GEN-XPERT® MTB/RIF Contribution in the Diagnosis of Pulmonary Tuberculosis with a Negative Bacilloscopy in Kinshasa (DRC)

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ABSTRACT

Background: Democratic Republic of Congo (DRC) is one of the 22 countries most affected by tuberculosis in the world. The diagnosis of tuberculosis is mainly based on direct microscopy after Ziehl-Neelsen staining. Cases with negative microscopy follows the algorithms adopted in the World Health Organization's guide.

Objective: To compare the contribution of GEN-Xpert® to culture for the diagnosis of tuberculosis among clinically diagnosed cases (TPM0).

Materials and Methods: In a cross-sectional study of all cases of smear-negative tuberculosis detected at two screening centers in the City of Kinshasa between July 2013 and May 2014. The samples were analyzed by GEN-Xpert® and cultured on Solid medium (LJ) used as standard Gold.

Results: The study included 347 smear-negative patients. Positivity rate was 29.1% (95% CI 33.9-43.3) for culture (LJ) and 32.9% (95% CI 36.7-46.6) for GEN-Xpert® MTB / RIF. The sensitivity of GEN-Xpert® by comparison was 93.1%. Rifampicin resistance and multidrug resistance were 9.9% (10). Sensitivity and specificity for detection of resistance to rifampicin was 100%.

Conclusion: The choice of a sensitive and rapid test increases the detection rate of TB cases in general and especially in subjects whose direct smear examination is negative.

Keywords: GEN-Xpert®; Diagnostic; Tuberculosis Smear Negative.

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INTRODUCTION

Democratic Republic of Congo (DRC) is among the 22 countries with the highest burden of tuberculosis in the world and ranks 3rd in Africa.[1]

National TB control programs are more concerned with the identification of bacilliferous cases in order to sterilize them. However, smear-negative tuberculosis, although less infectious, can also contribute to the increased incidence of tuberculosis and prevent the achievement of the global goal of tuberculosis elimination.

Previous studies have shown that contamination of patients 'or healthcare workers' surrounding was possible in the case of TPM0.[2,3] In 2014, DRC reported 116.894 cases of tuberculosis including 15386 (13%) cases of smear-negative tuberculosis.[1]

Ziehl-Nelseen based screening is facilitated by the fact that the lesions are excavated and very rich in bacilli.[2-5] However, it often happens that the lesions are paucibacillary making the detection of the bacilli difficult by direct examination.[1-3,5]

Moreover, the diagnosis by culture on the Lowenstein-Jensen medium, considered as a reference method, proves to be tedious and costly. It requires skilled personnel, appropriate infrastructure and biosecurity rules.[1-4] For example, the World Health Organization (WHO) recently recommended the use of molecular biology techniques, including GEN-Xpert®, which detects Mycobacterium tuberculosis directly in pathological products.[4] The results of the 2012-2013 survey by Mbonze and al. in DRC, determining the GEN-Xpert® impact in case reporting in TPM0 patients, were beneficial (14.3% positive).[6] It is therefore important to compare the contribution of this new approach to culture in confirmation of smear-negative tuberculosis and in early detection of resistance to rifampicin in our environment.

PATIENTS AND METHODS

Type and Location of Study

This is a prospective cross-sectional study that took place from July 10, 2013 to May 10, 2014 in Kinshasa for patients suspected of tuberculosis in order to compare the contribution of GEN-Xpert® to the culture. Sputum samples were collected at the Mother and Child Health Center in Ngaba and at Saint Alphonse Health Center in Matete, which are equipped with GEN-Xpert®.

Study Population

Only patients classified as TPM0 with at least 3 negative smears and with consent were included in the study.

Sample Collection and Analysis Sites

Two sputum specimens were collected on the same day from each patient at the Mother and Child Health Center in Ngaba (CMENgaba) and at the Saint Alphonse Health Center in Matete. A sample was analyzed on the site at the CSDTs by carrying out Ziehl-Neelsen and GEN-Xpert® and another was sent to the Mycobacteria Laboratory of the Faculty of Medicine of the University of Kinshasa for culture and antibiogram on Löwenstein-Jensens (LJ).

