Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Mutation in the Rifampicin Resistance Region in *Mycobacterium Tuberculosis* in Guinea

Moussa Condé[1], Alimou Camara[2,3], Abdoulaye Makanéra[4,5*], F. Nzabintwaly[6], Lansana Mady Camara[5,6] and Fodé Bangaly Magassouba[3]

[1]*National Reference Laboratory for Mycobacteria Ignace Deen National Hospital, Conakry, Republic of Guinea.*

[2]*National Public Health Laboratory, Conakry, Republic of Guinea.*

[3]*Department of Pharmaceutical and Biological Sciences, Faculty of Health Sciences and Techniques, Gamal Abdel Nasser University of Conakry, Republic of Guinea.*

[4]*Biomedical laboratory of the China-Guinea Friendship Hospital, Kipé, City of Doctors, Commune Ratoma 30 BP: 710 Conakry, Republic of Guinea.*

[5]*Department of Medicine, Faculty of Health Sciences and Techniques, Gamal Abdel Nasser University of Conakry, Republic of Guinea.*

[6]*Pneumophthisiology Department, Ignace Deen National Hospital, Conakry, Republic of Guinea.*

***Correspondence:**

Abdoulaye MAKANERA, Laboratoire biomédical de l'Hôpital de l'Amitié Sino-Guinéenne, Kipé, Cité des Médecins, Commune Ratoma 030 BP : 710 Conakry, République de Guinée, Tel. 00224 664 309523/ 00224 621015770

Received: 16 Dec 2022; **Accepted:** 15 Jan 2023; **Published:** 20 Jan 2023

Citation: Condé M, Camara A, Makanéra A, et al. Mutation in the Rifampicin Resistance Region in *Mycobacterium Tuberculosis* in Guinea. Microbiol Infect Dis. 2023; 7(1): 1-4.

ABSTRACT

Introduction: *In recent years, strains of Mycobacterium tuberculosis resistant to rifampicin have become frequently encountered in Guinea. This fact is regularly notified since the installation of GeneXpert automatons in hospital structures in Conakry and in the capital of the administrative regions as well as the various prefectures of Guinea. Objective: The aim of this present study was to determine the rifampicin resistance profiles of Mycobacterium tuberculosis strains at the Pneumophtisiology Department of the Ignace Deen National Hospital of Conakry.*

Methods: *We conducted a retrospective and analytical study lasting 22 months from January 2017 to June 2018 then from April to July 2019 on the files of patients received at the National Reference Laboratory for Mycobacteria of the Ignace Deen National Hospital of Conakry for examinations of samples on the GeneXpert.*

Results: *In 325 cases of rifampicin resistance, we found mutations associated with region E (42.15%), region D (35.38%), region B (15.08%), region A (1.53%) and that of C (0.92%). We also observed multiple mutations A-B (0.92%), A-D (3.69%), D-E (0.30%).*

Conclusion: *The proportion of E probes was the highest followed by D probes then B probes (512-518). We encountered multiple mutations, like A-B, A-D, D-E.*

Keywords
Mutation, Region of Resistance, Rifampicin, Guinea.

Introduction

The emergence of resistance to anti-tuberculosis drugs in a large number of countries has become a major public health problem and constitutes an obstacle to the effective control of tuberculosis [1].

In 2017, according to the WHO, there were 558,000 (483,000-639,000) new cases of rifampicin resistance (RR) worldwide, of which 82% were multidrug-resistant tuberculosis (MDR-TB) [1,2].

The Xpert® MTB/RIF (Cepheid) test is a fully automated, semi-quantitative, real-time polymerase chain reaction (PCR) assay for the rapid detection of *Mycobacterium tuberculosis* complex DNA and rpoB gene mutations associated with rifampicin resistance. Its principle is based on the amplification of an RpoB gene fragment containing the 81base pair (bp) region (codons 507-533) which is then hybridized into five molecular beacon probes. They are represented by Probes A (507–511), B (512–518), Probe C (518–523), Probe D (523–529) and Probe E (529–533) [3].

