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Original Research Article

Performance of Antibiogram on Slide in the Diagnosis of Multiresistant Tuberculosis in Kinshasa

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ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is a real public health threat globally. Its early diagnosis remains an important means of reducing morbidity and mortality. The Ziehl-Neelsen staining, commonly used in tuberculosis detection and treatment centers, cannot be used to diagnose MDR-TB.

Objective: The objective of this study was to evaluate the performance of antibiogram slide 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 slide technique with respect to the gold standard the proportion method on LJ medium and molecular method of Gen-Xpert® MTB / RIF in the detection of resistance to antituberculosis drugs.

Results: After analysis, resistance to Rifampicin was observed in 47.3% of samples (142) using slide antibiogram test, 47% (141) with Gen-Xpert® MTB / RIF and 46.3 % (139) by the proportional method. The rate of MDR-TB was 39% and 9% pre XDR-TB. The sensitivity and specificity of the antibiogram slide was 97.9 and 100% with a positive predictive value of 100%. In contrast to Gen-Xpert® MTB / RIF, it was 97.87% and 99.83% and the positive predictive value was 99.28%.

Conclusion: Antibiogram slide showed non-inferiority to LJ and Gen-Xpert® in detecting resistance to Rifampicin and / or second-line molecules (Ofloxacin and Kanamycin) after 10 days incubation. Its dissemination with a view of its validation deserves to be considered in a low-cost area.

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

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INTRODUCTION

The emergence of multidrug-resistant tuberculosis (MDR-TB) has become a major public health problem in several countries and a barrier to effective tuberculosis control worldwide.[1-4]

Nearly half a million MDR-TB cases are reported yearly due to insufficient investment in basic tuberculosis control activities, poor management of reserves and quality of TB medicines, inadequate treatment of tuberculosis patients.[1-4] This form is caused by bacilli resistant to at least the two most effective anti-tuberculosis drugs, Isoniazid and Rifampicin.[1-4]

According to World Health Organization (WHO) estimation in 2014, the number of MDR-TB in the world was 480000, of which 3.3% in new cases and 20% in reprocessing cases. In this group of multiresistant forms, 9.7% were ultra-resistant tuberculosis (XDR-TB), the cases resistant to Rifampicin and Isoniazid and at the same time to Fluoroquinolone and to one of the reserve aminoglycoside.[1]

HIV co-infection is a multiplier factor of mortality and morbidity in a population highly exposed to Mycobacterium tuberculosis.[1-4]

The Democratic Republic of Congo (DRC) is one of the 27 countries with a high rate of multidrug-resistant tuberculosis. In new cases, this rate was estimated to be 2.2% and 11% in reprocessing cases in 2014.[2] Two surveys conducted in Kinshasa on primary resistance in 2007 and 2009 by Kabedi and al. Showed resistance rates of 42.5% and 42.2%, respectively, with multiresistance around 5.3% for the first and 5% for the second study.[5,6]

To better understand this problem of TB drug resistance and MDR-TB, WHO had put in place the "Dots Plus" strategy to prevent the progression and spread of this new, highly lethal form.[1-4] The effective implementation of this approach nevertheless encounters several pitfalls, notably the unavailability in most affected countries of means of early diagnosis such as molecular techniques. The cost of these new tools limits their dissemination in many tuberculosis control programs.

This situation increases the risk of transmission of MDR-TB, due to the lack of interruption of the chain because of the delay in diagnosis.

The increasing rates of MDR-TB in the world require the development of innovative and effective alternatives in the detection, management and treatment of this form of tuberculosis with very high mortality.

Hence the interest of this work which exploits the antibiogram test on blade used in Bangladesh and at the Institute of Tropical Medicine (IMT), Antwerp with a more than satisfactory performance (Se = 96% and Sp = 98 %).[7,8]

The objective of this study is to evaluate the performance of the blade antibiogram technique against Gen-Xpert® MTB / RIF and culture on Löwenstein-Jensen medium in the detection of resistance to antituberculosis drugs.

