SPUTUM CULTURE AND DRUG SENSITIVITY TESTING OUTCOME AMONG X-PERT MTB/RIF POSITIVE, RIFAMPICIN RESISTANT SPUTA: A RETROSPECTIVE STUDY

Lebogang Jacktor Kenaope

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine

Johannesburg, 2016

Declaration

I, Lebogang Jacktor Kenaope declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

…….

……..day of……, 2016

Presentations arising from this study

1. This work has been submitted for presentation to the Public Health Association of South Africa conference 2016.

Abstract

Background

The introduction of the X-pert MTB/Rif has shortened the time to detection of rifampicin resistant tuberculosis, which is assumed to be a surrogate for multidrug resistant tuberculosis. In practice, therefore MDR TB treatment is usually initiated soon after a rifampicin resistance result on X-pert MTB/Rif, simultaneously with a second sputum specimen, taken for confirmatory culture and further drug susceptibility testing. In this retrospective review, we report the outcome of further drug susceptibility testing performed on the second sputum specimen.

Methods

This study was based at the Klerksdorp Tshepong Hospital Complex. We retrospectively reviewed clinical files of patients admitted to the hospital MDR unit with rifampicin resistant TB on X-pert MTB/Rif between April 2011 and February 2014. Data from 384 patients were analysed. Only drug susceptibility testing result on the first sputum after admission was considered.

Results

Of 384 individual patient files with X-pert Rif resistance, MDR TB was confirmed in the subsequent culture isolates of 182(47.4%) patients (this means 176 on MTBDR plus and 6 on phenotypic DST) and on raw sputa (MTBDR plus on a sputum smear) of 5(1.3%) patients. Therefore the total number of confirmed MDR TB cases was 187(49%). Rifampicin mono-resistance, isoniazid mono-resistance and drug sensitive TB were detected in 137(36%), 12(3%) and 48(13%) patients respectively. Half [37/74(50%)] of patients with a CD4 count less than 50 cells/mm^3 had rifampicin mono-resistance on culture and 4/74(5.4%) patients had isoniazid mono-resistance. Whereas patients with higher CD4 counts between 50 and 350 cells/mm^3, 58/181(32%) had rifampicin mono-resistance and 4/181(2.2%) had isoniazid mono-resistance (p-0.012).

Conclusion

Rifampicin resistance on X-pert MTB/Rif does not always mean multidrug resistant tuberculosis will be confirmed on sputum culture. Patients with lower CD4 counts who have rifampicin resistant TB on X-pert MTB/Rif may benefit from adding INH to the standardised MDR TB regimen while awaiting confirmatory tests to confirm or rule out MDR TB.

Acknowledgements

Doctor Neil Martinson, thank you for being such a great supervisor. Your support, guidance and challenging questions enabled me to work hard at this research.

Professor Ebrahim Variava, my heartfelt appreciation goes to you. Thank you for always showing and teaching me that we always need to strive towards excellence in what we do.

Doctor Hannetjie Ferreira, thank you for your support and for enabling me to gain access to the records.

To my wife, Wanani and my daughter Zwonaka, thank you for your continuing support and for being a source of strength to me.

Table of Contents

List of figures

List of tables

Protocol with extended literature review

Background

The World Health Organisation's global report on tuberculosis estimates that 8.7 million incident cases of tuberculosis occurred in 2011 globally with most of the estimated cases occurring in Asia and Africa at 59% and 26% respectively; 0.5 million were children, 2.9 million were women and South Africa was among the five countries with the largest number of incident cases at 0.4 million to 0.6 million cases[1]. It is worth noting that of the 8.7 million incident cases in 2011, 1.1 million (13%) were among people living with HIV and the highest proportion of these were in Africa[1]. Despite the overall decline in incident tuberculosis cases globally and in the Africa region shown in 2011, the incident tuberculosis cases were shown to be rising still in South Africa as a country[1]. There was an estimated 12 million prevalent cases of tuberculosis globally in 2011[1]. Mortality rates from tuberculosis were also shown to have declined since 1990 both globally and regionally with forecast tuberculosis mortality rates expected to decline further in the period 2012- 2015 including in South Africa as a country[1].

A breakthrough in the treatment of tuberculosis was reached in 1943 when the first effective anti-tuberculosis agent, streptomycin was isolated[2]. In 1948, thiacetazone and para-aminosalicylic acid, two new anti-tuberculosis agents came on the market[2]. Isoniazid was isolated in 1951, followed by pyrazinamide and cycloserine in 1952, ethionamide 1956, rifampicin 1957, and ethambutol 1962[2]. However the advent of every new drug resulted in the selection of mutations conferring resistance to it[2]. For example, trials showed a rapid onset of isoniazid resistance among patients receiving monotherapy and suppression of resistance when isoniazid was given in combination with streptomycin or para-aminosalicylic acid[2]. Multiple drug regimens were therefore developed[3].

Multidrug resistant (MDR) tuberculosis is defined as the resistance of the mycobacterium tuberculosis (MTB) to both isoniazid and rifampicin[4]. The WHO global TB report showed that globally, there were an estimated 630 000 multidrug resistant (MDR) tuberculosis cases out of the 12 million TB prevalent cases in 2011[1]. South Africa is one of the high burden multidrug resistant (MDR) tuberculosis countries with an estimated proportion of 1.8% (total 325 321) of new tuberculosis(TB) cases being multidrug resistant (MDR) tuberculosis and 6.7% (total 45 915) of retreatment cases in 2011[5]. The inpatient cost of multidrug (MDR) tuberculosis treatment per patient was estimated to be at more than 40 times the cost of drug susceptible tuberculosis[6].

Isoniazid (INH), only active against growing tubercle bacilli, is a pro drug that is activated by the enzyme called catalase peroxidase (KatG) encoded by *katG* gene and found in the mycobacterium tuberculosis (MTB)[7]. This activation results in the production of highly reactive species such as the isonicotinic-acyl radical or anion which then reacts with nicotinamide adenine dinucleotide [NAD(H)] and attacks the enoyl acyl carrier protein reductase (InhA enzyme) which is involved in mycolic acid synthesis[7].

Mutation in the *KatG* gene is the main mechanism of isoniazid (INH) resistance[7]. Resistance to isoniazid (INH) can also occur by mutations in the mabA/inhA promoter region causing over expression of the InhA enzyme or mutations in the InhA active site lowering InhA affinity to the isoniazid (INH)- nicotinamide adenine dinucleotide (NAD) adduct[7].

Rifampicin, active against both growing and stationary phase bacilli, interferes with RNA synthesis of the mycobacterium tuberculosis (MTB) by binding to the beta subunit of the RNA polymerase[7]. Rifampicin resistance is conferred by spontaneously occurring mutations in the *rpoB* gene[7].

Mycobacterial cell wall also confers resistance to drugs through its low permeability given by its components such as mycolic acid, arabinogalactan and peptidoglycan[7].

Efflux pumps belonging to different classes and present in the mycobacterium tuberculosis (MTB) have been shown to confer drug resistance by decreasing intracellular levels of drugs[8]. This mechanism of resistance is an object of study in the development of new drugs[8].

Mycobacteria also develop drug resistance through drug modifying enzymes such as the aminoglycoside 2'N-acetyltransferase described[9].

Previous tuberculosis (TB) treatment and default from treatment are important risk factors for drug resistance tuberculosis[10, 11].

On chest x-ray, studies have shown that in immune competent individuals, nodules, ground glass opacity and cavities are the predominant pattern in both extensively drug resistant and multidrug resistant tuberculosis[20]. On the other hand, immune compromised patients, especially those with very low CD4 T lymphocyte counts rarely present with cavities, but rather more commonly with hilar lymphadenopathies and consolidations in more than one lung zone[21].

