World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Contribution of Gene Xpert MTB/RIF and epidemiology in the diagnosis of pulmonary tuberculosis in PLWHIV seen at the Ignace Deen National Hospital in Conakry

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World Journal of Advanced Research and Reviews, 2022, 16(02), 300–308

Publication history: Received on 07 September 2022; revised on 31 October 2022; accepted on 03 November 2022

Article DOI: https://doi.org/10.30574/wjarr.2022.16.2.0942

Abstract

Introduction: Tuberculosis and HIV are real public health problems in the world and mainly in Africa. The current challenge is to be able to diagnose TB in all PLWHA, hence the interest in evaluating the effectiveness of GeneXpert MTB/RIF in the diagnosis of pulmonary TB.

Method: This is a prospective cross-sectional and descriptive study of HIV-positive patients who came for consultation during the survey period which lasted six months, from 1 February to 7 August 2021, at the Ignace Deen National Hospital in Conakry and at the Gamal Abdel Nasser University in Conakry. Data were collected and analysed using Epi-info version 7.0, Pack Office 2013 (Word, Excel and Power point).

Results: Out of 402 patients received, we recorded 112 positive cases, i.e. 28% and 290 negative cases, i.e. 72%. Fluorescence microscopy gave 16% and Ziehl-Neelsen microscopy gave 14% with sensitivities of 90%, 47% and 43% respectively.

104 sample patients were sensitive to Rifampicin, i.e. 92.86% against 08 cases of resistance, i.e. 7.14%. The male sex is more represented with 53% against 47% for the female sex. All the communes of Conakry were concerned, but the communes of Matoto and Ratoma were the most represented, respectively 31% and 23%. Patients from outside Conakry had a rate of 21%, the communes of Matam 11% and Dixinn 9%. Patients from the Commune of Kaloum were the least represented with 5%. Almost all socio-professional groups are affected. Commercial agents are the most represented with 31%, followed by workers with 22%, housewives with 15%, drivers with 10%, administrative agents with 9%, pupils/students with 7% and security agents with 6%. Also all age groups are affected by HIV-TB co-infection but with the highest prevalence in the 21 to 40 age group with 56% followed by the 41 to 60 age group with 30%. The age groups least affected are those under 20 and over 60 years of age, respectively 9% and 5%.

Conclusion: The present study confirms a significantly higher validity of GeneXpert MTB/RIF than microscopy in the detection of TB and its place in the prediction of multidrug resistance. Its systematic use coupled with microscopy would allow better control of tuberculosis in HIV-infected individuals.

Keywords: GeneXpert MTB/RIF; Microscopy; Tuberculosis-HIV co-infection; CHU Ignace Deen; Conakry

