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Analysis of Discordance between Genotypic and Phenotypic Assays for Rifampicin Resistant *Mycobacterium tuberculosis* Isolated from Healthcare Facilities in Mthatha

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Abstract: The study sought to determine the rate of discordant results between genotypic and phenotypic tests for the diagnosis of drug resistant tuberculosis (DR-TB). Sputum samples and cultured isolates from suspected DR-TB patients were respectively analysed for *Mycobacterium tuberculosis* by Xpert® MTB/RIF (Cepheid, USA) and Line Probe Assays (LPA) (Hain, Germany). Discrepant Rifampicin (RMP) resistant results were confirmed using BACTEC MGIT960 (BD, USA). Of 224 RMP-resistant by Xpert MTB/RIF, 5.4% were susceptible to RMP by LPA. MGIT960 showed 75% agreement with LPA. Discrepancy was attributed to either heteroresistance or DNA contamination during LPA testing in 58.3% of cases. In 25% of samples showing agreement in RMP resistance between Xpert MTB/RIF and MGIT960, discrepancy was attributed to laboratory errors causing false RMP susceptible results with LPA. Of 16.7% of cases, discrepancy was attributed to false RMP susceptible results with Xpert MTB/RIF. Of 224 isolates, susceptibility to isoniazid (INH) by LPA was performed in 73.7% RMP resistant isolates, of which, 80.6% were resistant. All RMP resistant isolates by Xpert MTB/RIF were confirmed in 98.5% by LPA if TB isolates were resistant to INH, but only confirmed in 81.3% if TB isolates were INH susceptible ($\varrho < 0.001$). In conclusion, Laboratory errors should be considered when investigating discordant results.

Keywords: Xpert MTB/RIF; Line-Probe Assay; MGIT 960 system; Mycobacterium tuberculosis; discrepant results

1. Introduction

Tuberculosis (TB) remains an important and one of the largest infectious diseases worldwide. According to the World Health Organization (WHO), there were an estimated of 10.6 million TB cases in 2021, among which 6.7% were reported among people living with human immunodeficiency virus (HIV) [1]. Majority of TB cases have been reported from the region of South-East Asia (45%), followed by the Africa region (23%), and the Western Pacific region (18%) in the third position [1]. During that same year, the total number of death reported from the WHO was 1.6 million; among which, 1.4 million deaths were reported among HIV non infected individuals and 187 000 deaths were reported among people living with HIV infection [1]. With the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant (XDR) strains, as well as the increased rate of direct transmission, TB has now become an even bigger threat. As per definition, MDR-TB refer to resistance to isoniazid and rifampicin, with or without resistance to other first-line drugs. However, XDR-TB is defined as resistant to at least isoniazid and rifampicin, as well as to any of the fluoroquinolone and any of the three second-line injectable drugs (amikacin, capreomycin, and kanamycin). The burden caused by MDR-TB is considerable; in 2021, the incidence of MDR rifampicin resistant TB (RR-TB) was at 450 000 cases worldwide and was associated to 191 000 deaths [1]. In south Africa (SA), TB disease remain among the leading cause of natural death caused by a single organism [2]. Up to 360000 individuals were reported to have had developed TB in 2019 in SA,

among which majority, or more than half of them (58%) were living with HIV infection and 17% of them was reported dead from the disease [3]. In 2015, MDR-TB in South Africa was at 2.08% from the global estimations [4].

