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Genotypic study of isolated resistance to isoniazid in the *Mycobacterium tuberculosis* complex in a Moroccan hospital

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Keywords : Tuberculosis , resistance , mutation , diagnostic, katG , inhA

Abstract:

Introduction:

Despite the introduction 40 years ago of effective and low-cost treatment for tuberculosis, morbidity and mortality from this disease remain substantial worldwide. The World Health Organization (WHO) estimates that 10.6 million people contracted tuberculosis, with 1.3 million deaths in 2022. The increasing number of multidrug-resistant strains of *Mycobacterium tuberculosis* raises concerns

Materials and Methods

This is a retrospective study conducted at the Bacteriology Department of Mohammed V Military Instruction Hospital over a period of 3 years. Data were collected via the laboratory information system. Clinical samples underwent treatment using both conventional bacteriological methods and molecular techniques. The study of resistance to major anti-tuberculosis drugs was performed using the reverse hybridization technique, specifically the HAIN method (GenoType® MTBDRplus by Hain Lifescience). Statistical analysis of the data was carried out using Excel software

Results

The study involved 464 patients treated for pulmonary and extrapulmonary tuberculosis, including both new cases and those previously treated with positive cultures. The mean age of the patients was 42.2 years, with a range from 8 to 88 years. There was a predominance of males at 74%, with a sex ratio of 2.8.

Pulmonary sputum samples accounted for 84.8% of the cases, whereas extrapulmonary samples representedonly15.2% , the positivity rates for direct examination and culture across all samples were74%and100%,respectively.

Isoniazid resistance had a prevalence of 9% (41/464). Genetic mutations observed indicated that 63% of the clinical isolates resistant to INH had mutations in the KATG gene, while 37% had mutations in the inhA gene

Conclusion

The increasing prevalence of Mycobacterium tuberculosis complex strains resistant to one or more first-line anti-tuberculosis drugs necessitates regular epidemiological surveillance to limit the spread of these strains in the general population

Data summary : no data were reused or generated in this study

INTRODUCTION

Tuberculosis is a transmissible disease ranked among the leading causes of ill health and is one of the foremost causes of death worldwide. Prior to the emergence of the COVID-19 pandemic, tuberculosis was the leading cause of death attributable to a single infectious agent, surpassing HIV/AIDS.

According to the World Health Organization (WHO), it is estimated that 10.6 million people contracted this disease, with 1.3 million deaths occurring in 2022.

In Morocco, a total of 29,327 cases were reported and treated in 2021 under the National Tuberculosis Control Program (PNLAT), and it is evident that the young population aged between 15 and 45 remains the most exposed.

According to the 2022 report from the World Health Organization (WHO), approximately 8.5% of newly diagnosed tuberculosis cases worldwide exhibit resistance to isoniazid.

In recent years, the diagnosis of tuberculosis has benefited from advances in immunopathology, molecular biology, interferon-based in vitro tests, and biomarkers that enhance both the sensitivity and, crucially, the speed of diagnosis

Currently, the issue of tuberculosis is far from being under control. It is complicated by three main factors: the HIV epidemic, the relative ineffectiveness of the BCG vaccine (Bacillus Calmette-Guérin), and the emergence of resistant strains of Mycobacterium tuberculosis, including multidrug-resistant strains.

At the national level, there are few studies focusing on isoniazid monoresistance. Therefore, this study aims to investigate isoniazid resistance in tuberculosis cases (pulmonary or extra-pulmonary, new cases, or previously treated) over a period of three years.

MATERIALS AND METHODS

This is a retrospective study conducted in the bacteriology department of the Mohammed V Military Hospital, spanning a period of 3 years, from March 2019 to December 2023, involving 464 patients who were followed for pulmonary or extra-pulmonary tuberculosis, either new cases or previously treated, with positive cultures.

The cultures were prepared by spreading various samples on slides, which were then stained using the Ziehl-Neelsen method. The slides were observed under an optical microscope at immersion (x100 magnification).

The molecular study for identifying *Mycobacterium tuberculosis* and detecting resistant bacilli was performed using the GenoType® MTBDRplus test from Hain Life Sciences: The GenoTypeMTBDRplus (Hain Lifescience GmbH, Nehren, Germany) is a second-line molecular test containing specific probes for the *M. tuberculosis* complex as well as probes for common mutations associated with rifampicin (RIF) and a subset of mutations conferring isoniazid (INH) resistance.

The test primarily aims to detect genetic mutations in the rpoB gene (associated with rifampicin resistance) and in the katG and inhA genes (associated with isoniazid resistance). Using PCR to amplify the target gene regions, the test then performs hybridization with specific probes on a membrane, thus detecting the present mutations.

These mutations are critical indicators of resistance to anti-tuberculosis drugs. Quality control was ensured using the reference strain *Mycobacterium tuberculosis* H37Rv. Data collection was done through the laboratory information system, and statistical data analysis was performed using Excel software.

RESULTS:

The study included 464 patients treated for pulmonary and extra-pulmonary tuberculosis, either new cases or previously treated, with positive cultures. The average age of the patients was 42.2 years, with a range from 8 to 88 years, and a predominance of males (74%), with a sex ratio of 2.8.

