Abstract

Background

The introduction of the X-perf 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-perf 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-perf 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-perf 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 spuma (MTBDR plus on smear) of 5(1.3%) patients. Therefore the total number of confirmed MDR TB cases was 187(48%). 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/mm³ 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/mm³, 58/181(32%) had rifampicin mono-resistance and 4/181(2.2%) had isoniazid mono-resistance (p=0.012).

Conclusion

Rifampicin resistance on X-perf 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-perf 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 vitro². Several gene mutations confer resistance to anti-
tuberculosis medications The more common mutation on the *katG* gene is a mechanism of isoniazid (INH) resistance.

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. Activation results in the production of highly reactive species such as superoxide, peroxide, hydroxyl radical, nitric oxide and isonicotinoyl-acetyl radicals. The isonicotinoyl radical 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.

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. Rif resistance is diagnosed by identifying mutations in the *rpoB* gene.

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.

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 diarrhea or infection with cryptosporidium. 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).

Globally, the frequency of MDR TB is 3.3% among new cases (primary resistance) with the majority found in Eastern Europe and Asia. The frequency of MDR TB among previously treated cases (secondary resistance) is 20%. In 2014, there was an estimated 480000 new MDR TB cases and approximately 190000 deaths from MDR TB worldwide. In South Africa, the South African Tuberculosis Drug Resistance survey 2012-2 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.

Of concern is the diagnostic gap where many MDR TB cases go undiagnosed. 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\(^3\).

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\(^6\).

Phenotypic 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\(^10\).

Xpert 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, Xpert MTB/Rif-Cepheid Inc. achieved a pooled sensitivity of 98% 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, Xpert 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 60% for smear negative culture positive sputum\(^12\). For rifampicin resistance detection, the pooled sensitivity was 94% with a specificity of 96%\(^12\). The higher the prevalence of rifampicin resistance, the lower the probability of the Xpert 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\textsuperscript{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\textsuperscript{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 Municipality 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-per 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-per 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-per 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-per 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 29th 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/mm³.

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/mm³, 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/mm³ were shown to be more likely to have MDR TB with 56.9% (103/181) in the CD4 count of 50-350 cells/mm³ category 0% 50.9% (29/57) in the CD4 count more than 350 cells/mm³ 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/mm³ were more likely to have rifampicin mono-resistance on confirmatory drug sensitivity testing.
In only half of patients with rifampicin resistance on X-per XTB/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-per 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 done15.

Similarly, in Cape Town, Osman et al found that rifampicin resistance on X-per 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 patients16.

Isoniazid mono-resistance was detected rarely in contrast to an initial report that isoniazid resistance is more common than rifampicin resistance17. 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-per. 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 CD4 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 monoresistance18-19.

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 TB20.

Due to INH resistance being polymorphic, MTB DR plus has been shown to misdiagnose certain cases as INH sensitive21, 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-per XTB/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-per XTB/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.
11% SIMILARITY INDEX