Sputum smear microscopy is an important tool first developed in the 1880's[12]. However, this technology only detects roughly half the number of active cases, it is highly operator dependant and takes days rather than hours to complete[12]. However, studies have shown that the sensitivity of smear microscopy is significantly lower in Human Immunodeficiency Virus (HIV) infected individuals than in HIV uninfected and those with unknown HIV status[13].

Culture of the mycobacterium tuberculosis, particularly in sputum sample, remains the gold standard for the diagnosis of both TB and drug sensitivity testing; the time period between sample collection and results availability depends on the culture medium used, up to seven days in liquid medium and up to 8 weeks in solid medium and a further 4-6 weeks for drug sensitivity testing (DST) with regard to the latter[14].

Drug sensitivity tests for tuberculosis are either phenotypic or genotypic[15].

Phenotypic drug sensitivity testing is a method involving the culturing of the M. tuberculosis in the presence of anti-tuberculosis drugs in order to detect growth (in terms of resistance) or inhibition(in terms of sensitivity)[15]. Direct phenotypic drug sensitivity testing involves the inoculation of a set of drug containing and drug free media with a concentrated specimen, whereas indirect testing involves inoculation of drug containing media with a pure culture grown from the original specimen[15]. The disadvantages of these methods are the time they take, technical complexity and the laboratory infrastructure they require[15].

Genotypic sensitivity testing involves detection of mutations that are responsible for drug resistance[15]. Examples are line probe assays that use PCR and reverse hybridization methods to detect rifampicin and isoniazid drug resistance[15]. The other type are beacon

assays that detect M. tuberculosis complex and associated rifampicin resistance directly from sputum samples using ultra-sensitive PCR[15].

The Genotype MTBDR plus is a commercially available molecular line probe assay containing probes specific for mycobacterium tuberculosis complex and probes for common rifampicin resistance conferring mutations as well as isoniazid (INH) resistance conferring mutations[16]. It showed a sensitivity of 48, 4% in the detection of M. tuberculosis among MGIT culture positive sputa and a specificity of 98, 9% for culture negative sputa, these were found in the same study conducted in a South African gold mine[16]. The Genotype MTBDR plus showed a very low sensitivity in the detection of M. tuberculosis from smear negative sputa with higher sensitivity from smear positive sputa[16]. For specimens positive for M tuberculosis by MTBDR plus, this assay showed a higher sensitivity of 85,7% and specificity of 96,6% for rifampicin resistance as compared to a sensitivity of 62,1% and specificity of 97,9% for isoniazid resistance, thus indicating that the sensitivity of genotype MTBDR plus depends on the bacillary burden[16]. However though the recent South African national data have shown that newer generation line probe assays are done off culture due to low smear positive rates[22].

X-pert MTB/RIF is an automated molecular test for Mycobacterium tuberculosis and resistance to rifampicin, it uses a semi nested real time polymerase chain-reaction assay to amplify an MTB specific sequence of the *rpoB* gene, which is probed with molecular beacons for mutations within the rifampicin-resistance determining region and it gives results within 2 hours[17].

In one review study of eighteen unique studies, the X-pert MTB/Rif achieved a pooled sensitivity of 88% and a pooled specificity of 98% when used as an initial test replacing smear microscopy[18]. As an add on test, following a negative smear microscopy, X-pert MTB/Rif achieved pooled sensitivity of 67% and pooled specificity of 98%[18]. For smear positive culture positive tuberculosis, the pooled sensitivity of X-pert MTB/Rif was 98%, and 68% for smear negative culture positive tuberculosis[18]. The pooled sensitivity was 80% in people living with HIV, and 89% in people without HIV[18]. For rifampicin resistance detection, the X-pert MTB/Rif achieved a pooled sensitivity of 94% and a pooled specificity of 98%[18]. In the same study, it was estimated that the lower the prevalence of rifampicin resistant tuberculosis, the higher the probability of the X-pert MTB/Rif wrongly identifying patients as being rifampicin resistant[18]. This test is therefore very relevant to South Africa as it is among the high burden multidrug resistant (MDR) tuberculosis countries[5].

According to the national guidelines, patients suspected to have drug resistant pulmonary tuberculosis who test X-pert MTB/Rif positive rifampicin resistant should be treated as multidrug resistant (MDR) tuberculosis while another sputum specimen is collected for drug resistant tuberculosis confirmation[19]. Confirmation is achieved through both or either one of the two routes, the first one is conventional sputum for tuberculosis culture and drug sensitivity testing, the second one is smear microscopy if positive followed by the MTBDR plus but if smear negative , conventional culture and then the cultured bacilli are subjected to line probe assay[19].

At Klerksdorp Tshepong Hospital Complex, of the patients who were admitted with rifampicin resistance on the X-pert MTB/Rif test between April 2011 and March 2013, a certain proportion of them turned out to either be true multidrug resistant (MDR), mono-resistant or

sensitive tuberculosis. In this study, we retrospectively determine the true proportion of samples that are true multidrug resistant (MDR) and the contributing factors to this as well as the statistical significance this has in our setting as this will influence our decision to treat or not to treat for multidrug resistant (MDR) tuberculosis when faced with rifampicin resistance on X-pert MTB/Rif.

OBJECTIVES

Overall aim

1. To determine if rifampicin resistance on X-pert MTB/Rif test infers multidrug resistance (MDR) when compared to the gold standard tests genotype MTBDR plus or MGIT culture and sensitivity.

Specific objectives

a) To identify the proportion of positive MGIT culture with multidrug resistant (MDR) tuberculosis and multidrug resistance (MDR) identified on the MTBDR plus and compare them to that identified as rifampicin resistant by X-pert MTB/Rif test.

b) To identify the proportion of samples that are positive for AFB on smear or negative for AFB out of all samples that tested positive for multidrug resistance on MGIT culture and sensitivity. c) To identify the proportion of mono-resistant and sensitive tuberculosis when X-pert MTB/Rif diagnoses rifampicin resistance.

d) To evaluate if HIV sero-status and CD4 count impacts on X-pert MTB/Rif positivity rates

METHOD

We will conduct a retrospective, cross sectional study at Tshepong hospital, an 800 bed hospital with a 76 bed MDR/XDR tuberculosis unit situated in a mining district of Dr Kenneth Kaunda. A referral unit for the surrounding districts in the North West province. With a null hypothesis that all rifampicin resistance on X-pert MTB/Rif means MDR tuberculosis. The study will be conducted on male and female patients aged 13 years and above, of all races and socioeconomic background for a period between April 2011 and February 2014.

Eligible patients are those patients who were admitted to Tshepong MDR/ XDR TB unit from home or referred from surrounding hospitals with rifampicin resistance on sputum X-pert MTB/Rif test. As the main researcher, I will abstract data by paging through the clinical records of patients admitted to Tshepong MDR/ XDR TB unit during the period of study mentioned above. The clinical files will be obtained by visiting the Tshepong MDR/XDR TB unit and pulling files from the unit's paper files, and abstracting will be done by filling in the printed data sheets so as to focus on obtaining variables mentioned above.

In case of a file missing, I will make use of the backup electronic database compiled by the medical officer in charge of the Tshepong MDR/XDR TB unit whose permission I also have. In case some electronic information is still missing, I will contact the referring hospital or local clinic or personally visit the institution concerned if it is within the Dr Kenneth Kaunda district. NHLS records will be accessed by myself electronically by entering specimen bar codes on to the old disa system or the new trackcare system depending on whether the sputum was taken before the new system was introduced or afterwards. I will then abstract the following variables from the NHLS: sputum expert MTB/Rif test result, smear and

microscopy result, phenotypic gold standard MGIT result, genotypic MTBDR plus result. Permission will be sought from the relevant authorities at NHLS and the Klerksdorp Tshepong Hospital complex prior to beginning this study.

