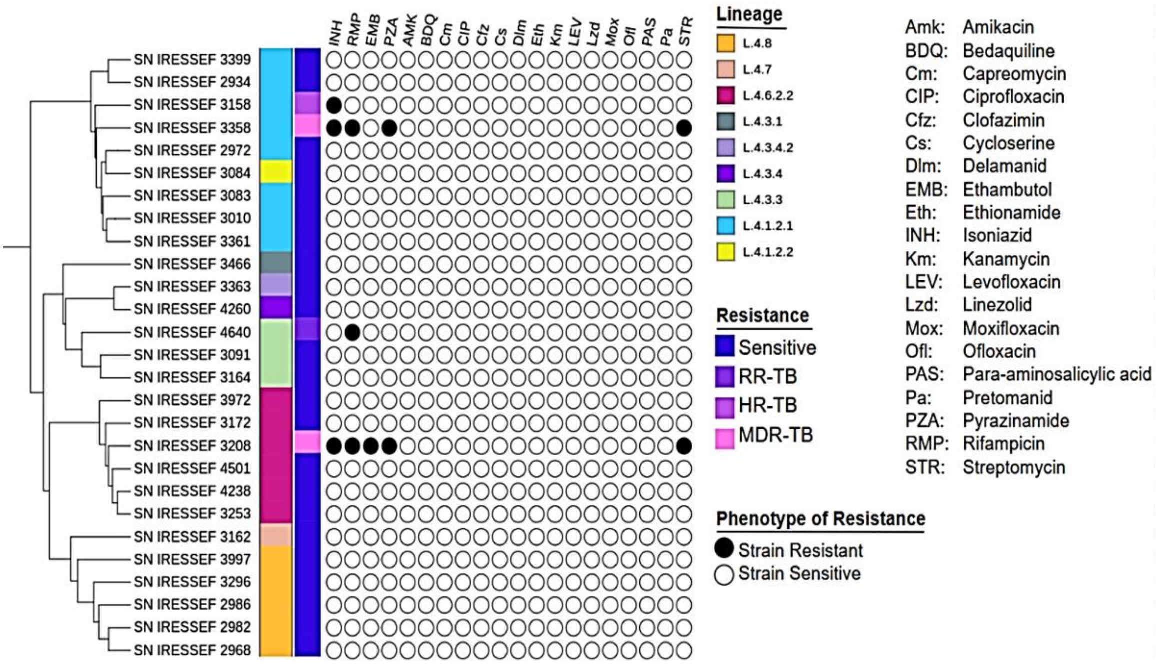

Figure 5. Lineage distribution and in Silico antibiogram for MTBC strains isolated from TB patients.

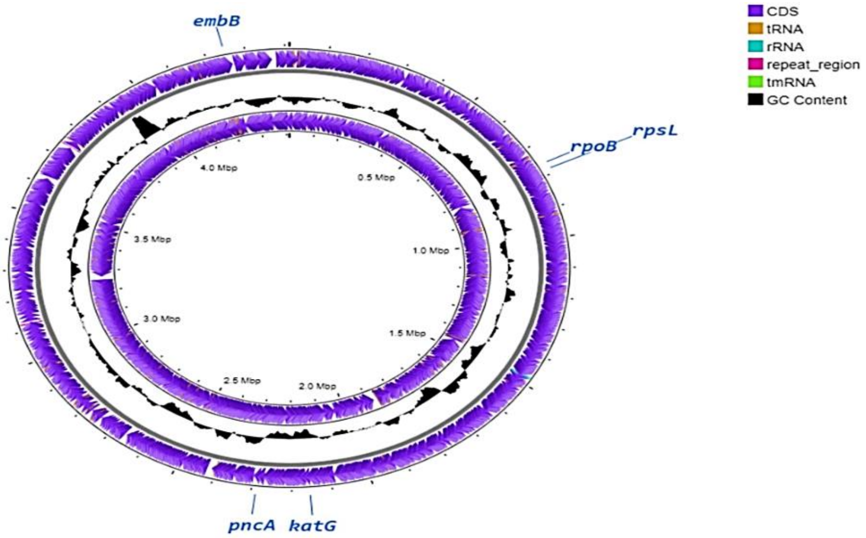

Figure 6. Resistance genes identified in TB new cases during 2023 pharmacoresistance Survey in Senegal.