Rapid and accurate laboratory diagnosis of drug resistant (DR) strains is vital for proper treatment and management, which might also be one of the most active approach to decrease transmission of DR-TB (MDR/XDR-TB). Phenotypic drug susceptibility testing (DST) of *M. tuberculosis* remain considered as the "gold standard" test, the use of BACTEC MGIT 960 is operational in many laboratories and considered as, a rapid liquid phenotypic DST method, but unfortunately requires a long period of time (4–6 weeks) to report results. The World Health Organization recommends the uses of rapid molecular tests Xpert® MTB/RIF assay (Cepheid, USA) and, WHO endorsed LPA "line probe assays" (Hain Lifescience, Nehren, Germany) for rapid screening of XDR-TB in MDR-TB patients [5]. Both tests are based on Polymerase chain reaction (PCR) amplification, followed by detection of mutations in its Resistance-Determining Region (RDR). In fact, the assay detects mutations in different genes: *rpo*B gene is detected for RIF resistance, *kat*G gene for high-level INH resistance, and *inh*A for low-level INH resistance [6]. Nevertheless, in few cases, Xpert MTB/RIF, LPA and phenotypic tests may show conflicting INH and RIF-susceptibility results; highly discordant results therefore have been reported for *M. tuberculosis* isolates, carrying specific resistance conferring mutations for some first-line drugs [7]. Discrepant results among XpertMTB/Rif and LPA usually occurs when GeneXpert report Rif resistant and LPA report Rif susceptible; Some factors which may be consider as responsible for discrepancy results include: bacterial population (repeated sub-culturing may lead to losing the slow-growing resistant population and false susceptible result), hetero-resistance, different growth kinetics, cross-contamination, Mixed infections, growth difficulties of some strains, and minimum inhibitory concentration (MIC) of some isolates which is closer to the critical concentration [8]. It was reported that mixed infections might also be an important mechanism underlying the change in drug susceptibility patterns through the presence or absence of antibiotic pressure, which determined the dominant growth of strains of mixed infections [9].

Phenotypic assays (MGIT 960) and genotypic assays (LPA) can provide data on all first-line and second-line drugs, Xpert MTB/RIF detect resistance to rifampicin only [10]. Resistance to rifampicin is a key determinant in treatment failure, and generally correlates well with MDR-TB as ~85% rifampicin-resistant clinical *M. tuberculosis* isolates worldwide are also additionally resistant to isoniazid [10].

Drug resistant TB (DR-TB) has emerged in South Africa by the 1980s, but was not thought to be a major problem [11]. The Eastern Cape Province has the 3rd largest population with close to 7 million people, is the 2nd poorest province in South Africa and has the 2nd lowest rate (10.2%) of individuals with access to a medical cover in the country [12]. In 2010, The Eastern Cape was the second worst affected province with drug resistant TB after KwaZulu-Natal with more than 10% of strains having resistance to at least one drug [12]. Therefore, the present study was conducted to investigate discrepancies of clinical *M. tuberculosis* isolates, using phenotypic and molecular methods in patients attending Gateway clinic in Mthatha, South Africa.

2. Materials and Methods

All patients were enrolled following an informed consent; and in order to protect privacy and confidentiality of the patients, no names were recorded and instead, a personalized research number was used for each patient and only the main investigator had access to the collected data.

Study design

This study was an observational descriptive study. Samples were prospectively collected for a period of 27 months.

Study setting and sample collection

Locate in a rural area of Mthatha, Gateway clinic in KSD has a satellite TB clinic that receive and manage all cases suspected of being drug-resistant tuberculosis (DR-TB) from KSD and Mhlontlo which was the 2 sub-districts where the study took place. Medical records from all DR-TB cases are

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also kept at Gateway clinic while all samples are sent to Mthatha at the National health laboratory service (NHLS) TB laboratory for analysis. Samples were collected by patients (Sputum) and by medical doctors (CSF, Pleural fluid) as part of the patients' routine management, and submitted to the NHLS microbiology laboratory.

On his own, Gateway clinic manages 57 primary health care (PHC) facilities: 33 facilities from KSD and 24 PHC facilities from Mhlontlo. Gateway clinic performs the following functions: Initiation of treatment, monthly monitoring & follow up, radiology service at Mthatha General, ECG done at Mthatha General, Mobile injection team & DOTS – Mthatha Hospice, Conducting DR-TB reviews on Wednesdays and Thursdays weekly.

Laboratory diagnosis of MDR-TB and XDR-TB

Molecular diagnosis

GeneXpert MTB/RIF

The geneXpert was performed directly on TB samples using the newer version (G4) of cartridges according to the manufacturer's recommendation (Cepheid, Sunnyvale, USA). Samples were decontaminated and reagent buffer containing NaOH and isopropanol was added at the ratio of 2:1, followed by incubation at room temperature for 15 minutes. Two milliliters of the final samples were then transferred into the Xpert MTB/RIF cartridge, and after mixing, the cartridge was loaded into the GeneXpert instrument. The software was automatically filling in the reagent lot ID, cartridge number, and expiration date. The results are usually generated after 90 – 120 minutes and were recorded. Results were reported as: *M. tuberculosis* negative or positive, and RMP resistant or susceptible.