From a prelimianry look at the records at the Klerksdorp Tshepong Hospital complex prior to embarking on the drafting of the protocol, it appears that over 300 patients are admitted to the hosptial every year. I plan to use the years from when the X-pert MTB/Rif was in use (mid 2011 – to the present). I will use data from June 2011 (when the X-pert was first routinely used to identify MDR TB at the site), and that I will include patients until February 2014, over 1000 MDR patients will be available. If only 30% were initially diagnosed by the X-pert MTB/Rif, conservatively it suggests there are 300 patients available for analysis. Assuming that 20% will have insufficient information (this is a retrospective study) then, conservatively, we will have approximately 240 patients for analysis. Assuming that 30% are either rifampicin resistant only or false positive and have no resistance noted, then the 95% CI intervals of this point estimate (30%) generated will be 0.24-0.36.

Similarly if 10% are false positive rifampicin resistant the 95%CI around this point estimate are 0.06-0.14. Both confidence intervals are sufficiently narrow for the purposes of this study. The sample size will be able to identify differences in proportions between sub groups at a prevalence of 0.1 and 0.3, respectively, or more at a power of 80 and alpha of 0.05.

DATA ANALYSIS

Data will be entered into the Microsoft excel spread sheet for cleaning and coding. Data cleaning will include looking for extreme cases, missing values and internal inconsistency. The Microsoft excel spread sheet will be imported to STATA version 12 for data analysis. For categorical variables frequencies and percentages will be computed and for numerical variables means and standard deviation will be calculated for normally distributed data, for abnormally distributed data median and interquartile ranges will be reported.

Kolmogorov Smirnov tests will be used to test the distribution of data.

Chi square test will be used to test for associations between two categorical variables for the first five specific objectives using contingency tables to represent them and determining the p value for each.

We will use the t test to test for significance difference between normally distributed binary categorical variables.

ETHICAL CONSIDERATIONS

Since this will be a retrospective study, an informed consent will not be sought from patients and the names of the patients will not be displayed on the research report.

An ethics approval will be sought from the ethics review panels at the university of the Witwatersrand, Tshepong hospital and the north west department of health.

Study timing

October 2013 : Protocol submission

March to May 2014 : Data collection and ethics application

June 2014 : Data analysis

July to August 2014 : Writing up of the report

FUNDING

Clinical records and results from the NHLS are those already done and paid for by the hospital.

Stationary, traveling and electronic resources will be paid for by me, the main researcher.

REFERENCES

1. World Health Organization, Global tuberculosis report 2011. Available at www.who.int/tb/publications/global_report/en/. Accessed 03 April 2012

2. Keshavjee S, Farmer P E. Tuberculosis, Drug Resistance and the history of modern medicine. N Engl J Med. 2012;367: 931-6

3. Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical research council tuberculosis units, 1946-1986. Int J Tuberc Lung Dis 1999;3:supplement 2: s231-s279

4. Tawanda Gumbo, 2010 'Chemotherapy of tuberculosis, mycobacterium avium complex disease and leprosy'. Goodman's and Gilman's. The pharmacological basis of therapeutics, chapter 56, 12th edition

5. World Health Organization. Tuberculosis country profiles. Available at http://www.who.int/tb/country/en/index.html. Accessed on 03 April 2012

6. Schnippel K, Rosen S, Shearer K et al. Cost of inpatient treatment for multidrug tuberculosis in South Africa. Tropical medicine and international health.2013; 18(1): 109-116.

7. Zhang Y, Yew W. Mechanisms of resistance in mycobacterium tuberculosis. Int J Tuberc Lung Dis, 13(11):1320-1330.

8. Machado D, Couto I, Perdigao J et al. Contribution of efflux to the emergence of isoniazid and multidrug resistance in mycobacterium tuberculosis. PLOSONE. 2012; 7(4).

9. Ainsa J A, Perez E, Pelicic V et al. Aminoglycoside 2'N-acetyltransferase genes are universally present in mycobacteria: characterisation of the aac(2')-Ic gene from mycobacterium tuberculosis and the aac(2')-Id gene from mycobacterium smegmatis. Molecular Microbiology.1997; 24(2): 431-441.

10. Weyer K, Brand J, Lancaster J et al. Determinants of multidrugresistant tuberculosis in South Africa: results from a national survey. S Afr Med J. 2007; 97.

11. Edlin BR et al. An outbreak of multidrug resistant tuberculosis among hospitalized patients with AIDS. N Engl J Med 1992; 326: 1514-21

12. Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics for developing countries. Lancet 2006; 367: 942-943

13. Lagrange PH, Thangaraj SK, Dayal R, Despande A, Ganguly NK et al(2012). A toolbox for tuberculosis diagnosis: An indian multicentric study (2006-2008): Microbiological results. PLOSONE 7(8): 43739.

14. Wood R. Challenges of TB Diagnosis and treatment in South Africa. Roche symposium, 3rd South African AIDS conference, Durban 5-8 June 2007

15. World Health Organization.TB Facts.org. Global Health Education. Available at http://www.tbfacts.org/ghe.html. Accessed on 03 April 2012.

16. Dorman SE et al. Genotype MTBDR plus for direct detection of mycobacterium tuberculosis and drug resistance in strains from gold miners in South Africa. Journal of clinical microbiology. 2012; 50(4): 1189-94

17. Catharina C. Boehme, M. D., Nabeta P, Hillemann D, et al. Rapid Molecular Detection of Tuberculosis and Rifampicin Resistance. N Engl J Med. Volume 363. Number 11. P1005-1015.

18. Steingart KR, Sohn H, Schiller I et al. X-pert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane database of systematic reviews. 2013;1. Article number: CD009593.DOI:10. 1002/14651858.

19. Department of health, Republic of South Africa. Management of drug resistant tuberculosis policy guidelines 2012. Available at http://www.tbfacts.org/ghe.html Accessed on 16 April 2013

20. Cha J, Yo Yun Lee, Kyung Su Lee et al. Radiological findings of extensively drug resistant pulmonary tuberculosis in non-AIDS adults: Comparisons with findings of multidrug resistant and drug sensitive tuberculosis. Korean Journal of Radiology. 2009. 10(3).

21. Burst JCM, Berman AR, Zalta B et al. Chest radiograph findings and time to culture conversion in patients with multidrug resistant tuberculosis and HIV in Tugela Ferry, South Africa. PLOSONE, 2013. 8(9). Accessed 24 November 2013.

22. Professor Wendy Stevens. RIF concordance by LPA or DST. E-mail, 10 June 2012.

Submissible Article

Title: Sputum culture and drug sensitivity testing outcome among X-pert MTB/Rif positive, rifampicin resistant sputa: A retrospective study

Lebogang Kenaope[1], Hannetjie Ferreira[2], Neil Martinson[3], Ebrahim Variava [3, 4]

Affiliations

1. Department of Internal Medicine, University of the Witwatersrand
2. Department of Internal Medicine, Klerksdorp Tshepong Hospital Complex, North West Department of Health, Matlosana, South Africa.
3. Perinatal HIV Research Unit (PHRU), MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, University of the Witwatersrand, South Africa.
4. Department of Internal Medicine, Klerksdorp Tshepong Hospital Complex, North West Department of health, University of the Witwatersrand, South Africa.

Corresponding Author

Lebogang Jacktor Kenaope
Mobile: 0027728406025
Email: jacktorlebo@yahoo.com
Postal address: 20 Storks Nest, Albertsdal, Alberton, South Africa, 1448.

Abstract

Background

The introduction of the X-pert MTB/Rif has shortened the time to detection of rifampicin resistant tuberculosis, which is assumed to be a surrogate for multidrug resistant tuberculosis. In practice, therefore MDR TB treatment is usually initiated soon after a rifampicin resistance result on X-pert MTB/Rif, simultaneously with a second sputum specimen, taken for confirmatory culture and further drug susceptibility testing. In this retrospective review, we report the outcome of further drug susceptibility testing performed on the second sputum specimen.

