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Original Research Article.

Performance of Antibiogram on Slide in the Diagnosis of Multiresistant Tuberculosis Persons Living with HIV / AIDS in Kinshasa

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is a real public health problem in the world. Its rapid and early diagnosis remains an important means of reducing its morbidity and mortality. Ziehl-Neelsen staining, which is common in tuberculosis detection and treatment centers, does not lead to the diagnosis of drug-resistant TB.

Objective: The objective of the study was to evaluate the performance of the slide antibiogram in the detection of resistance to rifampicin and to second-line molecules.

Methods: Prospective analysis of sputum smear from tuberculosis patients in therapeutic failure or relapse situations, comparing the performance of the antibiogram to slide technique against the gold standard, the method of proportion on medium Of LJ and the molecular method of Gen-Xpert® MTB / RIF in the detection of resistance to rifampicin.

Results: Resistance to rifampicin was observed in 66.3% of samples (53) using the slide antibiogram test, 67.5% (54) with Gen-Xpert® MTB / RIF and 61.3% (49) by the proportional method. The MDR-TB rate was 56.3%, 15% pre XDR-TB and 1.3% XDR-TB. The sensitivity and specificity of the antibiogram on slide was 100% and 87.1% with a positive predictive value of 92.5%. Gen-Xpert® MTB / RIF, however, was 100% and 83.9%, and the positive predictive value was 90.7%.

Conclusion: Slide antibiogram showed its performance against LJ and Gen-xpert in detecting resistance to rifampicin and / or second-line molecules (Ofloxacin and Kanamycin) after 10 days of incubation. Its popularization deserves consideration in resource-limited countries.

Key Words: Performance; Antibiogram on Slide; Multidrug-Resistant Tuberculosis; PVV; Kinshasa.

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INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is a serious threat to TB control programs.[1-3] This form is caused by bacilli resistant to at least the two most effective anti-tuberculosis drugs, isoniazid and rifampicin.[1-3]

According to World Health Organization (WHO) estimates in 2014, the number of MDR-TB worldwide was 480,000, of which 3.3% were among new cases and 20% were reprocessed. In this group of multidrug-resistant forms, 9.7% were highly resistant tuberculosis (XDR-TB), ie cases resistant to rifampicin and Isoniazid and at the same time to Fluoroquinolones and one of the aminoglycosides of reserve.[3]

HIV coinfection is a multiplier of mortality and morbidity in a population highly exposed to Mycobacterium tuberculosis (1-5). It is estimated at 12% in the world and 11% in the Democratic Republic of Congo (DRC).[3]

Drug-resistant tuberculosis (TB-PR) does not appear to be more frequent in HIV-infected patients than in non-infected patients. However, high mortality rates have been reported in HIV-positive patients with drug-resistant tuberculosis.[4,5]

The Democratic Republic of Congo (DRC) is one of the 27 countries with a heavy burden of multidrug-resistant tuberculosis.[3] In new cases, this rate was estimated to be 2.2% and 11% in reprocessing cases in 2014.[3] Surveys conducted in Kinshasa on primary resistance in 2007 and 2009 by Kabedi et al. Have shown that the DRC is affected by this drug resistance scourge with an MDR-TB rate around 5%.[6,7]

To address this problem, WHO adopted the "Dots Plus" strategy to prevent the progression and spread of the complicated form.[1,2]

The effective implementation of this approach, however, encounters several obstacles, notably the lack of availability in the most affected countries, of early diagnostic tools such as molecular techniques. The high cost of these new tools limits their systematic use in many tuberculosis control programs. This is justified by detecting only 123,000 MDR-TB cases out of the 480,000 expected in 2014.[3]

His situation, however, increases the risk of transmission of TB-PR, due to the lack of appropriate tools for diagnosis. This rapidly evolving drug resistance problem requires the development of innovative and effective detection alternatives to reduce morbidity and mortality. Therefore, the aim of this work is to evaluate the performance of the blade antibiogram technique in comparison with Gen-Xpert® MTB / RIF and the Löwenstein-Jensen proportion technique (LJ) in the detection of antituberculosis resistance in PVVIH in Kinshasa.

MATERIALS AND METHODS

Type and framework of study

The present study is prospective validity of the tests which took place from 08 March 2014 to 20 September 2015. The study was conducted in Elonga centers of Masina, mother and children of Ngaba and Saint Alphonse de Matete and University Clinics (CUK) in the city-province of Kinshasa (DRC). The first 3 centers were chosen because they have Gen-Xpert®.

Study Population

Any tuberculosis patient with therapeutic failure or relapse with informed oral consent was included in the study.