Line Probe assay: GenoType MTBDRplus

Molecular method was also done using line probe assay (LPA) and was performed according to the manufacturer's instructions (Hain Lifescience, Nehren, Germany). One type of LPA is the GenoType MTBDR*plus* and was designed for simultaneous detection of the most important *rpo*B mutations which confer RMP resistance, *kat*G and *inh*A mutations which confer high and low level INH resistance respectively; other genes included: *rrs* for Kanamycin/Amikacin (Km/Am) resistance, *tly*A for Capreomycin (Cm) resistance, *gyr*A/*gyr*B for Moxifloxacin/Ofloxacin (Mfx/Ofx) resistance. The test is based on DNA strip technology and has three consecutive steps, all three steps were performed as per the WHO recommendations: First, DNA extraction from *M. tuberculosis* isolates; next, multiplex PCR amplification of the resistance-determining region of the gene under question was performed using biotinylated primers. Following amplification, labelled PCR products were hybridized with specific oligonucleotide probes immobilized on a strip. If a mutation was present in one of the target regions, the amplicon will not hybridize with the relevant probe. After extraction and PCR amplification of the resistance-determining region of DNA, mutations were detected by the presence or absence of binding to "probes," indicated by the presence or absence of coloured bands on a strip. The assay was performed and the results were interpreted according to the manufacturer's recommendations.

Phenotypic DST

The automated BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 (Becton Dickinson, USA), is an in-vitro diagnostic instrument designed and optimized for the rapid detection of mycobacteria from clinical specimens (except blood). This system has a 960-tube capacity for nearly 8000 specimens per year and is useful in laboratories dealing with large specimen loads. The results indicating susceptibility or resistance were interpreted and reported automatically by the MGIT system using predefined algorithms that compare bacterial growth in the drug-containing tube with the growth in the drug-free control tube (Becton, Dickinson & Company). The growth unit (Gu) values of the drug-containing vials were evaluated. In other to interpret the results, when Growth control (GC) reached the value of 400 or more within 3 to 13 days with the instrument indicating that the test was complete. After scanning different tubes, an inventory report was printed, results for INH, RIF, Amk, Kan, Cm, Ofx, and Lfx were interpreted by the instrument as "S" for susceptible or "R" as resistant. When the GU of the drug-containing tube was <100, the result was reported as susceptible and when the GU of the drug-containing tube was ≥100, the result was reported as

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resistant strains. To ascertain that results were truly susceptible when GU was initially found to be <100, the test tube was incubated for a further 7 days and if it was still <100, the strain was then reported as a true susceptible.

Data analysis

Continuous variables were expressed as mean± Standard Error of the Mean (SEM) and categorical variables were expressed as proportions (%). The level of significance was fixed at $\varrho<0.05$, SPSS version 22.0 was used for all statistical analyses. Student t test was used to compare discrepant results between phenotypic and genotypic methods.

3. Results

Between June 2015 and September 2017, 224 patients suspected of having DR-TB were transferred from clinics located in KSD and Mhlontlo sub-districts to Gateway MDR-TB clinic in Mthatha, Eastern Cape. GeneXpert® MTB/RIF assay (Cepheid, USA) was performed on sputum samples obtained from those patients, and the presence of *Mycobacterium tuberculosis* with mutations in *rpo*B genes (resistance to RMP) was determined in all suspected 224 cases.

Following cultures, LPA tests were performed on 165 initial TB isolates (out of 224), of which, 133/165 (80.6%) isolates were resistant to INH and 157/165 (95.2%) were resistant to RMP. When classified by INH susceptibility results, all 165 RMP resistant isolates by Xpert MTB/RIF were confirmed in 98.5% by LPA if *M. tuberculosis* isolates were resistant to INH, but only confirmed in 81.3% if *M. tuberculosis* isolates were susceptible to INH ($\varrho < 0.001$) as describe in Table 1.

Table 1. Agreements between Xpert MTB/RIF and LPA by INH Susceptibility Results (n = 165).