Methods

This study was based at the Klerksdorp Tshepong Hospital Complex. We retrospectively reviewed clinical files of patients admitted to the hospital MDR unit with rifampicin resistant TB on X-pert MTB/Rif between April 2011 and February 2014. Data from 384 patients were analysed. Only drug susceptibility testing result on the first sputum after admission was considered.

Results

Of 384 individual patient files with X-pert Rif resistance, MDR TB was confirmed in the subsequent culture isolates of 182(47.4%) patients (this means 176 on MTBDR plus and 6 on phenotypic DST) and on raw sputa (MTBDR plus on smear) of 5(1.3%) patients.

Therefore the total number of confirmed MDR TB cases was 187(49%). Rifampicin mono-resistance, isoniazid mono-resistance and drug sensitive TB were detected in 137(36%), 12(3%) and 48(13%) patients respectively. Half [37/74(50%)] of patients with a CD4 count less than 50 cells/mm^3 had rifampicin mono-resistance on culture and 4/74(5.4%) patients had isoniazid mono-resistance. Whereas patients with higher CD4 counts between 50 and 350 cells/mm^3, 58/181(32%) had rifampicin mono-resistance and 4/181(2.2%) had isoniazid mono-resistance (p-0.012).

Conclusion

Rifampicin resistance on X-pert MTB/Rif does not always mean multidrug resistant tuberculosis will be confirmed on sputum culture. Patients with lower CD4 counts who have rifampicin resistant TB on X-pert MTB/Rif may benefit from adding INH to the standardised MDR TB regimen while awaiting confirmatory tests to confirm or rule out MDR TB.

Background

Multidrug resistant tuberculosis is defined by resistance of the mycobacterium tuberculosis (MTB) to both isoniazid and rifampicin (Rif)[1].

INH resistance occurs more frequent than for most anti-TB drugs at a frequency of 1 in 100000 to 1000000 bacilli in vitro[2]. Several gene mutations confer resistance to anti-tuberculosis medications The more common mutation on the *katG* gene is a mechanism of isoniazid (INH) resistance[2].

Isoniazid, active against growing tubercle bacilli, is a pro-drug and is activated by the enzyme catalase peroxidase (katG), encoded by the *katG* gene found in MTB[2]. Activation results in the production of highly reactive species such as superoxide, peroxide, hydroxyl radical, nitric oxide and isonicotinic-acyl radicals[2]. The isonicotinic- acyl radical then reacts with nicotinamide adenine dinucleotide (NAD [H]) and attacks the enoyl acyl carrier protein reductase (InhA enzyme) which is involved in mycolic acid synthesis.

Another mechanism of INH resistance occurs through mutations in the *mabA/ InhA* promoter region causing over expression of the InhA enzyme or mutation in the InhA active site lowering InhA affinity for the INH-NAD adduct[2].

Rif is active against both replicative and dormant bacilli, interferes with the ribonucleic acid (RNA) synthesis of MTB by binding to the beta subunit of RNA polymerase encoded by the *rpoB gene*[2]. Rif resistance is diagnosed by identifying mutations in the *rpoB gene*[2].

Factors identified with increased risk for mutations and development of MDR TB include a history of prior treatment for TB, inadequate TB treatment such as monotherapy, addition of a single drug to a failing regimen and poor adherence to treatment[3].

TB and human immunodeficiency virus co-infection may increase the risk for drug resistant TB through malabsorption of anti- TB drugs possibly as a result of chronic diarrhoea or infection with cryptosporidium[4-5]. TB drug resistance may be primary (either transmitted from someone with drug resistant TB or newly acquired) or secondary resistance (resistant mutations are selected by inadequate TB treatment)[6].

Globally, the frequency of MDR TB is 3.3% among new cases (primary resistance) with the majority found in Eastern Europe and Asia[1]. The frequency of MDR TB among previously treated cases (secondary resistance) is 20%[1]. In 2014, there was an estimated 480000 new MDR TB cases and approximately 190000 deaths from MDR TB worldwide[1].In South Africa, the South African Tuberculosis Drug Resistance survey 2012-2014 reports that the national MDR TB rate was 2.8% with MDR TB prevalence being 2.1% among new TB cases and 4.6% among previously treated cases[7].

Of concern is the diagnostic gap where many MDR TB cases go undiagnosed[1]. This then implies more transmission of the resistant strains and poor outcome for undiagnosed cases.

The recently developed End TB strategy calls for an early diagnosis of TB and universal drug sensitivity testing by 2035[1].

Diagnosis of Drug Resistant TB

TB DST can either be phenotypic or genotypic. Phenotypic DST involves the culturing of MTB in the presence of antibiotics with activity against TB[8].

Culture of the MTB remains the gold standard in diagnosis and determination of drug susceptibility of TB; culture on solid medium such as Lowenstein-Jensen slopes takes several weeks for an isolate to be positively identified as *M tuberculosis* with a further 4-6 weeks for DST whereas culture on liquid medium (BACTEC mycobacterial growth indicator tube 960, BD, Sparks, MD, USA) may take up to seven days[6].

Genotypic DST detects mutations conferring resistance to rifampicin and isoniazid namely, mutations in the *rpoB* gene for rifampicin and mutations in the *katG* gene and *InhA* promoter region for INH[9].It is tested in South Africa through the MTBDR plus (Hain Life science GmbH, Nehren, Germany).

Current diagnosis of MDR in South Africa uses a mix of molecular assays on raw sputum and molecular assays on culture isolates[10].

X-pert MTB/Rif (Cepheid Inc.) is an automated molecular test to detect MTB and rifampicin resistance, it uses a real time polymerase chain reaction to amplify the *rpoB* gene sequence which will be probed for mutations that confer rifampicin resistance[11].

In one review study of 18 unique studies, X-pert MTB/Rif- Cepheid Inc. achieved a pooled sensitivity of 88% and a pooled specificity of 98% when used as an initial test replacing smear microscopy[12].

As an add on test after a negative smear microscopy, X-pert MTB/Rif achieved a pooled sensitivity of 67% and specificity of 98%[12]. For smear positive culture positive sputum, sensitivity was 98% whereas it was 68% for smear negative culture positive sputum[12]. For rifampicin resistance detection, the pooled sensitivity was 94% with a specificity of 98%[12].The higher the prevalence of rifampicin resistance, the lower the probability of the X-pert MTB/Rif wrongly identifying some cases as rifampicin resistant as compared to areas of low rifampicin resistance prevalence[12].

Discordant results between the X-pert MTB/Rif and drug susceptibility testing (phenotypic or genotypic) have been reported where rifampicin resistant MTB was detected on the X-pert MTB/Rif and rifampicin sensitive MTB was detected on DST, this occurrence prompted guidelines to be put in place by the World Health Organization (WHO) on doing confirmatory tests of drug resistance by using DST[12].

In South Africa there is little data reporting discrepancies between initial X-pert MTB/Rif assay and more definitive results of resistance assays on cultured isolates. Clearly accurate assessment with appropriate initiation of treatment are essential especially as MDR TB treatment is lengthy, expensive and is linked to severe adverse events some of which are irreversible[13-14].

We therefore conducted a retrospective study to determine the laboratory based resistance result on sputum in patients with rifampicin resistance diagnosed on a sputum X-pert MTB/Rif assay. We also examined associations between discordant results and patient characteristics.

Objectives

Overall objective

To determine the proportion of patients with rifampicin resistance on initial X-pert MTB/Rif whose subsequent routine clinic sputum drug sensitivity testing on arrival at the MDR Unit was resulted as: MDR, rifampicin mono-resistance or other drug resistance using routine public sector laboratory processing to assess rifampicin and isoniazid resistance (MTBDR plus and/or liquid culture drug susceptibility testing).