The introduction of GeneXpert as a diagnostic method for tuberculosis has revolutionized the detection of tuberculosis, whether sensitive or multidrug-resistant.

Molecular detection of rifampicin resistance is used as a surrogate algorithm for MDR-TB in many countries because less than 10% of rifampin-resistant cases are not isoniazid-resistant. The different species of the *Mycobacterium tuberculosis* complex have similar resistance mutations (Ahmad S, Mokaddas E.) In Guinea, in 2017 the number of laboratories confirmed cases for rifampicin resistance was 265 cases. Of these, 3% of cases of extensively drug-resistant tuberculosis (XDR TB) were laboratory confirmed [2].

The objective of our study was to determine the frequency of mutation marked by the probes in the RpoB region in strains of the *Mycobacterium tuberculosis* complex at the National Reference Laboratory for Mycobacteria in Guinea.

Material and Methods

Our study took place at the National Reference Laboratory for Mycobacteria (LNRM) of the Pneumophtisiology department of the Ignace Deen National Hospital. The Molecular Biology Unit served as a field of study. The LNRM is located in the municipality of Kaloum more precisely at the premises of the Pneumology-phtisiology department of Ignace Deen. It is the national mycobacteria reference laboratory for the PNLAT (National Program for the Fight against Tuberculosis). It benefits from the support of the Global Fund through the PNLAT. Its purpose is research, training, supervision, quality assurance and control, biological monitoring and the application of national policy in the field of mycobacteriology.

Work Materials

Our study focused on all patients seen at the LNRM. As support we used:

• An individual survey form including study variables, examination request forms, GeneXpert registers, the computer database of the GeneXpert device, the FileMaker Pro Advance database

Methods

This is a 22-month retrospective and analytical study from January 2017 to June 2018 then from April to July 2019. It concerned all patients seen at the LNRM for GeneXpert examinations during the study period. The sampling was exhaustive and based on the determination of resistance to rifampicin by the GeneXpert with the MTB/RIF test.

The information on the examination sheets, in the GeneXpert database and then in that of file maker pro Advance, was recorded on a previously established survey sheet.

The data collected is saved in the Access software. The frequencies were determined with the Epi info 7.2.1 statistical software. by descriptive statistics methods and confidence interval estimates were used to show the results in the whole population.

Ethical and Deontological Aspects

The retrospective nature of our study meant that patient consents could not be collected. However, the data was collected anonymously and was only used for the purposes of this study.

Limit of the study

The retrospective nature of the study meant that certain risk factors were not analyzed because they did not appear on the examination request forms.

Results

Figure 1 shows the diagram of the results obtained on the mutation in the rifampicin resistance region in *Mycobacterium tuberculosis*.

Sociodemographic Aspects Related to Cases of Resistance to Rifampicin

The distribution of cases of resistance to rifampicin according to sex shows a clear predominance of the male sex with a sex ratio (M/F) equal to 2.78 (Figure 2).

Table 1: Distribution of rifampicin resistance cases by age.

Age group	Number	Percentage (%)	IC à 95%	
<=15	6	1.85	0.85	3.97
>15 – 30	**149**	**45.85**	**40.51**	**51.28**
>30 – 45	104	32.00	27.16	37.26
>45 – 60	55	16.92	13.24	21.38
>60	11	3.38	1.90	5.96
Total	**325**	**100**		

Mean age is 34 ± 13; Minimum age: 14 years old and Maximum: age 80 years old.