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1. Introduction

Tuberculosis (TB) is an infectious disease of human-to-human transmission caused by Koch's bacillus (BK). It is a major public health problem worldwide despite numerous tuberculosis control strategies (TCS) [1]. Tuberculosis associated with Human Immunodeficiency Virus (HIV) infection has a very special place in many areas of Acquired Immune Deficiency Syndrome (AIDS). Indeed, it is the most common opportunistic infection in AIDS. AIDS is a factor in the current resurgence of tuberculosis [2]. According to the World Health Organization (WHO), in 2019, there will be approximately 10 million cases of TB and 1.2 million deaths, of which 208,000 will be Human Immunodeficiency Virus (HIV)-positive [2]. In addition, drug-resistant TB remains a public health threat in 2019, with approximately half a million people worldwide having developed rifampicin-resistant TB (RR-TB) and 78% of these having developed multidrug-resistant TB (MDR-TB) [3]. In the United States (US), the 2019 TB survey found that 4.7% of people developed TB-HIV co-infection [3]. In France, the number of HIV-TB cases has decreased significantly with an incidence of 0.62 per 100,000 population [3]. Geographically, most HIV-related TB cases in 2019 were in Asia and Africa with 75336 and 318324 cases respectively [3]. In Guinea, 16390 new TB cases were detected in 2019 with 3341 people living with HIV or 23% [3]. In Guinea, 2019, Chad recorded 1696 cases of people living with HIV or 18% [3]. In addition, the methods of diagnosing TB in sub-Saharan Africa, where new cases are increasing, remain insufficient and rely essentially on microscopy, which has a low sensitivity in detecting new cases and an inability to prove drug resistance, which is becoming a real obstacle to TB control [4]. In addition, fluorescence microscopy using auramine staining has a better sensitivity than Ziehl Neelsen staining, with a gain of 10% [5]. In order to achieve the Millennium Development Goals, sustained efforts by WHO and many international organizations have been invested in the development and implementation of new diagnostic tests. The development of molecular biology methods, their standardization and automation are currently revolutionizing this diagnosis. The latest real-time gene amplification test Xpert MTB/Rif (Cepheid, Sunnyvale, CA, USA) allows rapid diagnosis of TB and rifampicin resistance in a few hours and has been approved for use by WHO since 2010 [5]. In Guinea, the guidelines of the National Tuberculosis Control Programme stipulate that Xpert/MTB/Rif testing is eligible for patients undergoing retreatment; contacts of multidrug-resistant tuberculosis cases; people living with HIV with symptoms suggestive of tuberculosis; prisoners and children with suspected tuberculosis [6]. Since 2020, Guinea, like other countries, has been using the GeneXpert as a first-line test. The National Reference Laboratory for Mycobacteria (NRLM) performs all Xperts MTB/Rif tests from the various tuberculosis reference centers (CATR) and screening and treatment centers (CDT) in the capital as part of the tuberculosis diagnosis. The current policy of the PNLAT is to expand the use of GeneXpert as a first-line test nationwide. However, in Guinea, there is no previous study on the contribution of GeneXpert compared to classical microscopy methods. In order to better evaluate the use of the molecular method in the diagnosis of pulmonary tuberculosis, we have chosen to conduct a study on Contribution of GeneXpert MTB/RIF and epidemiology in the diagnosis of pulmonary tuberculosis in PLWHIV seen in consultation at the CHU of the Ignace Deen National Hospital in Conakry.

2. Material and methods

The National Reference Laboratory for Mycobacteria (NRLM) and the Microbiology Laboratory of the Gamal Abdel Nasser University of Conakry served as the setting for our study. The LNRM is located in the pneumo-physiology department of the CHU of the Ignace Deen National Hospital in Conakry. This is a prospective cross-sectional and descriptive study lasting six months, from 1 February to 6 August 2021. The study population consisted of all HIV-positive patients of both sexes and of all ages who were seen at the Ignace Deen National Hospital during the study period. Sampling was simple random and the sample size (N=402) was calculated according to the Schwartz formula using the national prevalence of TB in the Republic of Guinea. All HIV-positive patients received in the laboratory with an examination form requesting Xpert and microscopy with patient information were included in our study. The bio-material consisted of sputum and gastric tube products from the patients.

2.1. Biological variables

Sputum, Gastric tubing products.

2.2. Epidemiological variables

Ages, Sexes, Occupation, Residence.

2.3. Clinical diagnosis

The diagnosis is based on the following functional signs

- Persistent cough without improvement (>14 days)

- Night-time Fever

Alteration of the general condition with break in the growth curve (weight/height), decrease in appetite, weight loss, etc.

2.4. Data collection and analysis

The data were collected on the pre-established survey forms, entered, processed and analyzed using Epi-info version 7.0, the 2013 office pack, Word and Excel.

3. Results

The application of the research methodology led to results in the form of graphs and tables which were interpreted, commented and discussed according to the available data from the literature.