MATERIAL AND METHODS

Type and Framework of Study

This prospective study of determination of non-inferiority, which took place from March 08 2014 to July 30 2015, was carried out at the following health centers: Elonga in Masina, Mother and Children in Ngaba, Saint Alphonse in Matete and at the University of Kinshasa Clinics (CUK) in Kinshasa (DR Congo). The three first health centers selected are equipped with Gen-Xpert® molecular analysis equipment.

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 samples were collected from the above-mentioned centers that perform molecular analyzes using Gen-Xpert®. Sputum cultures and antibiograms were performed on the Lowentein-Jensens (LJ) and 7H9 at the Mycobacteria laboratory of the Faculty of Medicine of the University of Kinshasa.

On the other hand, detection of Mycobacterium tuberculosis (M.t) nucleic acids was carried out at the three tuberculosis screening and treatment health centers (CSDT). The detection of the DNA of the M.t and of Rifampicin resistance gene was done as indicated by the manufacturer protocol.[9] A mixture of 1 ml of sputum with 2 ml of diluents was stirred vigorously 10 to 20 times until complete liquefaction. The product was then incubated for 15 minutes at room temperature.

Two milliliters of the mixture were taken up using a sterile transfer pipette provided with the kit and transferred to a cartridge with a sealable lid. The machine started up within minutes after 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. Results displayed on the screen two hours later, were logged. They were displayed according to manufacturer as: MTB- / RIF-, or MTB + / RIF or MTB + / RIF indeterminate or invalid or MTB + / RIF +.[9]

For positive Ziehl 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 carrying out culture and antibiograms.[8] The unfixed smears were stained with 0.025% (FDA) in a moist, dark room before incubation at 37° C for 30 minutes. They were then discolored with 0.5% acid alcohol for 3 minutes and restrained using 0.5% potassium permanganate.[8] Reading carried out with 25X objective, made it possible to show color green fluorescent bacilli.[7,8,10-12] Samples were then treated with N-acetylcysteine-sodium hydroxide (NALC-NaOH) for 15 minutes and then neutralized with 45 ml of phosphate buffer before centrifugation at 3000 rpm for 20 minutes. A 0.5 ml inoculum was sowed in a liquid medium containing 0.5 ml of the OADC and 0.8 ml of Panta while 0.2 ml was placed on the LJ medium slope.

The direct technique of the proportion method of Canetti et al. was used for the antibiogram on LJ.[13] Different molecules used were at the following concentration: Isoniazid (0.2µg/ml), Rifampicin (40µg/ml), Dihydrostreptomycin (4µg/ml), Ethambutol (2µg/ml), Kanamycin (30µg/ml) Ml) and Ofloxacin (2 µg/ml).[13] After dilution, they were incorporated into the media (LJ). Sowing of 0.2 ml of inoculum was carried out respectively on the tubes containing different molecules and on that containing exclusively pure LJ medium. On the other hand, inoculums dilution of 10^{-2} was sowed only on the tube containing the pure LJ medium.[13] Reading was done on the 28th day of incubation and took into account the growth on the control tube with a 10^{-2} dilution. For slide antibiogram, the following molecules and concentrations were used: Rifampicin (R1 = 0.5 µg/ml and R2 = 1.0 µg/ml); Ofloxacin (O1 = 2.0 µg/ml and 8.0 µg/ml) and Kanamycin (5 µg/ml and 2.0 µg/ml). Para nitrobenzoic acid (PNB = 500 µg/ml) and Nicotinamide (500 µg/ml) were used for the identification of Mycobacterium tuberculosis.[7, 8,10,11]

The smears of positive Ziehl and FDA samples were made on 10 half-slide sterile objects under microbiological hood and dried for one hour. Then two smears were inserted in two small vials containing OADC and 7H9 media and the remaining smears were introduced into small vials corresponding to the antibiotic used.[7,8,10,11]