Specific objectives

In patients identified as having rifampicin resistance on X-pert MTB/Rif and treated at Tshepong Hospital drug resistant TB facility:

- To report the proportion of MDR TB cases identified by subsequent phenotypic and genotypic (Hain Life science, GmbH, Nehren, Germany) drug susceptibility testing.
- To determine the proportion of non-MDR TB results (INH mono-resistance, rifampicin mono-resistance and drug sensitive TB) and their characteristics.
- To determine if HIV status and CD4 count are associated with DST outcome.

Methods

Tshepong hospital, located in Matlosana Municpality serving Klerksdorp and its surrounds, is a public sector hospital in the North West Province, South Africa. It has a dedicated drug resistant TB facility with 76 MDR beds. At the time of the study all patients with drug resistant TB were admitted to the facility for initiation of TB treatment and antiretroviral therapy if required. At the time of admission a baseline sputum sample was taken for confirmatory tests such as smear and microscopy, TB culture and drug sensitivity testing either on raw sputum (if smear positive) or on a cultured isolate by phenotypic and/or genotypic method. Other routine laboratory investigations are taken as required.

Data including patient age, gender, date of admission, HIV status, CD4 count, HIV viral load, antiretroviral drug history, history of TB treatment, sputum X-pert MTB/Rif date, sputum culture collection date, incubation time, drug susceptibility testing results, chest X ray report, were abstracted from clinical files. Sputum smear, mycobacterial culture and TB drug sensitivity. In cases where a patient had both phenotypic and genotypic drug susceptibility testing, only the phenotypic DST result will be considered for analysis because it is a gold standard test.

Other results including CD4 counts and HIV viral loads were obtained from the National Health Laboratory Services (NHLS) records which link patient tests to a unique laboratory number.

Three durations were collected:

1. Inter-specimen interval: duration from initial diagnostic X-pert MTB/Rif to the collection of the sputum within the first week of admission from which DST was obtained
2. Duration of TB treatment prior to sputum being taken for routine drug susceptibility testing

3. Time from specimen being placed in the liquid culture machine to the time the machine flagged the specimen as being culture positive (incubation time).

We used the following definitions:

1. MDR TB as MTB resistant to both INH and Rifampicin
2. Rif mono-resistance (mono) as MTB resistant only to Rifampicin
3. INH mono-resistance (mono) as MTB resistant only to INH
4. Sensitive TB as MTB that is sensitive to both INH and Rifampicin
5. Previous TB drug resistance detected as drug resistant TB diagnosed more than 2 years ago
6. Previous sensitive TB as sensitive TB diagnosed and treated for 2 months or more, more than a year ago

Data on treatment and inpatient outcome were also captured.

Patients aged at least 13 years admitted from the 1st of April 2011 to the 28th of February 2014 with an X-pert MTB/Rif demonstrating rifampicin resistance were eligible for inclusion. Additionally patients had to have an initial drug sensitivity testing result either on raw sputum or on sputum positive culture isolate within 6 months of X-pert MTB/Rif result showing rifampicin resistance.

Ethics approval

The Human Research Ethics Committee (Medical) of the University of the Witwatersrand granted permission for this study to be conducted.

Statistical analysis

Descriptive statistics such as the frequency distribution, mean, and standard deviation were used to summarise data. The mean was used to summarise metric variables such as patient age. Chi-square test of association was used to assess whether there was an association between two categorical variables such as drug sensitivity and HIV status. A Chi-square test p-value less than 0.05 is an indication that there is a significant relationship between the two variables while a p-value of greater than 0.05 is an indication of no association between the variables. Independent sample t-test was used to compare the mean values for patient age by drug sensitivity group. A p-value less than 0.05 is an indication that there is a significant difference between the two means while a p-value of greater than 0.05 is an indication of no significant difference between the mean values.

Results

In the period between the 1st of April 2011 and 28th of February 2014, a total of 650 patients were admitted to the drug resistant TB facility with a positive X-pert MTB/Rif test showing rifampicin resistance referred by primary health care providers (local clinics or primary care hospitals). The distribution of admissions per year between the above specified dates was 74(2011), 224(2012), 276(2013) and 76(2014).

A total of 604/650(92.9%) files of patients who came to the facility with rifampicin resistant TB diagnosed on the X-pert MTB/Rif were reviewed for eligibility, files of 46 patients were not found.

Of the 604 files with an initial X-pert MTB/Rif (GXP) reporting rifampicin resistance, two hundred and twenty (220) were excluded due to unavailability of drug resistance testing results, of whom 154 were untraceable, fifty six not done, eight patients had both sputum smear and culture negative, one grew a non-tuberculous mycobacterium (NTM) and one result was inconclusive. Data from 384 files was analysed (Figure 1).

Demographics

The average age of all patients was 36.74 years. There was no statistically significant difference in the average age across all categories of drug susceptibility test outcome (p-0.076). See table 1.

The majority of patients were HIV co-infected - 82% (315 patients) in this sample versus 69 (18%) HIV negative patients.

Of those with known CD4 count level, 181/312 (57.5%) had a count of 50-350 followed by 74 (23.5%) patients with a count less than fifty; 57 (18.1%) had a CD4 count greater than 350 cells/mm^3.

A total of 177 (56.2%) did not have a viral load taken because they were not on HAART on admission to the facility, for some, a viral load was not found.

Virtually all [375(97.7%)] patients were sputum culture positive and in most [209 (55%)], the confirmatory sputum for culture was collected within two weeks of the initial X-pert MTB/Rif specimen and in most cases [199 (52.2%)], within 2 days of admission to the Unit. The median incubation time in the patients who were culture positive was 13 days (IQR 10 days).

At admission, based on the initial X-pert MTB/Rif result and history of prior TB treatment, primary drug resistant TB was diagnosed in 197 (51.3%) patients.

Proportion of MDR TB cases

MDR TB was confirmed in 187/384 (49%), most by genotypic drug sensitivity assays, six were confirmed on phenotypic drug sensitivity testing (DST) and of these six, four had both genotypic and phenotypic DST, and there was a 100% concordance (they had MDR on both methods. (See figure 2 and table 2).

Characteristics of non-MDR TB cases

Table 1 also shows that 51.3%(101/197) non-MDR TB cases versus 44.9%(84/187) MDR cases had a history of prior TB more than a year ago and the majority of them did not have drug resistant TB detected previously (p-0.018, p-0.497 respectively).

The median incubation time in liquid culture for culture positive cases was 14 days for MDR cases, 14 days for rifampicin mono-resistant, 14 days for isoniazid mono-resistant and 12 days for sensitive TB cases. (Figure3).

Table 3 shows a significant association between the level of the CD4 cell count and the confirmatory drug susceptibility testing in this study. Among cases with a CD4 count of less than 50 cells/mm^3, the majority of them [50% (37/74)] had Rifampicin mono-resistant TB while the other half was distributed among MDR, INH mono-resistance and sensitive TB. Cases with a CD4 count of more than 50 cells/mm^3 were shown to be more likely to have MDR TB with 56.9% (103/181) in the CD4 count of 50-350 cells/mm^3 category and 50.9% (29/57) in the CD4 count more than 350 cells/mm^3 having MDR TB respectively (p-0.002).

Discussion

Our data suggests that a rifampicin resistant TB result on the X-pert MTB/Rif test does not always infer that MDR TB is present when patients are investigated subsequently and sputum is subjected to drug susceptibility testing. Moreover, it appears from our data that patients with a CD4 count below 50 cells/mm^3 were more likely to have rifampicin mono-resistance on confirmatory drug sensitivity testing.