Drug susceptibility profile	RMP resistant by GeneXpert n (%)	RMP resistant by LPA n (%)	p-value
LPA			<0.001
INH susceptible (n= 32)	32 (100)	26 (81.3)	
INH resistant (n=133)	133 (100)	131 (98.5)	

RMP: Rifampicin; INH: Isoniazid

At the end of the study, out of 224 RMP-resistant TB samples by Xpert MTB/RIF, 12 (5.4%) were found susceptible to RMP by LPA. Table 2, below shows demographic, clinical and outcomes information of the 12 participants whose samples displayed discrepant RMP results between LPA and Xpert® MTB/RIF.

Table 2. Demographic, Clinical and Outcomes Information about the 12 Participants whose samples showed discrepancies between LPA and Xpert® MTB/RIF.

Patient No.	Gender	Age	Sub-district of origin	HIV status	TB history	Final diagnosis	Outcomes
1	F	34	KSD	Neg	New	MDR	Still on anti-TB
2	M	66	KSD	Pos	PT	MDR	Still on anti-TB

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3	F	28	KSD	Neg	PT	INH-mono	Fav outcome
4	M	35	KSD	Neg	New	MDR	Fav outcome
5	F	55	KSD	Pos	PT	MDR	Still on anti-TB
6	F	30	KSD	Pos	PT	MDR	Poor outcome
7	F	40	KSD	Neg	PT	MDR	Fav outcome
8	F	42	KSD	Pos	New	MDR	Favo outcome
9	F	22	KSD	Pos	New	MDR	Fav outcome
10	M	18	Mhlontlo	Neg	New	Pre-XDR	Fav outcome
11	F	39	KSD	Neg	PT	MDR	Fav outcome
12	M	68	KSD	Neg	New	RMP-mono	Poor outcome

F= Female, M= Male, Neg= HIV negative, Pos= HIV positive, PT= Previously treated, ST= Still on treatment, Fav= Favourable, PO= Poor outcomes, MDR = Multiple drug resistant.

The 12 discrepant samples were further subjected to MGIT 960 drug susceptibility testing. The MGIT 960 results showed 75% agreement with LPA results. Table 3, describes the 12 discrepant isolates of *M. tuberculosis* and provides possible reasons for the occurrences of discrepancies between Xpert MTB/RIF, LPA and MGIT 960. Discrepancy was attributed to either hetero-resistance (mixed infections) or DNA contamination during LPA testing in 7 (58.3%) of the 12 discrepant cases. In 3 (25%) other samples that showed agreement in RMP resistance between Xpert MTB/RIF and MGIT960 (but disagreement with LPA), discrepancy was attributed mostly to laboratory error causing false RMP susceptible results with LPA (either sample mixed up or DNA contamination). For the remaining 2 (16.7%) cases, discrepancy was attributed mostly to laboratory error causing false RMP susceptible results with Xpert MTB/RIF (either Xpert readout errors with Ct value 4.1 – 4.9 or Sample transport delay).

Table 3. Analysis of discordance between genotypic and phenotypic assays among the 12 DR-TB cases.

Case No.	AFB Smear results	Xpert MTB/RIF (on sputum)	LPA (on isolates)	MGIT-960 (on isolates)	Possible reasons for discordant results	Possible errors that were investigated
1.	Negative	**Resistant**	**Susceptible** (All wild types are present, no rpoB mutation signal present)	**Susceptible**	Most likely laboratory error causing false resistant RMP result with GeneXpert OR Erroneous Xpert RMP result	• Sample mix up • DNA contamination for Xpert • Xpert readout errors • Delay in probes' hybridization • Ct value 4.1 – 4.9
2.	3+	**Resistant**	**Susceptible** (All wild types are present, no rpoB mutation signal present)	**Susceptible**	Most likely laboratory error causing false resistant RMP result with Gene Xpert OR Erroneous Xpert RMP result	• Sample mix up • DNA contamination for Xpert • Xpert readout errors • Delay in probes' hybridization • Ct value 4.1 – 4.9
3.	2+	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of	DNA contamination of LPA

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					Mtb isolates after inadequate treatment) OR DNA contamination for LPA	
4.	**Negative**	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR DNA contamination for LPA	DNA contamination of LPA
5.	Negative	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR	DNA contamination of LPA

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					DNA contamination for LPA	
6.	Negative	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR DNA contamination for LPA	DNA contamination of LPA
7.	3+	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR DNA contamination for LPA	DNA contamination of LPA