3.1. Variation of results according to biological diagnostic methods

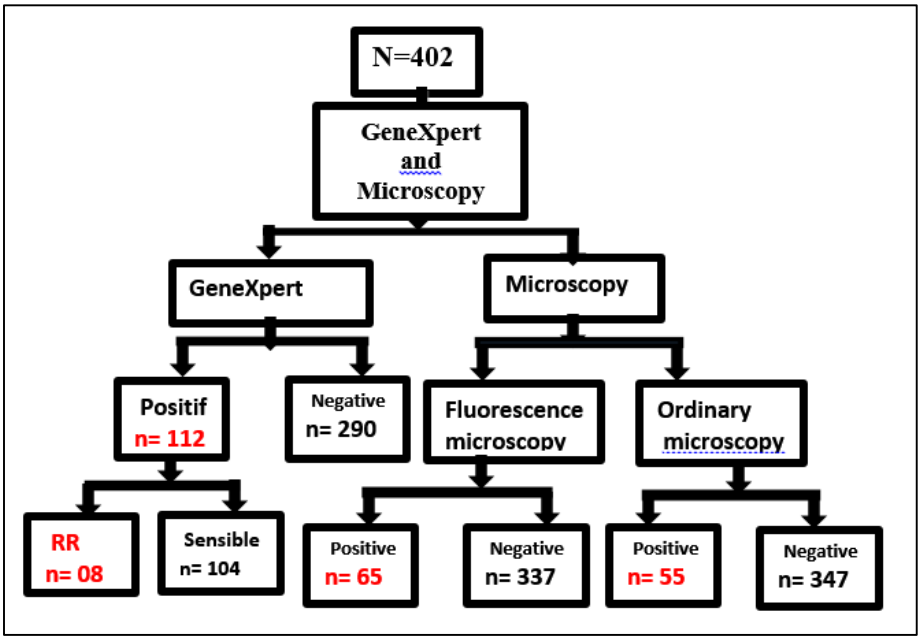

Figure 1 Flow chart of results by methods used

In this figure, we note that out of the 402 HIV positive patients examined, 112 were GeneXpert positive compared to 65 fluorescence microscopy positive and 55 ordinary microscopy positive, i.e. 28%, 16% and 14% respectively. These results show that the method of choice for a better diagnosis of tuberculosis is the GeneXpert method.

Table 1 Validity of the Ziehl-Neelsen test compared to GeneXpert MTB/RIF

	PCR GeneXpert MTB/Rif		**P- Value**
Ziehl-Neelsen	**Positive**	**Negative**	
Positive	48(42.86%)	7(2.41%)	0.01
Negative	64(57.14%)	283(97.59%)	0.01
Total	112(100%)	347(100%)	0.01

Table I shows the validity of the Ziehl-Neelsen compared to GeneXpert MTB/RIF shows that of the 112 GeneXpert positive patients, only 48 cases or 42.86% were Ziehl-Neelsen positive versus 64 cases or 57.14% Ziehl-Neelsen negative. Of the 347 GeneXpert MTB/RIF negative cases, 283 cases or 97.59% were also negative.

Table 2 Validity of the GeneXpert MTB/RIF test compared to Ziehl-Neelsen

	Ziehl-Neelsen		**P-Value**
PCR GeneXpert MTB/Rif	**Positive**	**Negative**	
Positive	48(87.3%)	64(18.4%)	0.01
Negative	7(12.7%)	283(8.6%)	0.01
Total	55(100%)	347(100%)	0,01

Of the 55 Ziehl-Neelsen positive patients, 48 cases or 87.3% were GeneXpert MTB/Rif positive compared to 7 cases or 12.7% that were GeneXpert MTB/Rif negative. Compared to the GeneXpert MTB/Rif test, the Ziehl-Neelsen had a lower sensitivity of 42.86% as the GeneXpert MTB/Rif had a maximum sensitivity of 87.3% and a specificity of 97.59%.