In only half of patients with rifampicin resistance on X-pert MTB/Rif was MDR TB subsequently confirmed. This contrasts to a study by Dlamini-Mvelase et al from Kwa-Zulu Natal (KZN), where rifampicin resistance on X-pert correctly predicted MDR TB in 130/180 (72.2%) patients when DST was done through MTBDR plus and in 81.4% when phenotypic DST was done[15].

Similarly, in Cape Town, Osman et al found that rifampicin resistance on X-pert MTB/Rif correctly predicted MDR TB in 88.6% of patients with genotypic DST (MTBDR plus) done on 159 patients and phenotypic DST for isoniazid resistance done on four patients[16].

Isoniazid mono-resistance was detected rarely in contrast to an initial report that isoniazid resistance is more common than rifampicin resistance[17]. This may be an under-estimate of the INH mono-resistant cases as the result is not a representation of all the mycobacterium tuberculosis cases because the entry point to this study was rifampicin resistance on the X-pert. The other reason for the under-estimate is that most of the confirmatory testing was reported on genotypic testing, therefore, other mutations that confer INH resistance may have not been covered by the test.

The high prevalence of rifampicin mono-resistance significantly associated with a low CD 4 count in this study agrees with observations made in the Western Cape and KZN of an emerging phenomenon of an increase in cases of rifampicin monoresistance[18-19].

A likely explanation may be malabsorption of TB drugs, especially of rifampicin in patients with diarrhoea and cryptosporidium as they showed that serum levels of rifampicin were lower than those of other anti-TB drugs measured after oral administration of these drugs,

although the study did not show that these patients developed MDR TB, malabsorption of anti-TB drugs may contribute to drug resistant TB[20].

Due to INH resistance being polymorphic, MTB DR plus has been shown to misdiagnose certain cases as INH sensitive[21], hence the importance of phenotypic DST of all the INH sensitive cases to confirm or rule out sensitivity to INH.

Limitations of this study include the fact that this is a retrospective study with missing data and lack of universal subjection of all specimens to both genotypic and phenotypic drug sensitivity testing as well as lack of information on resistance to second line anti-TB drugs due to the employment of the genotypic drug sensitivity testing. Another limitation is that for 210 cases, confirmatory drug susceptibility testing results were not available either because the specimen was lost or the test was not done, and this calls for improvement in the system of collection, transport, submission and tracing of the results as this will improve patient care.

Conclusion

Rifampicin resistance on X-pert MTB/Rif does not always mean the patient has multidrug resistant tuberculosis. Our findings need to be confirmed in a prospective study involving other sites in South Africa. However, a large proportion of patients with rifampicin resistant TB on X-pert MTB/Rif who have rifampicin mono-resistance should continue to receive isoniazid as part of their treatment regimen while awaiting confirmatory culture and drug susceptibility testing, especially if their CD4 count is less than 50.

Appendices

Figure1. Study diagram showing the number of X-pert MTB/Rif positive patients included and analysed in the study and their drug susceptibility outcome of initial confirmatory sputum.

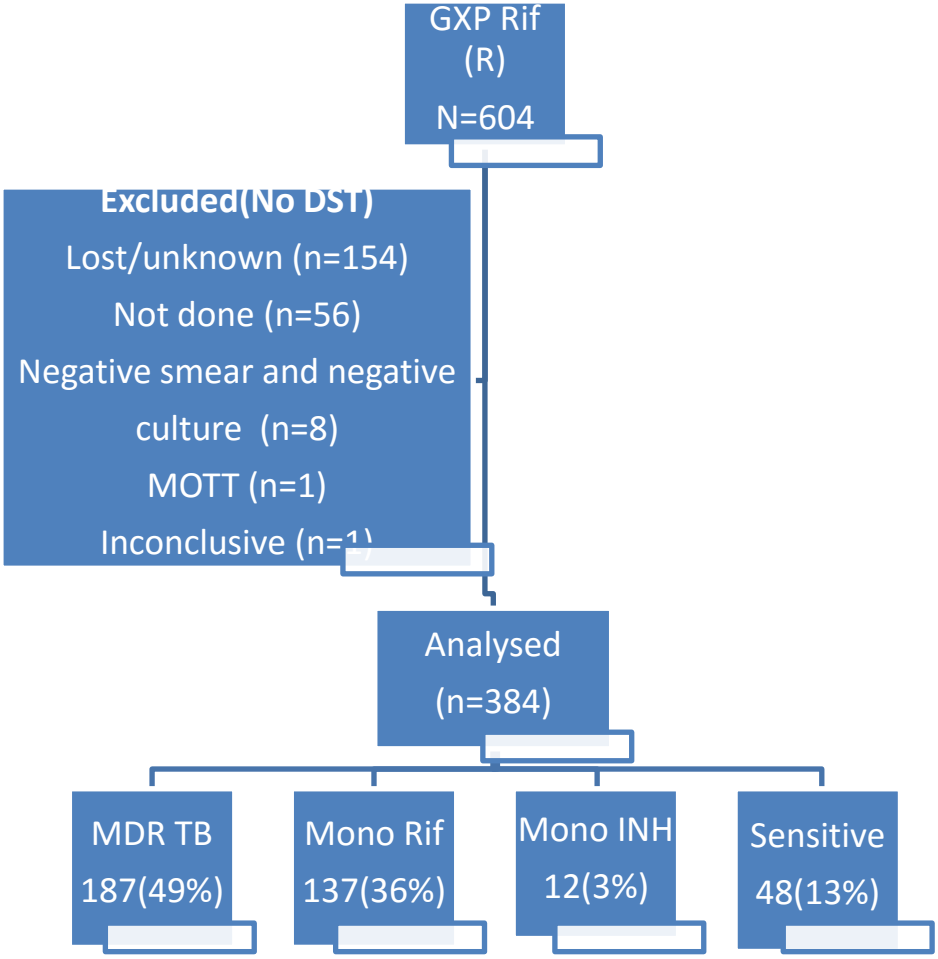

Table1. Patient characteristics and drug susceptibility results in 384 patients with initial X-pert MTB/Rif positive for rifampicin resistance.