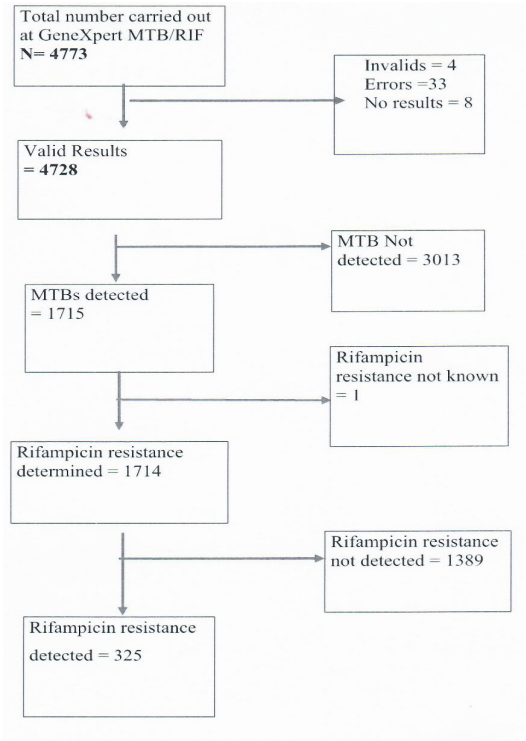

Figure 1: Diagram of results on the mutation in the rifampicin resistance region in *Mycobacterium tuberculosis.*

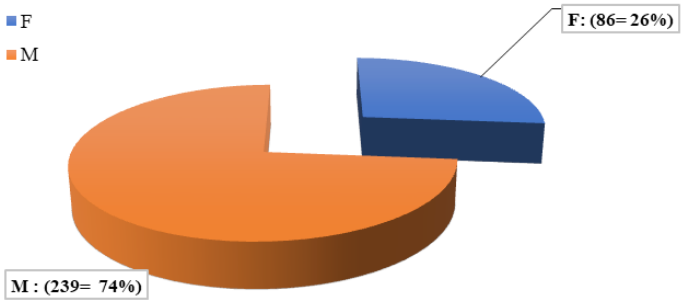

Figure 2: Distribution of rifampicin resistance cases by sex.
Sex ratio M/F = 2,78

Frequency of Rpob Probes in Strains of the *Mycobacterium Tuberculosis* Complex at the LNRM

Table 2: Breakdown of Rifampicin Resistance cases by probe

Probes	Number	Percentage	IC 95%	
Probes A	5	1.54%	0.66%	3.55%
Probe A-B	3	0.92%	0.31%	2.68%
Probe A-D	12	3.69%	2.12%	6.34%
Probe B	49	15.08%	11.60%	19.37%
Probe C	3	0.92%	0.31%	2.68%
Probe D	115	35.38%	30.38%	40.73%
Probe D-E	1	0.31%	0.05%	1.72%
Probe E	**137**	**42.15%**	**36.91%**	**47.58%**
Total	325	100.00%		

Discussion

In order to contribute to the understanding of the mutations that occur in the resistance markers of anti-tuberculosis drugs, particularly that of Rifampicin for an effective diagnosis, this study was carried out at the National Reference Laboratory for Mycobacteria of Guinea, on the files patients received in the period from January 2017 to June 2018 and from April to July 2019.

During the period of our study, the records of 4773 patients who received a GeneXpert MTB/RIF test were analyzed, of which 4728 results were valid, including 3013 (63.73%) negative samples and 1715 (36.27%) MTB detected. 325 (18.96%) cases of resistance to rifampicin were noted out of all the MTBs detected (see Results diagram).

Among the RR TB cases, 239 men were obtained, i.e. 73.54% (IC 68.49-78.04%), with a sex ratio M/F of 2.78 (Figure 2). Several studies show sex ratios ranging from 1.5 to 8 in favor of men [4]. In a study in Mumbai (India) published in January 2019, a slight male predominance is indicated by Kanade et al., then by Ahmad and Mokaddas in another study in Kuwait (2005) with the sex-ratios respectively of 1.06:1 and 3:2 [5,6]. Another study published in Senegal in 2014 by Touré N.O. et al indicated a sex ratio of 1.8 in favor of men [7].

The male predominance observed in tuberculosis patients with resistance can be explained by the fact that men are in the majority (with a sex ratio of 4/1) in this study on the one hand and on the other hand women are more observant in treatment and therefore less likely to receive inadequate first-line treatment, Smaoui Fourati S et al., 2015 [4]. Another study carried out in Bangladesh by Hamid Salim et al., in 2004 argues that socio-cultural factors in certain regions leading to lower exposure to infection could very well be responsible for the relatively low prevalence of tuberculosis among women [8].