Resistance Genes Identified and Mutations

Unexpectedly, we have identified mutations in new cases of tuberculosis. The genes identified were mainly those that induced resistance to first-line group A anti-tuberculosis drugs, such as *embB* (Ethambutol), *rpoB* (Rifampicin), *pncA* (Pyrazinamide) and *katG* (Isoniazid). We have also identified genes conferring resistance to group C anti-tuberculosis drugs, such as *rpsL* (Streptomycin) (Figure 6).

DISCUSSION

In this study, we used whole genome sequencing (WGS) technique to explore the diversity of lineages and sub-lineages of the *Mycobacterium tuberculosis* complex (MTBC) in Senegal. For the first time, this work has characterized the geographical distribution and population structures of resistant isolates from a pharmacoresistance survey conducted in 2023. The 27 samples analyzed reflect remarkable diversity, both in terms of genetic diversity and the distribution of drug resistance profiles.

The genomic data obtained from this study reveal a clear predominance of lineage 4 (Euro-American), with several sub-lineages represented among the *M. tuberculosis* isolates. This finding is consistent with previous studies conducted in West Africa, which have shown lineage 4 to be largely dominant in the region, further supporting its widespread presence across diverse geographical areas [14–15]. Among the identified sub-lineages, L4.1.2.1 (29.6%) and L4.6.2.2 (22.2%) were the most frequently observed, indicating their significant role in the regional transmission of tuberculosis. The third most common sub-lineage, L4.8, was found in 18.5% of the samples, contributing to the overall diversity of *M. tuberculosis* strains in Senegal. These results reinforce the need to monitor the evolution and spread of these sub-lineages to better understand their potential impact on tuberculosis transmission and drug resistance patterns in the region.

This genetic diversity reflects the evolutionary and epidemiological pressures influencing the distribution of *M. tuberculosis* lineages [12]. Major sub-lineages, such as L4.1.2.1, may be better adapted to local human populations or exhibit selective advantages related to their transmissibility. Conversely, the presence of less frequent sub-lineages, such as L4.3.4 and L4.3.4.2, highlights the potential for importations or specific epidemiological niches [14].

These results emphasize the critical need for a deeper understanding of the evolutionary dynamics of *M. tuberculosis* lineages both at regional and global levels. To effectively control the spread of tuberculosis, future studies should focus on uncovering the molecular mechanisms that drive the proliferation of dominant sub-lineages. Investigating how these sub-lineages evolve and adapt to selective pressures, particularly in relation to the development of drug resistance, is essential. Understanding the genetic factors that enable these strains to thrive and spread will provide valuable insights for developing targeted interventions and improving tuberculosis control strategies worldwide.

A detailed analysis of geographical distribution revealed remarkable diversity across the studied regions. Sub-lineages L4.1.2.1 and L4.6.2.2 were found in multiple regions, including Dakar, Thies, and Saint-Louis, while specific sub-lineages, such as L4.3.4 and L4.3.4.2 were confined to regions like Diourbel and Saint-Louis.

This distribution may reflect multiple factors, including population movements, social interactions, and internal or cross-border migration flows [15]. Dakar, as the capital a primary urban center, likely plays a central role in the transmission and dissemination of different sub-lineages throughout the country [16]. Additionally, peripheral regions, such as Fatick and Sédhiou, with less frequent sub-lineages, like L4.8, could represent local transmission areas with less interaction with major urban zones.

These observations have important implications for tuberculosis surveillance and control. A nuanced understanding of regional dynamics can guide targeted interventions, such as strengthening molecular diagnostic capacity in regions with higher genetic diversity or specific resistance profiles [17].