Table 3 Validity of the GeneXpert MTB/RIF comparative fluorescence microscopy

	PCR GeneXpert MTB/Rif		**Valeur de P**
Fluorescence microscopy	**Positive**	**Negative**	
Positive	53(47.32%)	12(4.14%)	0.01
Negative	59(52,68%)	278(95,86%)	0.01
Total	112(100%)	290(100,14%)	0.01

Table 3 shows the validity of Fluorescence Microscopy compared to GeneXpert MTB/RIF that among the 112 GeneXpert positive patients, only 53 cases or 47.32% were Ziehl-Neelsen positive versus 59 cases or 57.14% fluorescence negative. Among the 290 GeneXpert MTB/RIF negative cases, 278 patients were negative by fluorescence microscopy, i.e. 95.86%.

Table 4 Validity of the GeneXpert MTB/RIF test compared to fluorescence microscopy

	Fluorescence microscopy		**P-Value**
PCR GeneXpert MTB/Rif	**Positive**	**Negative**	
Positive	53(89.8%)	59(17.5%)	0.01
Negative	12(10.2%)	278(82.5%)	0.01
Total	65(100%)	337(100%)	0.01

Of the 65 fluorescence microscopy positive patients, 53 patients or 89.8% were GeneXpert MTB/Rif positive compared to 12 cases or 10.2% that were GeneXpert MTB/Rif negative. Compared to the GeneXpert MTB/RIF test, the fluorescence microscopy had a sensitivity of 47.32%, whereas the GenXpert test had a higher sensitivity of 89.8% and a specificity of 95.86%.

Table 5 Validity of Fluorescence Microscopy compared to Ordinary Microscopy

	Ziehl-Neelsen		**P-Value**
Fluorescence microscopy	**Positive**	**Negative**	
Positive	55(84.62%)	10(15.38%)	0.01
Negative	0(0.00%)	337(97.12%)	0.01
Total	55(13.68%)	347(86.32%)	0.01

In this table we see that the sensitivity of LED Microscopy was higher than that of ZN Microscopy at 85%.

3.2. Rifampicin susceptibility of HIV-TB co-infected patients

III.1 Susceptibility to Rifampicin among the 112 HIV-positive and GeneXpert MTB/RIF-positive patients, 104 cases were sensitive to Rifampicin, i.e. 92.86%, against 08 cases of resistance, i.e. 7.14%. This resistance, which remains high in HIV/TB co-infected subjects, could be due to the bioresistance of *Mycobacterium tuberculosis* to Rifampicin in these patients due to poor medication and poor nutrition.

3.3. Distribution of pulmonary TB patients by epidemiological variables

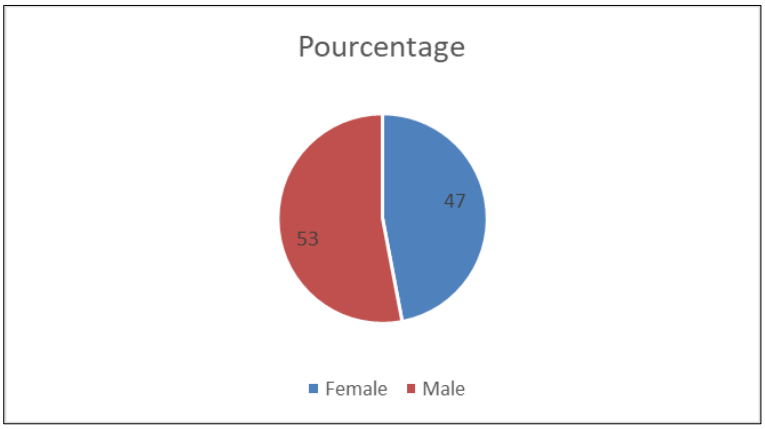

Figure 2 Gender distribution of HIV-TB co-infected patients

The Figure 2 shows that of the 112 HIV-TB co-infected patients, the male sex is more represented with 53% against 47% for the female sex.

This situation could be explained by the practice of vices such as smoking, alcoholism and polygamy among males.