Variable	Characteristic	All Patients	MDR	Mono (Rif)	Mono (INH)	Sensitive	P-Value
	n=	384	187	137	12	48	
Age (years)	mean years	36.74	37.12	37.58	30.50	34.42	0.076
HIV Status	Positive	315 (82.0)	154 (82.4)	114 (83.2)	11 (91.7)	36 (75)	0.474
	Negative	69 (18.0)	33 (17.6)	23 (16.8)	1 (8.3)	12 (25)	
CD4	< 50 cells/mm^3	74 (23.5)	20 (13.0)	37 (32.5)	4 (36.4)	13 (36.1)	0.012
	50 – 350 cells/mm^3	181 (57.5)	103 (66.9)	58 (50.9)	4 (36.4)	16 (44.4)	
	> 350 cells/mm^3	57 (18.1)	29 (18.8)	18 (15.8)	3 (27.3)	7 (19.4)	
	Unknown	3 (1.0)	2 (1.3)	1 (0.9)	0 (0.0)	0 (0.0)	
Viral Load	< 2 log	1 (0.3)	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)	0.685
	2 >= 2 log	93 (29.5)	39 (25.3)	38 (33.3)	4 (36.4)	12 (33.3)	
	Undetectable	44 (14.0)	20 (13.0)	19 (16.7)	1 (9.1)	4 (11.1)	
	Not done	177 (56.2)	95 (61.7)	56 (49.1)	6 (54.5)	20 (55.6)	
HAART	Yes	187 (59.6)	84 (54.5)	74 (65.5)	8 (72.7)	21 (58.3)	0.105
	No	124 (39.5)	70 (45.5)	36 (31.9)	3 (27.3)	15 (41.7)	
	Unknown	3 (1.0)	0 (0.0)	3 (2.7)	0 (0.0)	0 (0.0)	
Defaulted HAART	Yes	45 (23.6)	16 (18.8)	22 (28.6)	2 (25)	5 (23.8)	0.708
	No	145 (75.9)	69 (81.2)	54 (70.1)	6 (75)	16 (76.2)	
	Unknown	1 (0.5)	0 (0)	1 (1.3)	0 (0)	0 (0)	
Previous TB	Yes	185 (48.2)	84 (44.9)	80 (58.4)	6 (50)	15 (31.3)	0.018
	No	197 (51.3)	103 (55.1)	56 (40.9)	6 (50)	32 (66.7)	
	Unknown	2 (0.5)	0 (0.0)	1 (0.7)	0 (0.0)	1 (2.1)	
Previous TB treatment default	Yes	16 (8.6)	6 (7.1)	9 (11.3)	0 (0.0)	1 (6.7)	0.809
	No	169 (90.9)	79 (92.9)	70 (87.5)	6 (100.0)	14 (93.3)	
	Unknown	1 (0.5)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	
Previous TB drug resistance detected	Yes	7 (3.8)	2 (2.4)	5 (6.3)	0 (0.0)	0 (0.0)	0.497
	No	176 (95.1)	82 (97.6)	73 (91.3)	6 (100)	15 (100)	
	Unknown	2 (1.1)	0 (0.0)	2 (2.5)	0 (0.0)	0 (0.0)	
Previous sensitive	Yes	181 (97.8)	82 (97.6)	78 (97.5)	6 (100)	15 (100)	0.539
	No	2 (1.1)	2 (2.4)	0 (0)	0 (0)	0 (0)	
	Unknown	2 (1.1)	0 (0)	2 (2.5)	0 (0)	0 (0)	
Interspecimen interval	1 - 14 days	209 (55)	103 (55.4)	72 (52.9)	8 (66.7)	26 (56.5)	0.673
	> 14 days	127 (33.4)	61 (32.8)	45 (33.1)	3 (25)	18 (39.1)	
	Unknown	44 (11.6)	22 (11.8)	19 (14.0)	1 (8.3)	2 (4.3)	

Admission to culture collection	< 2 days	199 (52.2)	93 (49.7)	77 (56.6)	7 (58.3)	22 (47.8)	0.256
	>=2 days	139 (36.5)	73 (39)	40 (29.4)	4 (33.3)	22 (47.8)	
	Unknown	43 (11.3)	21 (11.2)	19 (14.0)	1 (8.3)	2 (4.3)	
Sputum culture collection to positive result interval	1 - 14 days	185 (48.2)	90 (48.1)	66 (48.2)	7 (58.3)	22 (46)	0.777
	> 14 days	140 (36.4)	69 (36.9)	50 (36.5)	4 (33.3)	17 (35.4)	
	Unknown	45 (11.7)	22 (11.8)	20 (14.5)	1 (8.3)	2 (4.3)	
	Median(IQR)	13(10)	14(11)	14(10.25)	14(11.5)	12(10.75)	
Mycobacterial Culture	Positive for *M tuberculosis*	375 (97.7)	183 (97.9)	136 (99.3)	12 (100)	44 (91.7)	0.046
	Negative	6 (1.6)	2 (1.1)	1 (0.7)	0 (0)	3 (6.3)	
	Not done /Unknown	2 (0.5)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	
	Lost	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)	
Hain on culture_1	MDR	176 (45.8)	176 (94.1)	0 (0)	0 (0)	0 (0)	0.000
	Mono (Rif)	136 (36)	0 (0.0)	136 (1)	0 (0)	0 (0)	
	Mono (INH)	12 (3.2)	0 (0.0)	0 (0)	12 (100.0)	0 (0)	
	Sensitive	44 (11.6)	0 (0.0)	0 (0.0)	0 (0.0)	44 (91.7)	
	Lost/ Unknown	4 (1.1)	2 (1.1)	0 (0.0)	0 (0.0)	2 (4.2)	
	Not done	3 (0.8)	1 (0.5)	0 (0.0)	0 (0.0)	2 (4.2)	
Auramine stain (Acid Fast Bacilli)	Positive	243 (63.3)	114 (61)	84 (61.3)	10 (83.3)	35 (72.9)	0.199
	Negative	141 (36.7)	73 (39)	53 (38.7)	2 (16.7)	13 (27.1)	
Hain on raw sputum	MDR	5 (1.3)	5 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.000
	Mono (Rif)	1 (0.3)	0 (0.0)	1 (0.8)	0 (0.0)	0 (0.0)	
	Sensitive	4 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (8.7)	
	Not done	363 (97.3)	179 (97.3)	130 (99.2)	12 (100.0)	42 (91.3)	
Phenotypic DST	MDR	6 (1.6)	6 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	0.000
	Mono (Rif)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Sensitive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Not done	378 (98.4)	181 (96.7)	137 (100.0)	12 (100.0)	48 (100.0)	

Figure2. MDR TB cases distributed according to the drug sensitivity testing method used.

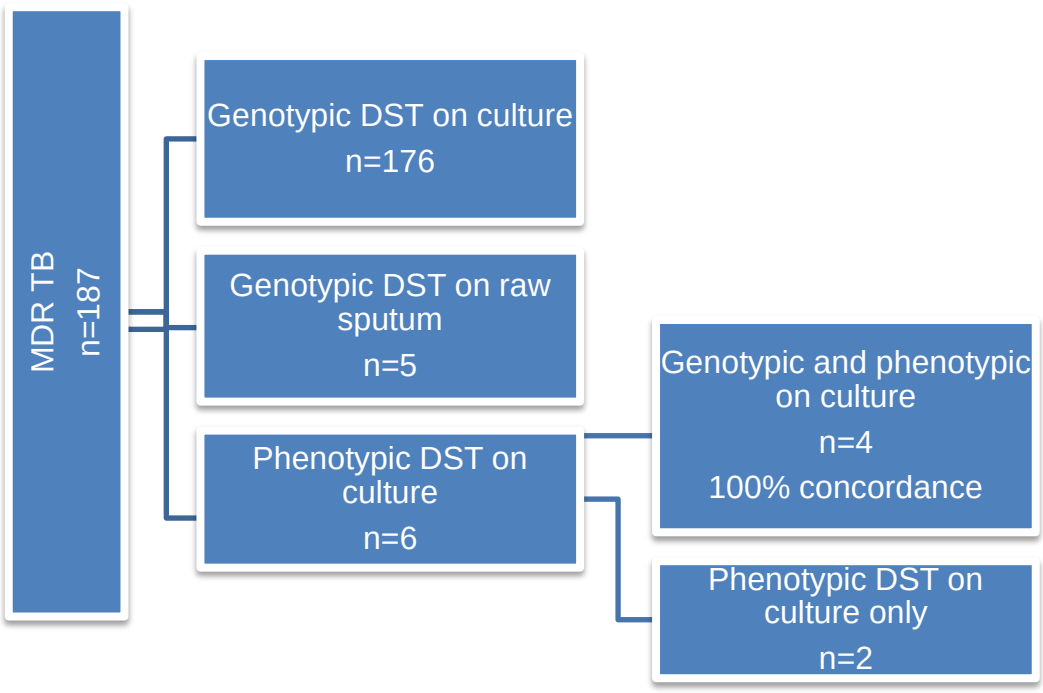

Table2. Results of confirmatory sputum TB drug susceptibility testing.

Category	Frequency	Percent
MDR	187	49%
Mono (Rif)	137	36%
Mono (INH)	12	3%
Sensitive	48	13%
Total	384	100%

Figure3. The median incubation time.