Laboratory Analysis

Ziehl-Neelsen, GEN-Xpert® and LJ culture were carried out according to WHO guidelines.[1,5-8] GEN-Xpert®MTB/RIF: For DNA detection of *M. tuberculosis* and the rifampicin resistance gene was done as follows: 1 ml of sputum was mixed with 2 ml of diluent and vigorously stirred for 10 to 20 times until complete liquefaction.[4,7] The mixture was incubated for 15 minutes at room temperature.[7] Using a sterile transfer pipette supplied with the kit, 2 ml of this mixture was collected and transferred into a cartridge.[7] The lid of the cartridge was closed tightly and cartridge inserted into the machine to start analysis. The machine was started within minutes of transferring the sample to the cartridge and left running until all parameters were displayed.[7] The patient's name, laboratory number, sample and sample type were entered into the machine. At the end, the results were displayed on the screen two hours later and recorded in a register as: MTB- / RIF-, MTB+ / RIF- or MTB+ / RIF undetermined or invalid and or MTB+ / RIF+.[7]

Culture and Susceptibility

The collected samples were placed in a refrigerated tank and carried to the Mycobacteria Laboratory of the Faculty of Medicine of the University of Kinshasa. These samples were treated with the N-acetyl-L-cysteine (NaLC) method.[8] After centrifugation, 0.2 ml of pellets were seeded on the LJ medium and both tubes were observed after 24 to 72 hours, for evidence of contamination.[8] Tubes were observed thereafter, once week to see the growth of cauliflower colonies and the culture was considered negative at the 8th week of incubation. The biochemical identification of the strains was performed using 2-thiophen carboxylic acid (2 µg / ml) and the Niacin test. After isolation and identification of the strain, the indirect method of the proportion technique of Canetti and al. was performed on LJ for the determination of the susceptibility of the strains.[8]

Reading took place after the 6th week of incubation. The following molecules and concentrations were used: Isoniazid (0.2 µg / ml); Rifampicin (40 µg / ml); Ethambutol (2µg / ml); Streptomycin (4 µg / ml). In addition, HIV serology was performed in TBSCs according to WHO and PATI IV guidelines to compare the detection rate of Mycobacterium tuberculosis between HIV + and HIV-.[9,10]

Statistical Analysis

Data recorded in the laboratory register were then transferred to the Excel software. They were analyzed using SPSS software version 20.0. This software allowed statistical processing of information. The mean and standard deviation were calculated for quantitative variables.

The results obtained with Gen-Xpert®MTB / RIF were compared with those obtained by the proportional LJ technique. Sensitivity, specificity, positive and negative predictive values and Kappa were calculated. The exact Fisher tests as well as the Pearson chi-square were used as needed. The significance level was set at 5% and the confidence interval (CI) at 95%.

Ethical Considerations

This study was approved by the Ethics Committee of the School of Public Health of the University of Kinshasa (N ° ESP / CE / 081/2010).

Table 1: Population Characteristics and Sex Diagnosis at CSDT (n = 347)

Sex	Number(%)	LJ+(%)	LJ-(%)	Cont*(%)	Xpert+(%)	Xpert-(%)	Invalid(%)
M	186(53.6)	56(16.1)	129(37.2)	1(0.3)	63(18.2)	121(34.9)	2(0.6)
F	161(46.4)	45(13)	114(32.9)	2(0.6)	51(14.7)	109(31.4)	1(0.3)
Total	347(100)	101(29.1)	241(69.5)	3(0.9)	114(32.9)	230(66.3)	3(0.9)

*Cont: Contamination

Table 2: Positivity according to serological HIV

Test	VIH+(n=52)%	VIH-(n=295)%	Total(n=347)%
LJ	20(19.8)	81(80.2)	101(29.1)
Gen-Xpert®	23(20.2)	91(79.8)	114(32.9)

RESULTS

Three hundred and forty-seven negative Ziehl samples from 347 patients, 52 of them were found HIV positive H. One hundred eighty nine (54.5%) were from CME Ngaba and 158 (45.5%) from CSDT St Alphonse. The average age of patients was 39 years old, with extremes ranging from 4 to 90 years. The age group 21 to 50 years old was the most affected (60%). There were more men (53.6%) than women (46.4%), but the difference was not significant (P = 0.19). The distribution of the characteristics of the study population and the diagnosis by sex is shown in Table 1.