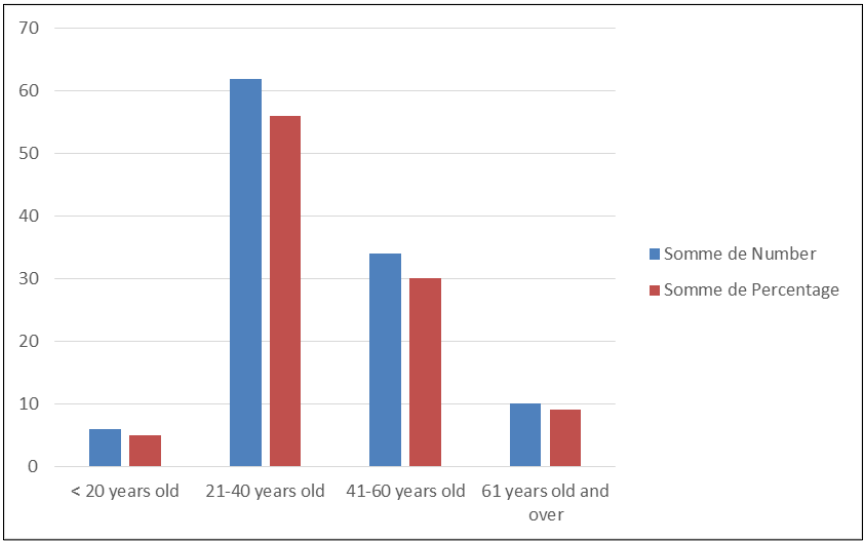

Figure 3 Distribution of HIV-TB co-infected patients by age group

In this figure, we see that of the 112 HIV-TB co-infected patients, all age groups are affected by the disease, but with the highest prevalence in the 21 to 40 age group with 56%, followed by the 41 to 60 age group with 30%. The least affected age groups are those under 20 and over 60 years of age, respectively 9% and 5%. The high prevalence in the 21-40 and 41-60 age groups could be explained by multiple sexual partners, alcoholism and smoking.

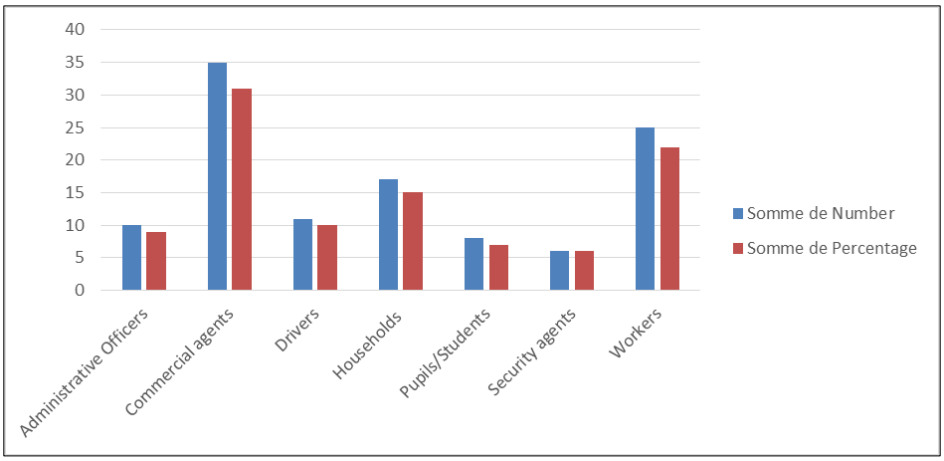

Figure 4 Distribution of HIV-TB co-infected patients by socio-professional category

In Figure 4, we note that of the 112 HIV-TB co-infected patients, almost all socio-professional strata are affected. Commercial workers are the most represented with 35 cases, i.e. 31%, followed by workers with 25 cases, i.e. 22%, housewives with 25 cases, i.e. 15%, drivers with 10%, administrative staff with 9%, pupils/students with 7% and security staff with 6%. The high prevalence among Commercial Agents and Workers could be due to promiscuity, polygamy, tobacco and alcohol abuse.

