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Evaluation of the *Xpert® MTB/RIF* Assay and Microscopy for Diagnosing Pulmonary Tuberculosis in Butembo, Democratic Republic of the Congo

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Authors' contributions

This work was carried out in collaboration between all authors. Author GKB designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AKN managed the literature searches and revised the first draft of the manuscript. Author AAM performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This study aimed to evaluate the efficacy of *Xpert® MTB/RIF* assay comparing to the acid-fast smear microscopy for diagnosing pulmonary tuberculosis cases in Butembo, Democratic Republic of the Congo.
Methodology: This was a retrospective study analyzing pulmonary tuberculosis cases that were diagnosed using both the *Xpert® MTB/RIF* assay and microscopy. Secondary data were collected

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from monthly report from the Microbiology department at GHR Katwa, Butembo, for the time period of February 2015 to September 2017.
Results: Three hundred and seventy-two specimens were collected from patients suspected of pulmonary tuberculosis. Of these, 98 (26.3%) were found to be positive for MTB by *Xpert® MTB/RIF* assay, out of which 73 (19.6%) were also found to be positive by microscopy. The remainder was negative. Two (2.04%) cases of resistance to rifampicin were detected. The sensitivity, specificity, positive predictive value and negative predictive value of Xpert assay were respectively 83.6%, 87.6%, 62.2% and 95.6%.
Conclusion: The use of the *Xpert® MTB/RIF* coupled with microscopy as part of routine assay, permits rapid diagnosis of pulmonary MTB and detection of rifampicin resistance. It may help in the epidemiological control of pulmonary MTB.

Keywords: Tuberculosis; Xpert® MTB/RIF assay; microscopy; diagnostic tests; rifampicin; DRC.

1. INTRODUCTION

Tuberculosis is one of the top ten causes of death worldwide and constitutes a serious public health problem. The World Health Organization (WHO) noticed 10.4 million of people who fell ill with tuberculosis and 1.7 million of deaths in 2016 (including 0.4 million among people with HIV) [1]. The morbidity and mortality due to tuberculosis remain high in low- and middle-income countries with over 95% of deaths [1], and the diagnostic methods for tuberculosis are inadequate and are essentially based on microscopy at primary level health care centres in these countries [2]. This constitutes a lagging factor for the control of tuberculosis in developing countries.

Microscopy with Ziehl-Neelson (ZN) staining is mostly used in developing countries as a diagnostic tool and it is associated with error in misdiagnoses, whereas the elimination and control of tuberculosis require improved diagnostic tools for early detection and initiating the treatment early [3,4]. This technique identifies the acid-fast bacilli and not directly the *Mycobacterium tuberculosis* (MTB). This may affect its specificity in settings with a low burden of tuberculosis or places with a high prevalence of non-tuberculosis mycobacteria [5]. The sensitivity of this method is also low (40-60%) [6,7].

Therefore, it is necessary to improve and possibly replace microscopy with simple, more affordable, and more accurate diagnostic methods [3,8]. Actually, diagnostic methods based on molecular biology are proposed. Two methods have been validated by the WHO in different programs in developing countries. It is the *GenoType MTBDR* plus assay (Hain Lifescience GmbH, Nehren, Germany) and the *Xpert MTB/RIF* assay (Cepheid, Sunnyvale, CA, USA) [9,10]. These molecular methods are rapid and have a high sensitivity and specificity [11]. But their high expenses are obstacles for their broad application as diagnostic method in low- and middle-income countries.

The *Xpert* assay is a system based on a nucleic acid amplification test that rapidly diagnoses and detects *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance in clinical specimens. It is a fully automated cartridge based-real time PCR based test which gives results in less than two hours [8,10,12]. This method showed a high sensitivity and specificity comparing to microscopic and culture methods in most recent meta-analysis and they are increasing the rate of new cases detection [11]. In 2016, 6.3 million new cases of tuberculosis were reported (up from 6.1 million in 2015), equivalent to 61% of the estimated incidence of 10.4 million [1].

Among countries leading the loading of tuberculosis at 80% worldwide, the Democratic Republic of the Congo (DRC) occupied the eleventh rank (in 2014) in spite of effort made by the National Program for Fighting against Tuberculosis [13] and the sixth rank among thirty countries with high burden tuberculosis reported in the global report tuberculosis 2017 by the WHO [1]. The same report reported that the annual incidence of tuberculosis in DRC reached 323 cases per 100,000 inhabitants (132,515 new tuberculosis cases) in 2016 with the case-fatality rate estimated at 0.25 [1]. Thus, for increasing the detection of tuberculosis cases for an early initiation of the treatment, the National Program for Fighting against Tuberculosis equipped main health centres/hospitals in the country with an automated *Xpert® MTB/RIF* machine. In Eastern DRC, the General Referral Hospital (GRH) of Katwa, located in Butembo, is among those

hospitals. Therefore, this study aimed to evaluate the efficacy of *Xpert® MTB/RIF* assay comparing to the acid-fast smear microscopy for diagnosing pulmonary tuberculosis cases in Butembo, DRC.