Each antibiotic was packaged in two vials at different concentrations (low and high). The flasks were incubated at 37° C and observed daily for signs of contamination. On the 10th day, they were placed in the oven at 90°C for 45 minutes to inactivate the germs.[7,8,10,11] The vials were then opened after inactivation under a microbiological hood in order to remove the half-slides containing the smears in the flasks before drying them. The half-slides loaded with the smears were fixed and stained with Ziehl-Neelsen.[7,8,10,11]

Reading was carried out with 10X objective to show the microcolonies according to their growth on each plate while taking into account growth on both control blades. The rating was expressed as: 1+, 2+, 3+ and 4+ for each concentration used.[7,8,10,11]

Operational definitions[14]

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

Primary Resistance: Resistance that occurs in subjects who have never received antituberculosis treatment or received it for less than 30 days.

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).

Statistical analysis

Data recorded in the laboratory register were transferred to the Excel software then analyzed using SPSS software version 20.0.This software made possible to statistically process information's. The mean and standard deviation were calculated for quantitative variables. The results of the antibiogram on slide and Gen-Xpert®MTB/RIF were compared to those obtained by proportional method on LJ. The proportion method on Löwenstein-Jensen medium was used as the reference test. Sensitivity, specificity, positive and negative predictive values and Kappa were calculated. The exact Ficher 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%.

RESULTS

Three hundred and ten positive Ziehl sputum specimens from 310 tuberculosis patients were recorded between March 2014 and July 2015 in the aforementioned CSDT. These samples were from 202 patients in category I, 103 from category II and 5 from category IV, according to the PATI IV classification.[14] After analysis, 7 cultures were eliminated for contamination, and 3 antibiograms for disability.

One hundred and thirty-three samples (43.3%) came from CSDT St Alphonse, 110 (36.7%) from CSDT Elonga, 51 (17%) from the Ngaba Mother and Children Center and 6 (2%) from the CUK. The mean age of patients was 36, 40 ± 14.8 years with extremes ranging from 7 to 83 years. The age group 18 to 50 was the most affected (77.7%). They were more male than female (60.7 % vs 39.3 %, p=0.002).

Three hundred strains of Mycobacterium tuberculosis were isolated on LJ, and 46.3% (n = 139) were resistant to Rifampicin. Among the resistant strains, 39% (n = 117) were both resistant to Rifampicin and Isoniazid (MDR-TB). At Gen-Xpert® MTB / RIF, resistance to Rifampicin (RR) was 47% (n = 141) and the slide antibiogram detected 47.3% (n = 142). Mono resistance to Kanamycin and Ofloxacin was 16.67% and 15.67%, respectively.

Of the 39% MDR-TB strains, 9% were preXDR-TB. The distribution of MDR-TB by category shows that 70.1% (n = 82) were in category I, 25.6% (n = 30) in category II and 4.3% (n =5) in category IV. However, the distribution of PreXDR-TB by category shows that 66.7% of PreXDR (n = 18) were in Category I, 18.5% (n = 5) in Category IV and 14.8 %(n= 4) in category II.

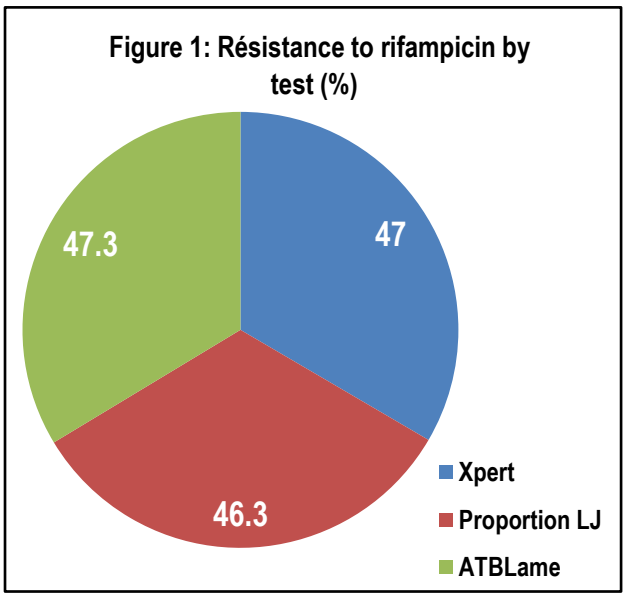
Table 1 illustrates near-equivalent sensitivities and specificities between the slide antibiotic and Gen-Xpert® (97. 99% and 100% vs 97. 87% and 99.83%).