The nature of the samples varied according to the location of the tuberculosis: 84.8% were pulmonary expectorations, 4% were lymph nodes, 3% bronchial aspirations, 2.5% pleural fluid, 2% biopsies, 1.5% pus, 0.6% urine, 0.6% cerebrospinal fluid, 0.5% bronchoalveolar lavage fluid, and 0.5% ascitic fluid. **Figure 1**

The positivity rate for direct smear and culture for all specimens combined was 74% and 100%, respectively. The molecular study involved 464 isolates tested with the GenoType® MTBDR plus assay by Hain Life Science. Among the 464 isolates, the prevalence of resistance to isoniazid was 9% (43/464) **Figure 2**

Among the patients with INH-resistant tuberculosis, the average age was 40 years. It is noteworthy that this group exhibited a marked male predominance: 37 men (86%) compared to

just 6 women (14%).The genetic mutations observed showed that 63% of INH-resistant clinical isolates had mutations in the katG gene, while 37% had mutations in the inhA gene. **Figure 3**

FIGURES:

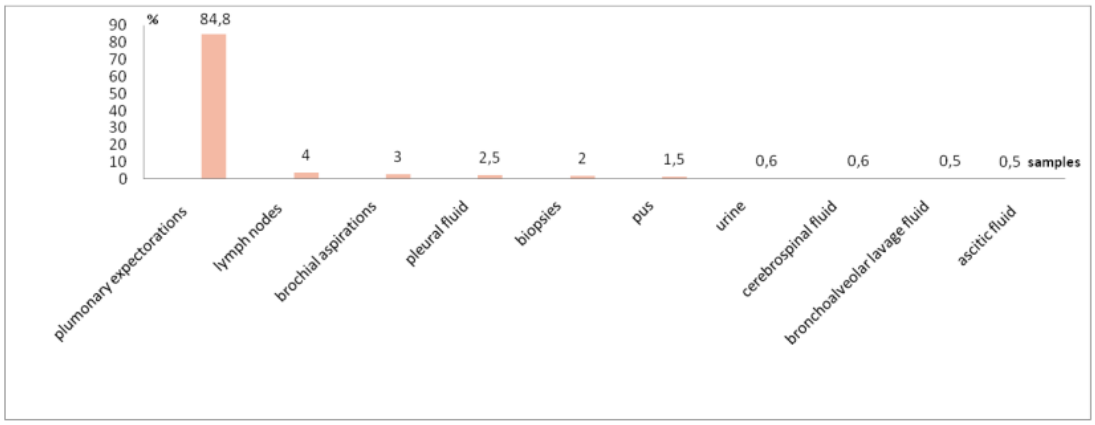

Figure 1: The nature of the sample collection varies depending on the location of the tuberculosis.

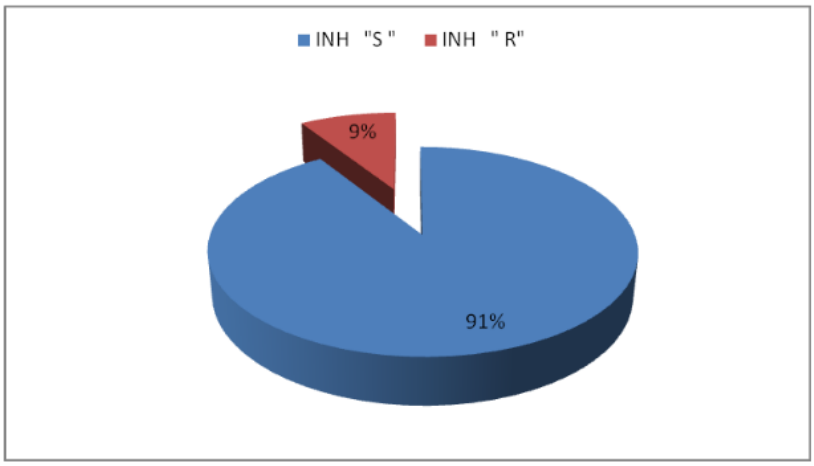

Figure 2: The prevalence of resistance to isoniazid

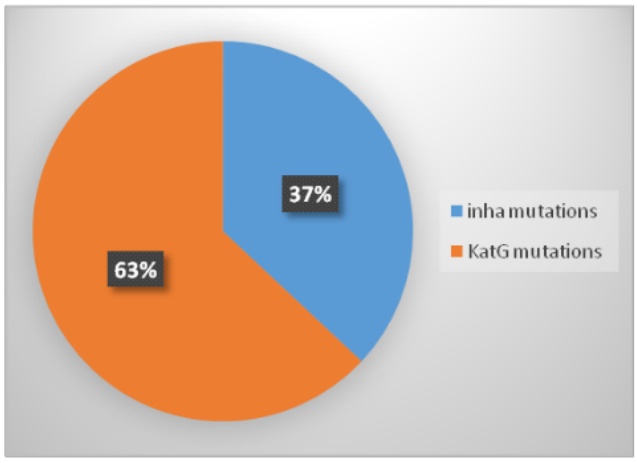

Figure 3: Resistance to INH (isoniazid)

DISCUSSION:

In recent decades, antibiotic resistance has become a growing global problem, severely affecting several deadly infectious diseases, such as tuberculosis and malaria. Despite the availability of a wide range of antibiotics, bacteria have rapidly developed resistance mechanisms, often easily and frequently. **[1]**

According to the World Health Organization (WHO), isoniazid resistance is a global issue with varying prevalence depending on the region. In 2022, global data indicated that the prevalence of isoniazid resistance was approximately 12%.