2. METHODS

2.1 Study Design and Setting

We retrospectively analysed pulmonary MTB cases diagnosed using both the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) and microscopy. We used secondary data from monthly report of the Department of Microbiology at GRH of Katwa from February 2015 to September 2017. The GRH of Katwa is the only hospital equipped with an automated *Xpert® MTB/RIF* machine for diagnosing MTB cases and resistance to rifampicin in Butembo city. Samples of patients suspected of pulmonary MTB, on clinical basis, were collected for analyses of both the *Xpert® MTB/RIF* assay and microscopy by using the Ziehl-Neelsen technique for sputum acid-fast bacilli smear. The total number of specimen collected from suspected patients was 372.

2.2 Direct Smear Microscopy

The conventional sputum acid-fast smear using the Ziehl-Neelsen staining method was performed to investigate the presence of acid fast bacilli. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli.

2.3 Gene Xpert MTB/RIF Assay

Two volumes of sample treatment reagent are added to each volume of sputum. The mixture is shaken until clear solution was made and then incubated at room temperature for 15 minutes. A mixture sample of 2 to 3 ml was transferred to the test cartridge using sterile dropper. All subsequent steps (DNA extraction, PCR presence inhibitors) occur automatically. Results were available in less than 2 hours and the user was provided with a printable automatic test result.

2.4 Definition of MTB Cases

In this study we considered as confirmed MTB cases all those cases with positive results in microscopy and in *Xpert® MTB/RIF* assay. For patients whose samples were Xpert-positive but microscopy-negative, probable MTB was diagnosed based on clinical and radiological findings, previous and/or current history of MTB treatment and treatment response.

2.5 Ethical Consideration

Ethical clearance was obtained from the local ethics review committee of the Faculty of Medicine at the Université Catholique du Graben, Butembo, DRC. The study was also approved by the Katwa Health Zone District Office. No individual consent was required as archived patient records were collected and no patient identification was used.

2.6 Data Analysis

The data was entered in *Microsoft excel* spread sheet and statistical analysis was done with the Statistical Package for the Social science System version 20.0. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Xpert assay were compared to the sputum acid-fast smear microscopy as a reference standard. *P* values less than 0.05 were taken as statistically significant.

3. RESULTS

3.1 General Characteristics of the Population

The Table 1 shows the general characteristics of patients suspected with pulmonary MTB. Among the 372 patients who have done acid-fast smear microscopy and *Xpert® MTB/RIF* assay, 208 (55.9%) were male and 164 (44.1%) females. The range ages of 31 to 45 years and 46 to 60 years represents major groups concerned with 31.5% and 24.5% respectively.

Table 1. General characteristics of the population (N=372)

Variables	n	%
Sex		
Male	208	55.9
Female	164	44.1
Age (years)		
≤15	42	11.2
16-30	57	15.3
31-45	117	31.5
46-60	91	24.5
>60	65	17.5

3.2 Biological Diagnosis of Tuberculosis

The positive diagnosis of pulmonary MTB was made in 98 (26.3%) cases by Xpert assay and in 73 (19.6%) cases by Acid-fast smear microscopy (Table 2). These results are significant (P<0.05). Among these positives cases, 61 were positive to both of the tests (Table 3). Thus, the prevalence of pulmonary MTB is estimated to 16.4%. Among the 98 positive cases by Xpert assay, 2 (2.04%) cases showed a resistance to rifampicin.

3.3 Comparison of the Performance of Xpert MTB/RIF Assay and Acid-Fast Smear Microscopy

The prevalence of MTB by using the Xpert diagnostic assay was 96 (26.3%) cases while it was 73 (19.6%) by acid-fast smear microscopy (Table 2). The Table 3 shows the respective ability of Xpert assay comparing to microscopy in diagnosing MTB cases. Among the 98 positive cases on Xpert assay, only 61 (62.2%) cases were positive on microscopy and 37 (37.8%) were negative. And among the 274 negative cases on Xpert assay, 262 (95.6%) cases were also negative by microscopy. Overall sensitivity, specificity, PPV and NPV of Xpert assay when acid-fast smear microscopy was taken as reference method is illustrated in Table 3.