A high frequency of probe D, ie 41.86% (Table 2) was observed in female subjects. There is no statistically significant association between gender and probes.

The average age of the subjects was 34 ± 13 years with extremes ranging from 14 years and 80 years. The most represented age group was that of 16-30 years with a frequency of 45.85% (CI 40.51-55.28%), followed by that of 31-45 years, i.e. 32.00% (CI 27.16-37.26%) (Table V). Our results can be superimposed on those of K. Horo et al., who found an average age of 33.37 years [9]. Some authors have reported a predominance of young subjects (between 25 and 44 years old) among patients affected by MDR-TB [4]. On the other hand, those Arevalo et al., found extremes ranging from 18 to 75 years in a study carried out in southern Spain [10]. The WHO reports these findings in its 2008 annual report, and argues that young people are most affected by MDR-TB. Indeed, the majority of infected subjects are between 25 and 44 years old. The predominance of young subjects can be explained by the non-compliance with hygiene measures in

this age group, given that they are very preoccupied with socio-economic activities; they are also more exposed to other resistance factors, including alcohol consumption [4]. Of 325 cases of resistance to rifampicin, a high frequency of mutations associated with the E region (529-533) or 42.15% (IC=36.91-47.58%) was found. A mutation occurs in the majority of cases on codon 531 of the RpoB gene (TGG to TTG) in which serine is replaced by lysine. The mutants associated with this region would be relatively resistant to physical conditions and would have a better capacity for adaptation and survival. The proportion of region D (523-529) was 35.38% (IC=30.38-40.73%) and that of region B (512-518) was 15.08% (IC=11)., 60-19.37%). Low frequencies of mutations associated with regions A (507-511) and C (518-523) were noted with respectively 1.54% (IC=0.66-3.55%) and 0.92% (CI=0.31-2.68%). We also found multiple mutations A-B, A-D, D-E with respectively 0.92% (IC=0.31-2.68%); 3.69% (IC=2.12-6.34%) and 0.31% (IC=0.05-1.72%) (Table 2).

Our results are close to those of Ochang et al., in Nigeria in 2016 who found 60.3% E-probe associated mutations, 13.8% B-probe associated mutations; 3.4% mutations associated with Probe A, but found only one type of multiple mutation B-D different from the one we found and no mutation on C [3]. On the other hand, our results are very different from those of Kanade et al., in India in 2019, who found mutation frequencies of 83.82% associated with E, 3.93% with D and 3.79 % to B. However, these authors, having found no mutation associated with A, nevertheless reported a multiple ADE mutation, i.e. 0.14% [6].

Low levels of rifampicin resistance are caused by mutations at codons 511 (probe A), 516 and 518 (probe B), 522 (probe C) and 533 (probe E). High levels of rifampicin resistance are caused by mutations at codons 513 (probe B), codon 526 (probe D) and 531 (probe E). The high frequency of E probes could be explained by the fact that these probes are involved in both low and high levels of rifampicin resistance.

Multiple A-B mutations; A–D; D-E encountered during the present study confer an ability of mycobacteria to adapt to exposure to antituberculous drugs as affirmed by Kanadé et al., in their study in 2019 in Mumbai (India) [6].

Conclusion

In Guinea, mutations associated with the E probe (42.15%) also called codons 531 and 533 are the most common RpoB gene mutations identified by the Xpert MTB/Rif test. Moreover, it was noted the presence of multiple mutations such as A-B; A–D; D-E which are means used by M. tuberculosis to adapt to physical conditions but also to protect itself from exposure to anti-tuberculosis drugs.

However, showing a correlation between the different mutations in the RpoB gene and the history of the occurrence of multidrug-resistant tuberculosis, such as cases already treated, patients living with HIV, could make it possible to better understand the mechanism of development of resistance of strains of *Mycobacterium tuberculosis*.

Aknowledgement

Our thanks go to:

- Laboratory technicians for carrying out GeneXperts examinations;
- Managers of the National Reference Laboratory for Mycobacteria to facilitate the work;
- The National Tuberculosis Control Program and its partners, including the global fund for the provision of GeneXpert reagents.

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