Table 6 Distribution of HIV-TB co-infected patients by residence

Residence	Number	%
Matoto	35	31
Ratoma	26	23
Outside Conakry	24	21
Matam	12	11
Dixinn	10	09
Kaloum	5	05
Total	112	100,00

In this table, we note that HIV-TB co-infected patients from the Communes of Matoto and Ratoma are the most represented, with 35 cases (31%) and 26 cases (23%) respectively. They are followed by patients from outside Conakry with 24 cases, i.e. 21%, from Matam 11% and from Dixinn 9%. Patients from the Commune of Kaloum are the least represented with 5%. The high prevalence in the communes of Matoto and Ratoma could be due to the large number of patients coming from these communes for consultation during our survey period and the fact that they are the most populated in the city of Conakry.

4. Discussion

The main objective of this study is to compare the effectiveness of GeneXpert, Ordinary Microscopy (Ziehl-Neelsen) and Fluorescence Microscopy in the diagnosis of pulmonary tuberculosis. The aim is to participate in the improvement of the health care of PLWHA at the University Hospital in Conakry.

The present work noted a frequency of pulmonary tuberculosis of 28% among 402 clinically suspected HIV patients. The positivity was significantly higher for GeneXpert than for Fluorescence Microscopy and Ordinary Microscopy. Of the 112 GeneXpert MTB/Rif positive patients, 8 cases or 7% had Rifampicin resistance. The frequency of tuberculosis found in this study is similar to that reported by other authors. Indeed, Zeka AN et al. noted a frequency of tuberculosis of 26% in Turkey [7]. The frequency found by Gounder A et al. was relatively low, at 18.7% in a sample of 415 patients in Fiji [40]. This variation could be explained by the difference in the methodology applied and especially by the risk factors associated with HIV infection. According to the WHO, of the 10 million new cases of TB worldwide, 8.2% were

HIV positive in 2019 [3]. In our series, the prevalence of HIV/TB co-infection was relatively higher than that of the WHO. This confirms the strong correlation between HIV/TB co-infection and HIV prevalence in a region.

In our study, there was a male predominance of 53% with a male/female sex ratio equal to 1.39; the difference being statistically significant (p = 0.03). HE Hassan et al, in a study carried out in Morocco in 2021, reported 58.1% for male sex [9]. This male predominance could be explained by the fact that professionally, men occupy more sectors of activity than women, which exposes them more to contagious diseases such as tuberculosis. The average age of our patients was 39 years, with extremes ranging from 2 to 80 years. Our results correspond to those of HE Hassan et al, who also found an average age of 40 years [9].

The most represented social stratum is that of traders with a frequency of 31%, followed by workers with 22%, with a link between the predominance of the male sex and housewives with 15%. Our results corroborate those found by ABOU SAID in his doctoral thesis in medicine that the most represented social stratum is that of the liberals with a frequency of 28.18% [10].

We note that patients from the Communes of Matoto and Ratoma are the most affected, respectively 31% and 23%. They are followed by patients from outside Conakry 21%, Matam 11% and Dixinn 9%. Patients from the Commune of Dixinn are the least affected and less represented, at 5%. This high prevalence in the Communes of Ratoma and Matoto can be explained by the fact that they represent the two largest and most populated communes in Conakry. More patients come from these two communes than from other communes in Guinea.

The 21-40 age group, 56%, was the most represented, followed by the 41-60 age group, 30%. David Lupande et al, in their study in Bukavu, Democratic Republic of Congo (DRC), found that the 21-39 (57.3%) and 40-59 (23.2%) age groups were the most common [11].