The analysis of pharmacoresistance profiles revealed that resistant isolates were found in 4 out of the 8 regions studied (Dakar, Thiès, Louga, and Saint-Louis), with an overall prevalence of 14.81%. MDR strains represented the majority of pharmacoresistance cases (7.4%), with isolates predominantly associated with the L4.1.2.1 and L4.6.2.2 sub-lineages. These results suggest a possible genetic association between these sub-lineages and mutations conferring resistance to isoniazid and rifampicin.

These findings highlight a relatively low prevalence of pharmacoresistance and also underscore the presence of MDR strains, which pose a significant public health threat [18]. The presence of pre-MDR strains, characterized by resistance to isoniazid or rifampicin, is concerning as these isolates could evolve into more complex resistances, such as XDR, in the absence of appropriate treatment [18]. To prevent this, early diagnostic strategies and personalized treatments must be rapidly implemented.

In Silico antibiogram analyses also provided valuable insights into specific mutations associated with drug resistance. Mutations identified in the rpoB, katG, and gyrA genes were frequently associated with resistance to rifampicin, isoniazid, and fluoroquinolones, respectively [19]. This information is crucial for developing rapid molecular diagnostic tools that could directly identify these mutations in clinical samples.

Resistant isolates were detected in four of the eight regions studied: Dakar, Thiès, Louga, and Saint-Louis, suggesting that drug resistance is not confined to a specific geographical area but is widely distributed across the country. This widespread occurrence of resistant strains underscores the need for a more robust national strategy to monitor and control drug resistance.

The presence of susceptible isolates in other regions, such as Diourbel, Fatick, Ziguinchor, and Sedhiou, indicates that public health interventions may have been effective in limiting resistance in these areas. However, this also highlights the importance of reinforcing surveillance systems and control measures nationwide to prevent the further spread of resistant strains, ensuring that progress made in controlling tuberculosis remains sustainable and effective.

A key observation from this study is the association between certain sub-lineages and pharmacoresistance profiles. For example, sub-lineages L4.1.2.1 and L4.6.2.2 had a higher proportion of resistant isolates, suggesting a possible genetic or epidemiological correlation. These results require further investigation to determine whether these sub-lineages possess intrinsic characteristics that promote resistance or if their association with pharmacoresistance is merely the result of environmental selective pressures [20].

Identifying these associations is crucial for the clinical management of tuberculosis. Patients infected with sub-lineages associated with higher rates of pharmacoresistance may benefit from faster, more targeted care using therapeutic regimens based on predicted susceptibility profiles [21].

Study Limitations

Although this study provides valuable insights into the molecular and resistance profiles of *M. tuberculosis* in Senegal, it is important to acknowledge some limitations that may affect the generalizability of the findings. One of the primary limitations is the relatively small sample size, which may not fully represent the broader diversity of *M. tuberculosis* strains circulating across the country. While the study includes samples from eight regions, it only captures a snapshot of the geographic and genetic diversity of tuberculosis in Senegal. Expanding the sample size and incorporating samples from additional, underrepresented regions could provide a more accurate and complete picture of the tuberculosis landscape across the country. Furthermore, the study's geographical coverage was confined to select areas, which may not capture regional variations in drug resistance and lineage distribution that could be critical for developing targeted interventions. By including more regions, especially those with a high burden of tuberculosis, future research could uncover previously overlooked patterns and trends. Additionally, integrating clinical and epidemiological data into future studies would offer a more holistic understanding of the factors driving tuberculosis transmission and resistance patterns. Information, such as patient demographics, treatment histories, comorbidities, and socioeconomic factors could provide important context for interpreting resistance profiles and understanding the impact of local healthcare infrastructure and policies. This multidimensional approach could lead to more informed public health strategies, ultimately aiding in the development of more effective and targeted interventions to control tuberculosis and mitigate the spread of drug-resistant strains in Senegal and beyond.