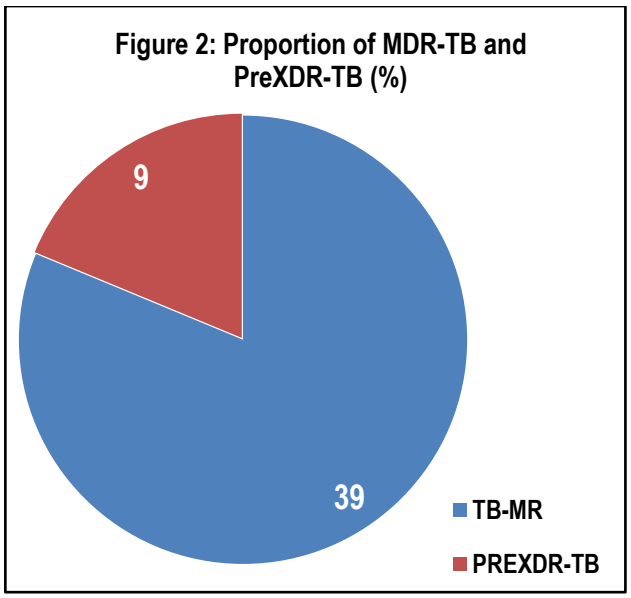
Contrary to LJ medium reading results carried out on the 28th and 42nd days respectively for 197 and 103 strains, those of the slide antibiogram were done on the 10th day of incubation, while Gen-Xpert® reading is possible after 2 hours.

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%)	VG% (IC95%)	Kappa%	L
ATBlame	97.99 (93.95-99.56)	100 (99.39-100)	100 (97-38-100)	99.5 (98.55-99.90)	99.6 (98.82 -99.91)	98.68	∞
GEN-Xpert®	97.87 (93.90 -99.56)	99.83 (99.1-99.9)	99.28 (96.1 -99.98)	99.5 (98.55 -99.90)	99.46 (98.62-99.85)	98.24	0.17

ATBlame: Antibiogram on slide; VPP: positive predictive value;VPN: negative predictive value; GL: Total value; L: Likelihood value





DISCUSSION

The main objective of this study was to evaluate performance of the slide antibiogram in the determination of anti-tuberculosis sensitivity in relation to LJ culture data and molecular data. The proportion method on Löwenstein-Jensen medium was used as the reference test. It shows essentially a strong resistance to Rifampicin but almost equivalent for the three tests (47.3% on blade, 47% Gen-Xpert and 46.3% on LJ). Resistance to second-line anti-tuberculosis drugs was (16.67% vs. 15.3% on LJ) for Ofloxacin, 15.67% on the slide and 14.5% on LJ for Kanamycin.

The sensibility of slide antibiogram was 97.99% (95% CI: 93.95-99.56) with specificity at 100% (95% CI: 99.39 -100), a VPP and VPN of 100% (97.38-100) and 99.5% (98.55-99.90) respectively. The Kappa value was 98.68.

Concerning the characteristics of the data analyzed, 202 samples were from category 1 patients, reflecting a higher risk of primary resistance. The unequal number of samples between the different centers could likely induce a selection bias; it is nonetheless due to the conditions of accessibility and attendance of different centers as well as the lack of molecular techniques at the CUK.