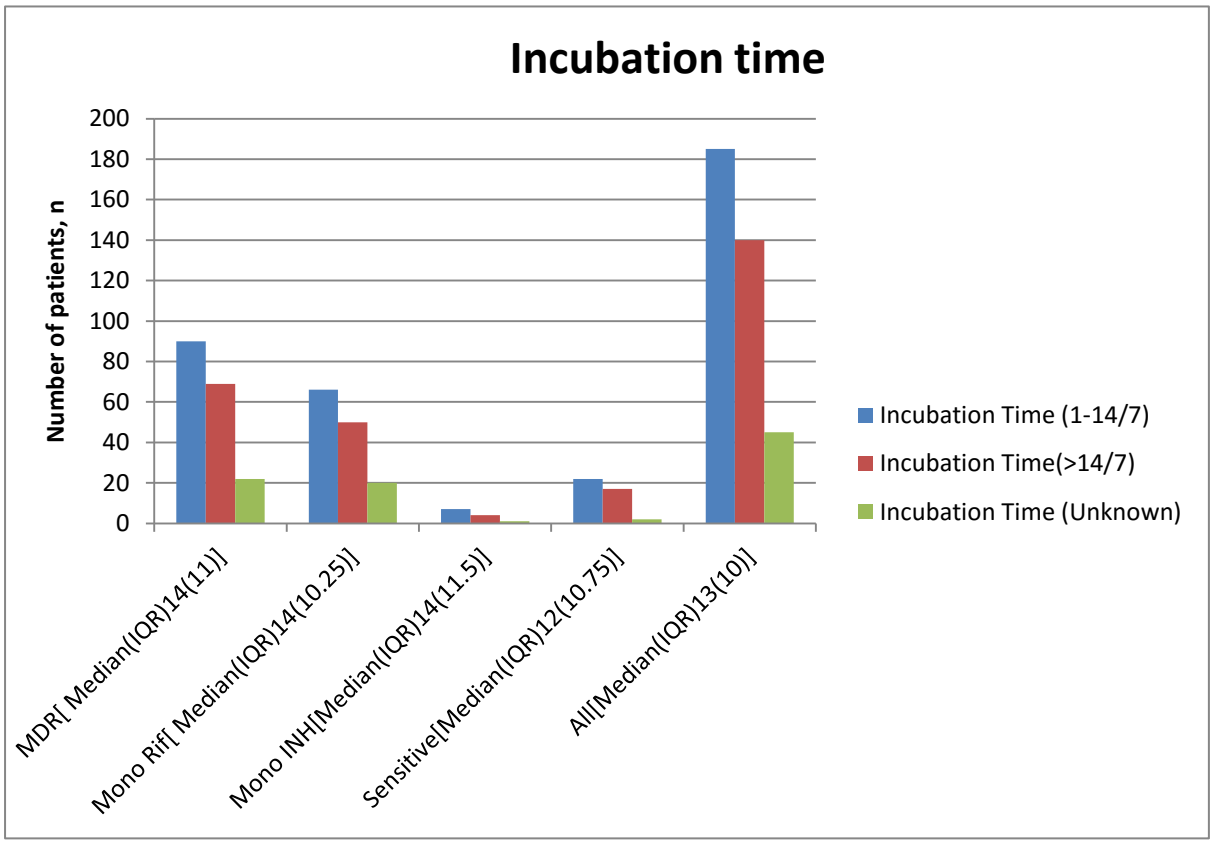

Table3. Association between CD4 count and sputum drug susceptibility testing outcome.

	CD4			Total	P - Value
	< 50	50 - 350	> 350		
MDR	20 (27.0)	103 (56.9)	29 (50.9)	152 (48,7)	
Mono (Rif)	37 (50.0)	58 (32.0)	18 (31.6)	113 (36,2)	0,002
Mono (INH)	4 (5.4)	4 (2.2)	3 (5.3)	11 (3,5)	
Sensitive	13 (17.6)	16 (8.8)	7 (12.3)	36 (11,5)	

References

1. World Health Organization (2016). Drug- resistant tuberculosis. Available at: http://www.who.int/tb/areas-of-work/drug-resistant-tb/en/. [Accessed 07 April 2016].

2. Zhang Y Yew W. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis. 2009; 13(11):1320-30.

3. Sharma SK, Mohan A. Multidrug resistant tuberculosis, a menace that threatens to destabilise tuberculosis control. CHEST. 2006; 130 (1): 261-272.

4. Wells CD, Cegielski JP, Nelson L J et al. HIV infection and multidrug resistant tuberculosis- The Perfect Storm. J Infect Dis. 2007; 196(1): S86-S107.

5. Gurumurthy, P, Ramachandran, G, Kumar AKH et al. Decreased Bioavailability of Rifampin and Other Antituberculosis Drugs in Patients with Advanced Human Immunodeficiency Virus Disease. Antimicrob Agents Chemother. 2004; 48 (11): 4473–4475.

6. Conaty SJ, Hayward AC, Story A et al. Explaining risk factors for drug resistant tuberculosis in England and Wales: contribution of primary and secondary drug resistance. Epidemiol Infect. 2004; 132 (6): 1099–1108.

7. SA TB DRS (2012-14). 12 July 2016.

8. Wood, R. Challenges of TB Diagnosis and treatment in South Africa (2007). S Afr J HIV Med. 2007; pp 44-48. Available at: http://www.ajol.info/index.php/sajhivm/article/download/ [Accessed 07 April 2016].

9. World Health Organization.TB Facts.org. Global Health Education. Available at: http://www.tbfacts.org/ghe.html [Accessed on 03 April 2012].

10. Department of health, Republic of South Africa. Management of drug resistant tuberculosis policy guidelines. 2012. Available at: http://www.doh.gov.za/docs/policy/2012/TBpolicy.pdf. [Accessed 16 April 2013].

11. Boehme CC, Nabeta P, Hillemann D et al. Rapid Molecular Detection of Tuberculosis and Rifampicin Resistance. N Engl J Med. 2010; 363 (11):1005-1015.

12. Steingart KR, Sohn H, Schiller I et al. X-pert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (2013). Cochrane database of Syst Rev. 2013: 31(1).

13. Schnippel K, Rosen S, Shearer K et al. Costs of inpatient treatment for multi-drug-resistant tuberculosis in South Africa. Tropical Medicine and International Health. 2013; 18(1): 109-116.

14. Jacobs TQ, Ross A. Adverse effects profile of multidrug-resistant tuberculosis treatment in a South African outpatient clinic . S Afr Fam Pract. 2012; 54(6):531-539.

15. Dlamini-Mvelase NR, Werner L, Phili R, Cele LP, Mlisana KP. Effects of introducing X-pert MTB/RIF test on multi-drug resistant tuberculosis diagnosis in KwaZulu-Natal

South Africa. BMC Infectious Diseases. 2014; 14(442). Available at http://www.biomedcentral.com/ [Accessed 07 April 2016]).

16. Osman M, Simpson JA, Caldwell J et al. GeneXpert MTB/RIF Version G4 for Identification of Rifampin-Resistant Tuberculosis in a Programmatic Setting. J Clin Microbiol.2014; 52 (2): 635–637.

17. Weyer K, Brand J, Lancaster J et al. Determinants of multidrug-resistant tuberculosis in South Africa: results from a national survey. S Afr Med J. 2007; 97(11). 1120-1128.

18. Mukinda FK, Theron D, van der Spuy GD et al. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. Int J Tuberc Lung Dis. 2012; 16:196–202.

19. Coovadia YM, Mahomed S, Pillay M et al. Rifampicin mono-resistance in Mycobacterium tuberculosis in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. PLoS One 2013, 8(11):e77712.

20. Gurumurthy P, Ramachandran G, Kumar A K H et al. Decreased Bioavailability of Rifampin and Other Antituberculosis Drugs in Patients with Advanced Human Immunodeficiency Virus Disease. Antimicrob Agents Chemother. 2004; 48 (11): 4473–4475.

21. Ling DI, Zwerling AA, Pai M. Genotype MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. Eur Respir J. 2008; 32: 1165–1174.