8.	Negative	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR DNA contamination for LPA	DNA contamination of LPA
9.	3+	**Resistant**	**Susceptible** (All wild types are present, rpoB mutation present)	**Susceptible**	Heteroresistance (presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment) OR DNA contamination for LPA	DNA contamination of LPA
10.	1+	**Resistant**	**Susceptible**	**Resistant**	Most likely laboratory error causing false	• Sample mix up • DNA contamination of LPA

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			(All wild types are present, no rpoB mutation present)		susceptible RMP result with LPA	
11.	3+	**Resistant**	**Susceptible** (All wild types are present, no rpoB mutation present)	**Resistant**	Most likely laboratory error causing false susceptible RMP result with LPA	• Sample mix up • DNA contamination of LPA
12.	2+	**Resistant**	**Susceptible** (All wild types are present, no rpoB mutation present)	**Resistant**	Most likely laboratory error causing false susceptible RMP result with LPA	• Sample mix up • DNA contamination of LPA

AFB: Acid-fast bacillus, LPA: line probe assay, Mtb: *Mycobacterium tuberculosis*, DNA: deoxyribonucleic acid, Ct: cycle threshold.

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4. Discussion

According to WHO, an effective treatment regimen depends on optimal susceptibility testing of *Mycobacterium tuberculosis* to anti-TB drugs [4]. Over the past years, new technologies have been introduced to shorten and improve methods for detection of anti-TB drug resistance, notably at molecular and phenotypic levels. As the use of such molecular and phenotypic assays increases, discordance between results has been encountered.

We had cases with discrepancy results between LPA and GeneXpert when testing for Rifampicin resistance in our study. In many cases, discrepancy often occur due to: bacterial population (repeated sub-culturing may lead to losing the slow-growing resistant population and false susceptible result), hetero-resistance, cross-contamination, mixed infections, growth difficulties of some strains, and minimum inhibitory concentrations (MIC) of some isolates which are closer to the critical concentration [8].

In this present study, we found that Rifampicin discrepancy rate between GeneXpert and LPA was very low (1.5%) if INH was subsequently resistant whilst a very high discrepancy rate (19.7%) was observed if INH was subsequently susceptible (P<0.001). So, we hypothesised that susceptibility to INH also might play a role in Rifampicin discrepancy results between GeneXpert and LPA. However, further studies with a large number of participants are required in order to draw a final conclusion.

In this present study, from the 12 discrepant results identified, three main reasons for discordance were observed:

1.Technical laboratory errors causing false resistant RMP result with GeneXpert was considered due to the fact that RMP resistance was identified only on Xpert MTB/RIF while LPA and MGIT 960 indicated RMP susceptibility.

In this study we used Xpert MTB/RIF version G4 of cartridges (Cepheid, USA) that was introduced in order to reduce false RMP resistant results as compared to the G3 version. The Xpert MTB/RIF assay detects *M. tuberculosis* and RMP resistance by PCR amplification of the rifampin resistance-determining region (RRDR) of the *M. tuberculosis rpoB* gene and subsequent probing of this region for mutations that are associated with RMP resistance. Although the study by Helb et al., established that the Xpert MTB/RIF assay has a limit of detection (LOD), defined as the minimum number of bacilli that can be detected with 95% confidence) of 131 CFU per ml of clinical sputum, this was not observed in our study [13]. Of our 12 discrepant samples (all with positive Xpert MTB/RIF results), 5 had negative smear microscopy results and 1 sample had scanty AFB observed. The remaining 6 samples had moderate to many AFB observed. General troubleshooting for the presumed laboratory errors were performed, and included verification of samples, review of the used technique that could allow the occurrence of cross-contamination, reagents' quality control, Xpert read-out errors, dropout or delay in probes' hybridization by ensuring that we did not have ΔCt value between 4.1 – 4.9 on Xpert. Drop-out was excluded since none of the probes had a Ct value of zero, and delta Ct max value for each test was not between 4.1 – 4.9 hence Xpert readout errors were excluded. Although cross-contamination was not fully excluded, it is high likely that sample mix-up was the reason for discrepancy in our 2 results out of 12. Cross-contamination is very rare with Xpert MTB/RIF since it operates on a close system.