The study shows that the age group 18 to 50 years old was the most concerned (77.7%). This age group of active population is subject to greater mobility.[1,2]

The vulnerability of the economically most active layer is a fact reported in numerous WHO statements, that tuberculosis has a definite social impact and is related to poverty.[1-4]

The preponderance of male sex observed in this survey (60.7% vs39.3%, p = 0.002) is in line with some authors who argue that women tend to consult less frequently than men in our African society because of to some socio-cultural barriers.[15,16]

The relatively high rate of MDR-TB strains on the 300 examinee on LJ (39%) and that of preXDR-TB (9%) are of concern, suggesting a significant risk of circulating resistant bacilli in the community. They represent a permanent threat to the tuberculosis control program in our environment by the spread of these dangerous strains. The rate of 39% of MDR-TB strains found in the study is, however, less than the 47% from a retrospective cohort study in Belarus by Rusovich and al.[17] These authors used national data and compared Gen-Xpert® MTB / RIF to the culture. Hoang and al., found a much lower rate (10.5%, 340/3224 strains) in MDR-TB patients in Vietnam.[18] The disparities between these reports may be due to different methodologies and objectives of each study, but also to the non-uniform size of the samples analyzed. The distribution of MDR-TB strains by category shows that the largest number (¾ of the strains) belong to category I. This reflects the expected trend in most tuberculosis control programs where category 1 is the most frequent form encountered at the time of diagnosis. It is important to note, however, that literature reports a higher risk of MDR-TB in case of reprocessing, thus in category 2 patients.[1-4] A possible selection bias could explain this observation. With a sensitivity of 97.99% (95% CI: 93.95-99.56) and a specificity of 100% (95% CI: 99.39-100), slide antibiogram in this study was found at less no inferior to data reported in India by Yadav and al.[19]

Indeed, these authors evaluated the performance of the molecular test MTBDR plus in relation to Canetti and al., method of proportion in patients suspected of MDR-TB. The transverse survey found a sensitivity and specificity of 97 and 100%, respectively.[19] This performance of the slide antibiogram in this work is also supported by the calculated Kappa value, which is 98.68% with a likelihood ratio that was infinite.

The choice of Gen-Xpert® and culture on LJ as comparison media for the validation of slide antibiogram is based on previously reported evidence.[1-4,7-10] In our study, the sensitivity and specificity of Gen-Xpert® to detect resistance to rifampicin were 97.87 (95% CI: 93.90 -99.56) and 99.83% (95% CI: 99.1-99.9); with no significant difference compared to the LJ method, with a Kappa value of 98.24 and a likelihood value of 0.17.

WHO reports through studies in suspected MDR-TB patients in South Africa, Azerbaijan, India and Peru[20] have shown non-inferiority of Gen-Xpert® to LJ in the detection of resistance to Rifampicin (Se = 97.6% and Sp = 98.1%). Similar reports for a survey in Lithuania by Pimkina and al. in 2015 which described a sensitivity of 100% and a specificity of 98.2% in a multicentre study.[21] In relation to waiting time for the result, Gen- Xpert® is very advantageous because reading is possible within two hours, whereas slide antibiogram on the plate requires 10 days which is less than the 28 to 42 days required for LJ results using the direct method. Indeed, the antibiogram on slide is feasible on sputum and allowed after a shorter time period than a culture on LJ to detect resistance to Rifampicin in 47% of strains, 15.67% for Kanamycin and 16.67 % for Ofloxacin.

However, it is important to take into account certain limitations in the interpretation of the results obtained. The first limit is related to the inability of the antibiogram on slide to determine sensitivity to Isoniazid, a first-line molecule also used in treatment. Secondly, the antibiogram on slide is only feasible on Ziehl-positive specimens, which limits the diagnosis spectrum by excluding smear-negative tuberculosis.

The last limitation is that analysis concerned only 4 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 strength of the results is supported by the quality of the statistical tests which made it possible to determine the non-inferiority of the technique. The protocol presented could serve as a basis for further analysis on greater sampling for robust validation.

CONCLUSION

The present study showed the 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 disseminated, with the aim of accompanying tuberculosis control programs in the reduction of mortality due to resistant forms.

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