Table 2 shows that the positivity rate was increased in both groups using Gen-Xpert®.

Table 1 shows that the positivity rate for GEN-Xpert®MTB / RIF is higher (32.9%) compared to the LJ culture (29.1%) and men are more concerned (16.1%). In total, one hundred and one *M. tuberculosis* strains were isolated from 347 negative Ziehl samples cultured on LJ. The absolute difference between Gen-Xpert® and the LJ culture was 3.8 (95% CI: 2.1-6.5) with a ratio of 1.1 (95% CI: 0.9-2.2). Comparison of the results of the tests used shows that Gen-Xpert® is 1.1 times better than the culture.

The sensitivity of Gen-Xpert® compared to the LJ culture was 93.1% (95% CI: 0.86-0.97) and specificity at 96.3% (95% CI: 0.93-0.98). Positive predictive value (PPV) and negative predictive value were respectively 91.3% 95% CI: 0.88-0.93) and 97.1% (95% CI: 0.93-0.99).

Table 2 shows that the positivity rate was increased in both groups using Gen-Xpert®. In HIV positive group, the positivity to culture was 19.8% (20) and 20.2% (23) in Gen-Xpert® (p = 0.55) with a Kappa coefficient of 0.008. In the HIV-negative group, the positivity rate was 80.2% (81) on culture and 79.8% (91) on Gen-Xpert® (p = 0.37) and the Kappa coefficient was 0.0073. The HIV-TB co-infection rate was 6.6% (23/347).

Resistance to Rifampicin in Gen-Xpert® was 9.9% and multiresistance by the Canetti proportion method was around 9.9%. Therefore, Gen-Xpert® agreed to 100% (10/10) with the detection of anti-tuberculosis resistance by the proportional method on LJ.

DISCUSSION

The present study, initiated to compare Gen-Xpert's in relation to culture on the solid medium in the diagnosis of smear-negative tuberculosis, shows that 101 samples (29.1%) were positive for LJ culture whereas 114 samples (32.9%) were positive for Gen-Xpert®. Of the 347 patient in this study, 52 were HIV-positive and the tuberculosis-HIV co-infection was 6.6%. Resistance to Gen-Xpert® and the proportional method was 9.9% with a multidrug resistance rate of 10%. The sensitivity of Gen-Xpert® to the culture was 93.1% (95% CI: 0.86-0.97) and specificity at 96.3% (95% CI: 0.93-0.98).

On the other hand, positive predictive value (PPV) and negative predictive value were respectively 91.3% (95% CI: 0.88-0.93) and 97.1% (95% CI: 0.93-0.99). As for the origin of the patients, a slight difference is observed which is not statistically significant (p = 0.11). The slightly higher proportion of males found in this study is similar to that found by some authors who believe that TB cases in women are insufficiently reported in developing countries.[11]

The study shows that the most represented age group was 21-50 years (60%). This corroborates numerous WHO statements that TB affects more active layer of population.[1-3] This explains the mobility of young people.[1]

The 29.1% of positive culture obtained in this study shows Ziehl-Neelsen's weakness in detecting tuberculosis bacilli. The positivity rate obtained in this study is higher than those of 18.5% and 15.9% respectively described by Barnard and al. in South Africa and Alaghbari and al. in Yemen.[12,13] On the other hand, our rate is lower than those 100% and 34.5% respectively reported by Kim and al. South Korea and Morse and al. in Botswana in cases classified as TPM0.[14,15]

Hepple and al. described also similar finding in various studies, comparing the performance of microscopy and culture in the diagnosis of tuberculosis from induced sputum.[16] The disparity in rates could be explained by the size of the samples, the methodology used and the immunosuppressive state of the patients. This survey confirms the literature's findings that failure to detect bacilli under microscopy can be palliated by culture and molecular testing (101 strains on LJ vs 114 Gen-Xpert® detection).[1-5,7-20] The culture is much more sensitive than microscopic examination and it improves the diagnosis of smear-negative pulmonary tuberculosis even though its use is limited in our low resource countries.[1,2]

The study showed also that the detection rate was higher in Gen-Xpert® MTB / RIF than in culture (101 strains on LJ vs 114 Gen-Xpert® detection). Sensitivity and specificity were 96.7% and 100%, respectively. The absolute difference between Xpert® and culture in our study was 3.8 (95% CI: 2.1-6.5) with a ratio in favor of Xpert® of 1.1. This result illustrates that Xpert® is 1.1 times more sensitive than culture for the detection of *M. tuberculosis*.