CONCLUSIONS

This study demonstrates the value of whole genome sequencing in characterizing the genetic diversity and resistance profiles of *M. tuberculosis* strains in Senegal using next generation sequencing (NGS). The results provide a solid scientific foundation for guiding tuberculosis control policies while also highlighting gaps that need to be addressed for more effective disease management. A concerted effort involving governments, research institutions, and international organizations will be required to achieve global goals for the elimination of tuberculosis as a public health problem.

PERSPECTIVES

The findings of this study pave the way for future research that could focus on improving screening and treatment strategies, particularly through the development of faster and more accessible molecular diagnostic tests for early detection of resistant mutations. It is also crucial to further investigate the molecular mechanisms underlying resistance to better understand the factors that drive the transmission and evolution of resistant sub-lineages. In the long term, integrating genomic data into epidemiological surveillance strategies will enable a more targeted and personalized response to tuberculosis while strengthening local capacities for disease management. Continuous monitoring and enhanced collaboration among West African countries, supported by international partnerships, will be essential to eradicate tuberculosis and address the global challenges posed by resistant forms.

Data Availability

- Newly generated sequences have been deposited in ENA under the project.
- Accession: PRJEB83383 through accession numbers SAMEA117493692.
- SAMEA117493666.

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Author Contributions

A.P., M.G., Y.M.D., and S.M. conceived and designed the study. Y.A.D., S.N., M.S., A.L.S., N.A.D and M.S.G., performed the experiments. A.P., S.N., M.G., F.B., A.M., A.S.D., B.D., N.N.C., and P.A.D., recruited study participants and collected data. A.P., A.M., S.N., A.S.D., O.D. and O.S.D., and M.N. analyzed and interpreted the data. S.M. and IRESSEF institute contributed to reagents/materials/analysis tools. A.P., S.N. and M.G. participated to study design. A.P., M.G., Y.M.D., and S.M. participated in study coordination. A.P., S.N., M.N., M.A.G, D.O.E.A. and R.G.A. wrote/drafted the manuscript. A.P., S.M. and R.G.A., critically reviewed the manuscript for important intellectual content. A.P., Y.M.D, R.G.A. and S.M. approved the final version to be published. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

This study was approved by the National Ethics Committee for Health Research of Senegal under the following number: 00000273/MSAS/CNERS/SP. Free and informed consent is provided by each adult individual who participated in this study.

Conflicts of Interest

Authors declared no conflicts of interest.