2. Heteroresistance was considered since there was a simultaneous presence of all rpoB wild types (wt) and specific rpoB mutation signals in LPA in the presence of RMP susceptible by MGIT 960 but RMP resistant on Xpert MTB/RIF

We identified possible 7 (3.1%) out of 224 cases of hetero-resistance in this study; those are usually defined as the coexistence of susceptible and resistant *M. tuberculosis* strains in the same patient [14]. Similar result was found in a study done in Ethiopia by Mekonnen and his colleagues reporting 8 (1.9%) cases of RMP hetero-resistance [15]. In our study, the level of hetero-resistance was lower compared to studies done in Uzbekistan (South Central Asian union) by Hofmann-Thiel and colleagues with 20% of hetero-resistance and another study done in India with 34% cases of hetero-resistance [14] [16].

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Hetero-resistance might be the result of several factors such as the presence of both resistant and susceptible Mtb isolates; or endogenous development of two sub-populations of Mtb isolates after inadequate treatment since in the same patient with different drug susceptibility patterns, several sub-populations may co-exist [17]. It is also believed that Hetero-resistance develop during treatment of DR-TB [18]. Since we had many patients on anti-TB treatment and also having the history of previous exposure to anti-TB drugs, the possibility of endogenous development of sub-populations was therefore raised among our cohort of patients who displayed hetero-resistance results.

Direct transmission of hetero-resistance of both susceptible and resistant bacterial populations from drug resistant patients to previously untreated cases could also happen. Hetero-resistance has been proven so far to occur mostly in high TB incidence locations. For a country such as South Africa where the prevalence of DR-TB strains is very high, the level of patients directly infected with resistance is therefore high. In different parts of the world, hetero-resistance was found to be low at 1.4% in Italy [19], 1.9% in Russia [20], and 1.9% in Pakistan [21]. The possibility of DNA sample contamination during LPA tests was also investigated but could not be fully excluded.

3. Technical laboratory errors causing false susceptible RMP result with LPA since RMP resistance in Xpert was confirmed using MGIT 960 performed on positive cultures (while LPA indicated discordant results).

Molecular techniques such as LPA have revolutionized the diagnosis of DR-TB. Contamination of DNA samples during LPA testing is not an uncommon phenomenon observed in LPA laboratories. Factors such as laboratory air and surfaces, tools and equipment are potential sources for contaminating DNA during a pre-LPA testing procedure [22]. More sources of DNA contaminations could also be molecular biology grade water, LPA reagents, and DNA extraction kits since they have been all reported as major sources of DNA contamination by many studies [23] [24] [25]. Nevertheless, contamination during LPA can also be caused by direct transfer of contaminating DNA from an analyst or any person in the laboratory to the sample ready for LPA testing. DNA contamination can also be due to an object used in the premise of the laboratory and afterward from this object to the sample. Therefore, laboratory and personal equipment may consequently act as a vector for DNA contamination.

In a TB laboratory, it is essential for personal protective equipment (PPE) to be applied appropriately, hence equipment such as masks, hats, gloves and lab coats are worn in order to prevent contamination. But unfortunately, if not appropriately used, PPE could also be a vector for DNA contamination.

In addition to possible DNA sample contamination, troubleshooting also included verification of sample identities in order to exclude sample mix up cases. Since our laboratory receives many samples for possible diagnosis of DR-TB, there is a high workload with possibility of samples being mixed up, resulting in discrepant results being reported.

5. Conclusions

Discordance between genotypic and phenotypic tests are increasingly recognized and are becoming a concern mostly in a country such as South Africa where the incidence of tuberculosis is still high. For 1st line anti-TB agents, discrepancy between LPA and GeneXpert was significantly associated with INH susceptibility. Laboratory errors such as sample mix up and LPA contamination, as well as cases of hetero-resistance were among the predominant reasons for discrepant results between the two genotypic tests and the used phenotypic method. Findings from this present study are important for regional TB control program managers, who need to double evaluate the performance of the Xpert MTB/RIF before rolling it out in the DR-TB control programs.

Limitations for this study include: (i) small number of discordant results; (ii) absence of the cohort of susceptible TB for comparison with DR-TB since we worked in DR-TB clinic only; and (iii) missing of some clinical and/or laboratory findings that led to the exclusion of some cases during analysis.

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