Sample Collection and Processing Sites

Sputum specimens were collected from the three above-mentioned centers which perform molecular analyzes by Gen-Xpert® and CUK. Sputum cultures and antibiograms were performed on the Lowenstein-Jensens (LJ) and 7H9 media in the Mycobacteria laboratory of the Faculty of Medicine. On the other hand, the detection of Mycobacterium tuberculosis (M.t) nucleic acids was carried out at the three tuberculosis screening and treatment centers (CSDTs) selected and designated above. Detection of the Mt DNA and the rifampicin resistance gene followed the protocol indicated by the manufacturer.[8] The mixture of 1 ml of sputum with 2 ml of diluent was then stirred vigorously 10 to 20 times until complete liquefaction was obtained. The product was then incubated for 15 minutes at room temperature.

Two milliliters of the mixture were aspirated using a sterile transfer pipette provided with the kit and transferred to a cartridge with a sealable lid. The machine started up within minutes of loading the cartridge until all parameters were displayed. The identification of the sample was thoroughly monitored, including the name of the subject, laboratory number, type and sample number. The results were displayed on the screen two hours later, and were logged.

They indicated MTB- / RIF-, or MTB + / RIF-, or MTB + / RIF indeterminate, or invalid or MTB + / RIF + according to the instructions of the firm.8 For Ziehl positive samples, the viability of Koch bacilli was confirmed by fluorescein Diacetate (FDA) staining according to the recommendation of the Antwerp Supra National Laboratory (IMT) before cultivation and susceptibility testing.[9,10]

The unfixed smears were stained with 0.025% (FDA) in a moist, dark chamber before incubation at 37 ° C for 30 minutes. They were then decolorized using 0.5% acid alcohol for 3 minutes and recolored using 0.5% potassium permanganate. The reading was carried out at objective 25X, making it possible to demonstrate the fluorescent bacilli which are colored green.[9-11]

The samples were then treated with N-acetylcysteine-sodium hydroxide (NALC-NaOH) for 15 minutes and neutralized with 45 ml of phosphate buffer before centrifugation at 3000 rpm for 20 minutes. A 0.5 ml inoculum was seeded in a liquid medium containing 0.5 ml of the OADC (Oleic acid, Albumin, Dextrose, Catalase), and 0.8 ml of Panta, while 0.2 ml was deposited on The slope of the middle LJ. The direct method of proportional technique of Canetti et al. was used for the antibiogram on LJ.[12] Isoniazid (0.2µg / ml), Rifampicin (40µg / ml), Dihydrostreptomycin (4µg / ml), Ethambutol (2µg / ml), Kanamycin (30µg / ml) and Ofloxacin (2µg / ml).[12]

After dilution, they were incorporated into the media (LJ). The seeding of 0.2 ml of inoculum was carried out respectively on the tubes containing the different molecules and on that containing exclusively the pure LJ medium.

On the other hand, the inoculum dilution 10-2 was seeded only on the tube containing the pure LJ medium.[12] Reading took place on the 28th day of incubation and took into account growth on the control tube with dilution 10 -2. For the antibiogram on slide, the following molecules and concentrations were used: Rifampicin (R1 = 0.5 µg / ml and R2 = 1.0 µg / ml); Para-nitrobenzoic acid (PNB = 500 µg / ml) and Nicotinamide (500 µg / ml), Were used for the identification of Mycobacterium tuberculosis.[9] The smears of the positive Ziehl and FDA samples were carried out on 10 half-slides containing sterile objects under the microbiological hood and dried for one hour.[9,10] At that time, two smears were inserted into two small vials containing the OADC (Oleic acid, Albumin, Dextrose, Catalase) and 7H9 media and the rest of the smears were introduced into the small vials corresponding to the antibiotic used.[9,10,13]

Each antibiotic was packaged in two vials at different concentrations (low and high). The flasks were incubated at 37°C and observed each day for signs of contamination. On the 10th day, they were placed in the oven at 90°C. For 45 minutes to inactivate the germs. The flasks were then opened after inactivation under the microbiological hood in order to remove the half-slides containing the smears in the flasks before drying them. The half-slide loaded with the smears were fixed and colored at the Ziehl-Neelsen.[9,10,13]

The reading was carried out at the objective 10X to demonstrate the micocolonies according to their growth on each plate and taking into account the growth on the two control plates. The rating was 1+, 2+, 3+ and 4+ for each concentration used.[9,10,13]

Operational definitions[14]

PreXDR: TB-MR strains resistant to either Kanamycin or Ofloxacin.

HIV serology

Furthermore, HIV serology was performed, using the test to Dettermin® and Unigold, in all tuberculous patients, after informed consent in accordance with the recommendations of PNLT (PATI V) and WHO.[3-5,14]

Ethical Considerations

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

Statistical Analysis

The data recorded in the laboratory register were then transferred to the Excel software. They were analyzed using SPSS software version 20.0.This software made it possible to statistically process the information. The mean and standard deviation were calculated for the quantitative variables. The results of the antibiogram on slide and Gen-Xpert® MTB / RIF were compared to those obtained by the proportional method on LJ and the molecular method of Gen-Xpert®MTB / RIF. Sensitivity, specificity, positive and negative predictive values, and Kappa and likelihood were calculated. The exact Ficher test 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%.