A study conductedby the Pasteur Institute of Casablanca revealed that the prevalence of isoniazid resistance was 12.5% among new tuberculosis cases in 2018.

According to these results, the prevalence of isoniazid resistance in our study aligns with national and international trends, fluctuating around 10-15%.

Furthermore, katG gene mutations, notably the S315T mutation, are common in Morocco among isoniazid-resistant strains. They account for approximately 60-70% of isoniazid resistance cases in the country. **[2]** In contrast, mutations in the inhA gene are less frequent compared to katG mutations in Morocco, representing about 20-30% of isoniazid resistance cases. **[3]**

Based on this data and our results, these trends are consistent with international observations where katG mutations are also most frequently associated with isoniazid resistance.

The resistance of *Mycobacterium tuberculosis* to isoniazid primarily develops through mutations that alter the target, and less frequently, through the activity of efflux pumps. This bacterial species possesses at least 46 efflux pumps, a remarkably high number given the size of its genome. **[4]**

Genetic studies on clinical strains resistant to isoniazid and rifampicin have revealed overexpression of genes associated with efflux pumps. **[5]**

Isoniazid, a prodrug also known as isonicotinic acid hydrazide, requires activation by the catalase-peroxidase enzyme encoded by the katG gene.**[6]** Once activated, the active principle (isonicotinyl acyl radical or anion) reacts with nicotinamide adenine dinucleotide (NAD), forming the INH-NAD complex, which inhibits the InhA enzyme, thereby disrupting the synthesis of mycolic acid essential for the cell wall. Isoniazid is effective only against active forms of *M. tuberculosis* and does not work against dormant bacilli. **[7]**

Tolerance to isoniazid in dormant mycobacteria may result from the action of the mycobacterial DNA-binding protein 1 (MDP1), analogous to histones, which regulates katG transcription and can induce isoniazid tolerance. **[7]**

Resistance to this drug is a complex process involving mutations in several genes, including katG, ahpC, inhA, kasA, and ndh, all associated with isoniazid resistance. Mutations in the katG

gene represent the primary mechanism of resistance to isoniazid (INH). **[8, 9, 10]** The katG S315 mutation is the most common among INH-resistant strains, present in 50 to 95% of resistant clinical isolates. In contrast, mutations in the inhA gene or its promoter region, though less frequent than katG mutations, are generally associated with lower-level resistance, with minimum inhibitory concentrations (MICs) ranging between 0.2 and 1 g/L**. [11]**

inhA mutations not only confer resistance to INH but also cause cross-resistance to ethionamide (ETH), a structurally similar drug.**[12]** Other genes, such as kasA, ahpC, ndh, and the intergenic region within ahpC-oxyR, have been linked to INH resistance, although their specific impact on resistance in clinical isolates remains uncertain**.[13,14]**

There is ongoing debate over whether patients with INH-resistant bacilli can still be treated with INH. **[15]** Some experts believe that high concentrations of INH may have a beneficial effect on bacilli with low-level INH resistance (> 1% of bacilli resistant to 0.2 mg/mL but sensitive to 1 mg/mL of INH) but not on bacilli with high-level resistance (> 1% of bacilli resistant to 1 mg/mL). **[16]**

This consideration is based on the fact that the peak serum concentration of INH (3 to 5 mg/mL) exceeds the MIC of so-called low-level resistant strains (generally with an MIC of 0.5 mg/mL). However, since the distinction between high and low levels of resistance is not generally made in clinical trials, it is difficult to know whether this assumption is confirmed in reality.

There are indirect arguments to estimate INH activity on low-level resistant strains. For instance, authors have studied the initial bactericidal activity of INH based on its dosage and the generated serum concentrations**. [17]** It appears that optimal bactericidal activity is achieved with 5 mg/kg, generating a peak serum concentration of 3 mg/mL. When the peak serum concentration is reduced by a factor of 10, the bactericidal activity is halved. Based on these elements, it can be considered that a low-level resistant strain with an MIC (0.5 mg/mL) ten times higher than that of a wild-type strain corresponds to a wild-type strain treated with a dosage ten times lower, which would result in a bactericidal activity initially reduced by half. Thus, at a maintained dosage, INH would have a bactericidal activity on low-level resistant strains, reduced by half compared to that on sensitive strains.

CONCLUSION:

The increasing prevalence of *Mycobacterium tuberculosis* complex strains resistant to one or more first-line anti-tuberculosis drugs necessitates regular epidemiological surveillance to limit the spread of these strains in the general population.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL : We collected the data while respecting patient anonymity and the confidentiality of their information after approval .Consent for publication Not applicable.

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