Previous studies reported a similar finding demonstrating the performance of Gen-Xpert® in the detection of M. tuberculosis directly in the pathological product. Mbonze and al. in DRC, reported a positivity rate of 14.3% in TPM0 patients using Xpert®.[6] It is the same for Barnard and al. in South Africa, using molecular tests (Xpert® and MTBDR plus) reported a positivity rate of 18.9% (52/248) while Kim and al. in South Korea reported a 100% positivity rate for 39 TPM0 samples.[12,14] Pimkina and al. in Lithuania, also reported a sensitivity of 82.5% for negative smear samples.[17] The difference in rates may be due to the immune status of the patients and the number of samples analyzed.

The present study revealed that of the 347 Ziehl negative patients (TPM0), 52 had positive HIV serology and the tuberculosis-HIV co-infection was 6.6%. On the other hand, Mbonze and al. reported a high rate of coinfection (14.4%) in their series.[6] The difference in rate may be due to the sample size (347/6920).

By comparing the detection rate with HIV status, the positivity rate was increased in both groups using Gen-Xpert®. In HIV positive group, the rate was 19.8% (20) on culture and 20.2% (23) at Gen-Xpert® (p = 0.55) with a Kappa coefficient of 0.008. In the HIV negative group, the positivity rate was 80.2% (81) on culture and 79.8% (91) on Gen-Xpert® (p = 0.37) with a Kappa coefficient of 0.0073. The HIV-TB co-infection rate was 6.6% (23/347).

This result shows that the difference was not significant (p = 0.55) and there was no concordance between culture and Gen-Xpert.

This association has been described by several authors in the world.[6,15,18] It is the case of Morse and al. in Botswana and Wilson and al. who reported a rate of 81.4% and 100% respectively.[15,18] The diversity of the numbers is probably related to the recruitment of patients and the difficulty of systematically performing HIV serology in all patients.

The sensitivity of Gen-Xpert® compared to the culture was 93.1% (95% CI: 0.86-0.97) and specificity at 96.3% (95% CI: 0.93-0.98) despite an analyzed smear. These values are higher than those reported by Boehme and al., Theron and al. and Pimkina and al., with sensitivity respectively of 72.5, 60 and 82.5% when considering a single smear.[17,19,20] They showed that sensitivity increased with the number of smears examined in patients with negative smear and positive culture.[17]

Our survey also shows that primary resistance was 38.9% with multiresistance around 9.9%. Sensitivity and specificity for detection of resistance to rifampicin was 100% in our series. On the other hand, a study by Boehme and al. shows that sensitivity

and specificity of the set of true negative for detection of resistance to rifampicin were 97.6 and 98.1%[19], respectively. Pimkina and al. reported a sensitivity and overall specificity for the detection of resistance to rifampicin, calculated in relation to the phenotypic results, of 100% and 98.2%, respectively.[17] These results show that the threat to efficient control of tuberculosis is a serious problem because multidrug-resistant strains (MDR-TB) circulate in the community. The culture contamination rate (0.9%) obtained in this study is less than that of 3% recommended by the WHO[1,4,8] including invalid results in Gen-Xpert®.

Although the study showed the contribution of Gen-Xpert® to the rapid detection of M. tuberculosis directly in the pathological product with rapid information on resistance to rifampicin, it has also a limit of having examined a sample after a series of 3 negative samples in Ziehl-Neelsen. According to literature, the probability of detecting bacilli depends on the quality and repetition of sampling as well as on samples transport to the laboratory.[8]

CONCLUSION

The study showed that culture remains a reference examination test because it makes possible to improve diagnosis of tuberculosis cases under negative microscopy. In contrast, Gen-Xpert® MTB / RIF is a powerful tool for its sensitivity and speed in the diagnosis of TPM0 and provides information on resistance to rifampicin after two hours for good monitoring.

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