4. DISCUSSION

In this retrospective study, we have evaluated the efficacy of *Xpert® MTB/RIF* assay to diagnose pulmonary MTB cases and resistance to rifampicin and compared it with the acid-fast smear microscopy which was taken as gold standard.

This study showed a prevalence of 16.4% of pulmonary MTB among 372 suspected patients. This result is close to those reported by other authors [2,13]. In fact, Lupande et al. reported a prevalence of 16.3% among a sample of 452 patients in Bukavu [2], a city located in a neighbour province to the one in which this study was conducted. Meanwhile, a high prevalence (26%) of tuberculosis is noted in Turkey [14]. This difference may be explained by the methodology and approach applied. For the study done in Turkey, pulmonary and extra pulmonary tuberculosis were considered.

In the current study, there were more males (55.9%) than females (44.1%) and the range ages of 31 to 45 years and 46 to 60 years represents major groups concerned with 31.5% and 24.5% respectively but the age group below 15 years were concerned only in 11.2%. Mavenyengwa et al. [3] found a slightly more females than males in Namibia and the age groups ranged between 20 and 60 years had the highest MTB infection. The age distribution in our study may be explained by two facts. On one hand, the low infection rate in the younger age group could possibly be explained by this age group's literacy. The incorporation of hygiene and disease prevention courses in the levels of primary and high school may allow them to effectively receive educational information on MTB prevention from schools and the media. On the other hand, the high rate of pulmonary MTB infection in age groups between 31 and 60 years could be due to the fact that these groups are sexually active, therefore encountering sexual partners in whom both MTB and HIV are both prevalent.

Table 2. Biological diagnosis of tuberculosis

Variables	Positive, n (%)	Negative, n (%)	*P*-value
Xpert MTB/RIF assay	98 (26.3)	274 (76.7)	0.03
Acid-fast smear	73 (19.6)	299 (80.4)	

Table 3. Comparison of *Xpert MTB/RIF* assay and acid-fast smear microscopy

Variables		Acid-fast smear microscopy			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative	*P*-value				
Xpert assay	**Positive, n=98**	61 (62.2)	37 (37.8)	0.0000	83.6	87.6	62.2	95.6
	Negative, n=274	12 (4.4)	262 (95.6)					

The Xpert assay detected 98 (26.3%) cases of pulmonary MTB versus 73 (19.6%) cases detected by microscopy. The difference in MTB diagnosis between the two methods was statistically significant (P<0.05). Higher detection of MTB by Xpert assay comparing to microscopy has been demonstrated by several studies [15-17]. Literature has proved that microscopy has limited diagnostic benefits as it is susceptible to human error and other factors beyond control that can result in false negatives. Those factors may include poor quality specimens, not obtaining the proper portion of the specimen during smear preparation or the effect of MTB-HIV co-infection [18]. Among the positive cases on Xpert assay, 2 (2.04%) cases showed resistance to rifampicine. Zeka et al. [18] have found also 1 case of resistance to rifampicine among 89 positive cases on Xpert assay in Turkey. Taking the microscopy as gold standard, the sensitivity, specificity, PPV and NPV of Xpert assay were respectively 83.6%, 87.6%, 62.2% and 95.6%. These values are higher compared to the sensitivity of microscopy (40-60%) reported by other authors [6,7]. A systematic review of 27 studies showed that Xpert assay for pulmonary MTB diagnosis had a pooled sensitivity of 89% (95% CI: 85%–92%) and specificity of 99% (95% CI: 98%–99%) in the diagnosis of pulmonary MTB [11]. The findings in this study are slightly low to these values. This may be explained by the low number of the sample studied.

5. LIMITATIONS OF THIS STUDY

The present study was retrospective, implying all inherent insufficiencies to its methodology notably the lack of some information on the follow-up and the evolution of the patients once the diagnosis is done, especially for cases with resistance to rifampicine. The culture of the complex MTB was not feasible in the laboratory of Microbiology. It did not allow using it as a reference gold standard in the present study. HIV patients were not notified, thus the Xpert assay was not evaluated at cases of MTB-HIV co-infection. In spite of these limits, this study had the merit to have evaluated for the first time the *Xpert® MTB/RIF* assay since it was started to be used in Butembo.

6. CONCLUSION

This study confirms significantly the validity of the Xpert® *MTB/RIF* assay compared to acid-fast smear microscopy in the detection of the pulmonary *Mycobacterium tuberculosis* and its place in the detection of rifampicin resistance. Its adoption coupled with microscopy as part of routine assay will improve the detection of pulmonary MTB and its control as the Xpert assay provides results in about 2 hours, with information on rifampicin resistance, which is highly associated with multidrug-resistance and poor treatment outcomes using conventional MTB treatment regimens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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