RESULTS

Eighty-two positive Ziehl sputum specimens from 390 tuberculosis patients registered between March 2014 and September 2015 in the above-mentioned CSDTs. All of these patients had positive HIV serology. The samples were from 20 patients in category I, 50 from category II and 12 from category IV, according to the classification in PATI V.[14] After analysis, 2 cultures were eliminated for contamination.

Thirty-six samples (45%) were from CSDT St Alphonse, 32 (40%) from CSDT Elonga, 10 (12.5%) from the Mother and Children Center in Ngaba and 2 (2.5%) from the CUK. The mean age of patients was 36, 50 ± 14.1 years with extremes ranging from 12 to 66 years and the age group 22 to 51 years was the most affected (73.8%).

There were more men than women (63.8% vs 36.2%, p = 0.000). Eight strains of Mycobacterium tuberculosis were isolated on LJ and 61.3% (n = 49) were resistant to rifampicin. Of these resistant strains, 56.3% (n = 45) were both resistant to rifampicin and Isoniazid (MDR-TB). At Gen-Xpert® MTB / RIF, resistance to rifampicin (RR) was 67.5% (n = 54) and the antibiogram on the slide detected 66.3% (n = 53). Kanamycin and Ofloxacin were 13% and 15%, respectively.

Of the 56.3% of MDR-TB strains, 15% were preXDR-TB (12) and 1.3% XDR-TB (1). The distribution of MDR-TB by category shows that 11.1% (n = 5) were in category I, 62.2% (n = 28) in category II and 26.7% (n = Category IV. On the other hand, the distribution of pre-XDR-TB by category shows that 16.7% (n = 2) were in category II and 83.3% (n = 10) in category IV. The 1.3% (n = 1) of XDR-TB was in Category IV.

Table 1 illustrates near-equivalent sensitivities and specificities between the Antibiogram on slide and Gen-Xpert® (100% and 100% vs 87.10% and 83.87%).

Unlike the LJ results on the 28th and 42th days respectively for 55 and 25 strains, that of the slide antibiogram data was performed on the 10th day of incubation. Reading on Gen-Xpert® was possible after 2 hours of time.

Table 1: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Global Value, Kappa and Likelihood Ratio of Different Tests Used

TESTS	Sensibility % (IC95%)	Specificity % (IC95%)	VPP % (IC95%)	VPN % (IC95%)	Kappa%	L+
ATBlame	100 (92,75-100)	87,10 (70,17-96,37)	92,45 (81,79-97,90)	100 (87,23-100)	0,89	7,75
GEN-Xpert®	100 (97,50 -100)	83,87 (66,27-94,55)	90,74 (79,70 -96,92)	100 (86,77 -100)	0,86	7,69

ATBlame: Antibiogram on slide; VPP: positive predictive value; VPN: negative predictive value; GL: Total value; L: Positive likelihood value.

DISCUSSION

The main objective of this study was to evaluate the performance of slide antibiogram in the determination of anti-tuberculosis sensitivity as compared to LJ culture data used as a gold standard and molecular data in PVVIH. It essentially showed a co-infection rate of 21% (82/390) and a high resistance to rifampicin for the three tests (66.3% on the blade, 67.5% on Gen-Xpert® and 61.3% on LJ). Second line anti-tuberculosis drug resistance was 15% on ATBlame vs 13% on LJ for ofloxacin, 13% on ATBlame and 13.5% on LJ for kanamycin. The sensitivity of the antibiogram on the slide was 100% (95% CI: 92.75-100) and specificity at 87.10% (95% CI: 70.17 -96.37) with a PPV and VPN of 92, 45 (81.79-97.90) and 100 (87.23-100). The Kappa value was 0.89.

The unequal distribution of the samples considered between the different centers is likely to induce a selection bias; It is nevertheless attributable to the conditions of accessibility and attendance of different centers as well as the lack of molecular techniques at the CUK.

The survey shows that the age group 22 to 51 years was the most concerned (73.8%). This age group is that of the working population and is subject to greater mobility.[3]

The vulnerability of the economically most active layer is reported in many WHO reports, which show that TB is a social illness that often reflects the degradation of the living conditions of the population.[1-3]

The preponderance of the male sex observed in this study (63.8% vs. 32.2%, p = 0.002) agrees with some authors who argue that women tend to consult less frequently than men in our African settings, Because of certain socio-cultural barriers.[3,15,16]

The 21% co-infection rate reported in this survey is slightly higher than the 19% found by Kabedi et al. In a study on the evaluation of the Fluorescein Diacetate staining technique in the bacteriological follow-up of tuberculosis patients in Kinshasa.[11] However, Lora et al. in Bolivia reported a much higher rate (45%) than ours in a study on the evaluation of the MODS technique versus the LJ culture in PVVIH.[17] The difference in proportions

could be explained by the size of the sample, the degree of immunosuppression of the patients and the prevalence of the disease.