REFERENCES

1. Lapierre SG, Fellag M, Magan C, Drancourt M. Mycobacterium malmoense pulmonary infection in France: a case report. BMC Res Notes. 2017 Aug 31;10(1):436.
2. Km M, Sv G. Mycobacterium tuberculosis complex members adapted to wild and domestic animals. Adv Exp Med Biol [Internet]. 2017 [cited 2023 Mar 3];1019. Available from: https://pubmed.ncbi.nlm.nih.gov/29116633.
3. Harouna Hamidou Z, Padane A, Zgheib R, Mamadou S, Drancourt M, Saad J. Two-decade distribution of Mycobacterium tuberculosis lineages in sub-Saharan Africa: a genome-based review. Soumis à South Afr J Sci.
4. Agence Nationale de la Statistique et de la Démographie [Internet]. Available from: https://www.ansd.sn/recensement/rgph-5-20231.
5. Agence Nationale de la Statistique et de la Démographie [Internet]. Available from: https://www.ansd.sn/sites/default/files/2024-11/Projections-demographiques_2023-2050.xlsx.
6. World Health Organization. Rapport sur la tuberculose dans le monde 2021. Geneva: World Health Organization; 2022. 25 p.
7. TD09-22.pdf [Internet]. [cited 2023 Mar 6]. Available from: https://beep.ird.fr/collect/eismv/index/assoc/TD09-22.dir/TD09-22.pdf.
8. Dia ML, Affolabi D, Ba F, Sanoussi N, Gueye PAL, Kâ R, et al. Genetic diversity and resistance profile of mycobacterial strains isolated in Senegal. Acad J. 2019 Nov 14. Available from: https://academicjournals.org/journal/JBR/article-full-text-pdf/C2B880B62434.
9. Diallo AB, Walbang OG, Camara M, Lo S, Ayorinde A, Niang A, et al. Molecular genotypes of Mycobacterium tuberculosis strains circulating in Dakar, Senegal. Afr J. 2016.
10. Iketleng T, Lessells R, Dlamini MT, Mogashoa T, Mupfumi L, Moyo S, et al. Mycobacterium tuberculosis next-generation whole genome sequencing: opportunities and challenges. Tuberc Res Treat. 2018 Dec 9;2018:1298542.
11. Mukherjee F, Bahekar VS, Prasad A, Rana SK, Kanani A, Sharma GK, et al. Isolation of Mycobacterium tuberculosis from Antelope cervicapra and Gazelle bennettii in India and confirmation by molecular tests. Eur J Wildl Res. 2015 Oct 1;61(5):783–7.
12. Cancino-Muñoz I, López MG, Torres-Puente M, Villamayor LM, Borrás R, Borrás-Máñez M, et al. Population-based sequencing of Mycobacterium tuberculosis reveals how current population dynamics are shaped by past epidemics. eLife. 2022 Jul 26;11:e76605.
13. Anthonia C, Adebowale AS, Umeokonkwo CD, Balogun MS, Nguku P, Lawson L. Patterns of resistance to first-line anti-tuberculosis drugs among patients in Abuja, Nigeria: 2009–2012. J Interv Epidemiol Public Health. 2024 Feb 15;7:Article 5.
14. Chihota VN, Niehaus A, Streicher EM, Wang X, Sampson SL, Mason P, et al. Geospatial distribution of Mycobacterium tuberculosis genotypes in Africa. PLoS One. 2018 Aug 1;13(8):e0200632.
15. Chekesa B, Singh H, González-Juarbe N, Vashee S, Wiscovitch-Russo R, Dupont M, et al. Whole-genome sequencing-based genetic diversity, transmission dynamics, and drug-resistant mutations in Mycobacterium tuberculosis isolated from extrapulmonary tuberculosis patients in Western Ethiopia. Front Public Health. 2024 Aug 9;12:1399731.
16. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM. Deciphering drug resistance in Mycobacterium tuberculosis using whole-genome sequencing: progress, promise, and challenges. Genome Med. 2019 Jul 25;11(1):45.

17. Li YF, Yang Y, Kong XL, Song WM, Li YM, Li YY, et al. Transmission dynamics and phylogeography of Mycobacterium tuberculosis in China based on whole-genome phylogenetic analysis. Int J Infect Dis. 2024 Mar;140:124–31.
18. World Health Organization. Tuberculosis in the WHO African Region: 2023 progress update. Geneva: WHO; 2023.
19. Jones RC, Harris LG, Morgan S, Ruddy MC, Perry M, Williams R, et al. Phylogenetic analysis of Mycobacterium tuberculosis strains in Wales by use of core genome multilocus sequence typing to analyze whole-genome sequencing data. J Clin Microbiol. 2019 May 24;57(6):e02025-18.
20. Sow KD, Yanogo P, Ndiaye M, Kane M, Sawadogo B, Otshudiandjeka J, et al. Profil épidémiologique de la tuberculose, Sénégal, 2009–2018. J Interv Epidemiol Public Health. 2021;4(3).
21. Rostamian M, Kooti S, Abiri R, Khazayel S, Kadivarian S, Borji S, et al. Prevalence of Mycobacterium tuberculosis mutations associated with isoniazid and rifampicin resistance: a systematic review and meta-analysis. J Clin Tuberc Other Mycobact Dis. 2023 Jun 1;32:100379.