Positivity was significantly higher for GeneXpert MTB/RIF than Ziehl-Neelsen and auramine staining specifically in HIV-positive individuals. Compared to the GeneXpert MTB/Rif test, the Ziehl-Neelsen had a low sensitivity (42.86). However, compared to the Ziehl-Neelsen, the sensitivity of the GeneXpert MTB/Rif was not maximum (87.3%) and specificity was 97.59%. Our results differ from those found in Rwanda by Jean Claude Semuto Ngabonziza et al who reported a sensitivity of 55.1 for Ziehl-Neelsen and 77.6% for Xpert [12].

Compared to the GeneXpert MTB/RIF test, fluorescence microscopy had a sensitivity of 47.32%, whereas fluorescence microscopy compared to GenXpert had a sensitivity of 89.8% and a specificity of 95.86%. These results are consistent with the results of recent reports, which showed that Xpert had a higher sensitivity for the detection of TB cases. Chakravorty et al (2017), reported a similar overall sensitivity of 88% for Xpert MTB/Rif. However, this high sensitivity of Xpert MTB/Rif led to a specificity of 95.86% [13]. The use of GeneXpert MTB/Rif allowed early detection of rifampicin resistance in 7.14% of cases with rapid adaptation to multidrug resistant treatment.

Finally, we performed a systematic review to summarise the accuracy of fluorescence microscopy compared to conventional microscopy. In the HIV-infected group, the sensitivity of LED microscopy was higher than ZN microscopy at 85%. This table shows a contribution of fluorescence microscopy which is 10 patients or 15.38% compared to Ziehl-Neelsen. This frequency seems significantly higher than those found in other studies and far exceeds the WHO estimate of 10%. Our results differ from those found by Ngabonziza et al. who also demonstrated an overall sensitivity of ordinary microcopy of 55% and LED microscopy of 37% [12].

5. Conclusion

At the end of our study on the contribution of GeneExpert MTB/RIF and epidemiology in the diagnosis of pulmonary tuberculosis in PLHIV at the National Reference Laboratory for Mycobacteria (LNRM) of the Donka University Hospital and the Microbiology Laboratory of the Gamal Abdel Nasser University of Conakry, we have drawn the following conclusion:

Out of 402 patients received, we recorded 112 positive cases, i.e. 28% and 290 negative cases, i.e. 72%. Fluorescence microscopy gave 16% and Ziehl-Neelsen microscopy gave 14% with sensitivities of 90%, 47% and 43% respectively.

104 patient samples were sensitive to Rifampicin, i.e. 92.86% against 08 cases of resistance, i.e. 7.14%. The male sex was more represented with 53% against 47% for the female sex. All the communes of Conakry were concerned, but the communes of Matoto and Ratoma were the most represented, respectively 31% and 23%. Patients from outside Conakry had a rate of 21%, the communes of Matam 11% and Dixinn 9%. Patients from the Commune of Kaloum were

the least represented with 5%. Almost all socio-professional groups are affected. Commercial agents are the most represented with 31%, followed by workers with 22%, housewives with 15%, drivers with 10%, administrative agents with 9%, pupils/students with 7% and security agents with 6%. Also all age groups are affected by HIV-TB co-infection but with the highest prevalence in the 21 to 40 age group with 56% followed by the 41 to 60 age group with 30%. The least affected age groups are those below 20 years and above 60 years, respectively 9% and 5%.

The present study proves that the GeneXpert MTB/RIF method is the best method for the diagnosis of pulmonary tuberculosis in comparison with Microscopy (Fluorescence and Ordinary) and plays a major role in the prediction of multidrug resistance of Mycobacterium tuberculosis. Its systematic use in conjunction with microscopy would allow better control of TB in HIV-infected individuals.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the managers and laboratory technicians of the National Reference Laboratory for Mycobacteria (LNRM) of the CHU de Donka and the Microbiology Laboratory of the Gamal Abdel Nasser University of Conakry for their support in carrying out this work.

Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

confidentiality was respected throughout the data collection process and the results were used for strictly scientific purposes.

Statement of informed consent

Prior to the study, patients gave their consent to participate in the study.

Authors' contributions

All authors have contributed to this work and have read and approved the final version of the manuscript.

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