The relatively high levels of MDR-TB strains in the 80 examined on LJ (56.3%), preXDR-TB (15%) and XDR-TB (1.3%) are of concern. They suggest a significant risk of circulating resistant bacilli in the community and represent a permanent threat to the TB control program in our environment by spreading these dangerous strains. The rate of 56.3% of the MDR-TB strains in this survey is lower than the 82.3% (94/109) reported by Noor et al.[13] They evaluated the slide antibiogram compared to the method of proportions on LJ in Banglandesh. On the other hand, our rate is higher than the 47% obtained from a retrospective cohort study in Belarus by Rusovich et al.[18]

These authors used national data and compared Gen-Xpert® MTB / RIF to the culture. Hoang et al. found a much lower rate (10.5%; 340/3224 strains) in patients suspected of MDR-TB in Vietnam.[19] Lora et al. in Bolivia also reported a low rate of 9% (4/48) in a study on the evaluation of the MODS technique compared to the LJ proportional technique in PVVIH patients.[17] Differences between these reports may be due to different methodologies and objectives in this work, but also to the non-uniform size of the samples analyzed. The 1.3% XDR-TB and 15% preXDR-TB show a significant threat that could reverse the current progress in TB control.

The distribution of MDR-TB strains by category shows that 26.7% of strains belong to category IV and 62.2% to category II. On the other hand, 83.3% of preXDR-TB strains were in category IV and 16.7% in category II. 1.3% XDR-TB in Category IV. This finding is consistent with data from the literature showing high risk of resistant strains in Category II and IV.[1-3]

With a sensitivity of 100% (95% CI: 92.75-100) and a specificity of 87.10% (95% CI: 70.17 -96.37), the antibiogram on slide in this study proved to be effective Compared with the data reported in India by Yadav et al.[20] Indeed, these authors evaluated the performance of the molecular test MTBDR plus compared to the method of proportion of Canetti et al. In patients suspected of MDR-TB. The transverse survey found a sensitivity and specificity of 97 and 100%, respectively.[20] This performance of the slide antibiogram in this work is also supported by the calculated Kappa value, which is 0.89% with a likelihood ratio of 7.75.

The choice of Gen-Xpert® and the proportional technique on LJ (Gold Standard) for the validation of the antibiogram on a slide is based on previously reported evidence.[3,9,17-21] In our study, the sensitivity and specificity of Gen-Xpert® to detect resistance to rifampicin were 100% (95% CI: 97.50 -100) and 83.87% (95% CI: 66.27- 94.55); With no significant difference compared to the LJ method, with a Kappa value of 0.89 and a likelihood value of 7.75.

WHO reports from studies in suspected MDR-TB patients in South Africa, Azerbaijan, India and Peru showed non-inferiority of Gen-Xpert® compared to LJ in detecting Resistance to rifampicin (Se = 97.6% and Sp = 98.1%).[21] The same applies to a survey in Lithuania by Pimkina et al. in 2015 who described a sensitivity of 100% and a specificity of 98.2% in a multicentre study.[22]

Regarding the waiting time for the result, Gen-Xpert® is very advantageous, reading is possible within two hours, whereas the antibiogram on the blade requires 10 days; But less than the 28 to 42 days required for results on LJ medium using the direct proportional technique.

It is true that the antibiogram on slide is feasible on sputum and allowed after a shorter time (10 days) than the culture on LJ to detect Rifampicin resistance in 56.3% of the strains, Kanamycin in 13% And Ofloxacin in 15%.

It is important, however, to take into account certain limitations in the interpretation of the results presented. The first limitation is related to the inability of the antibiogram on slide to determine the sensitivity to Isoniazid, a first-line molecule and also used in the management of patients. Secondly, the slide antibiotic is only feasible on Ziehl-positive specimens, which limits the spectrum of diagnosis by excluding smear-negative tuberculosis.

The last limitation is that the analyzes involved only 3 tuberculosis screening centers and the conclusions can not reflect the reality at Community level. The survey is nevertheless strengthened by its prospective nature and the power of the results is supported by the quality of the statistical tests which have made it possible to determine the performance of the technique.

CONCLUSION

The present study showed good performance of the slide antibiogram in the determination of resistance to Rifampicin and second-line antituberculosis drugs. This easy and inexpensive technique deserves to be popularized with the aim of accompanying tuberculosis control programs.

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