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Evolving rifampicin and isoniazid mono-resistance in a high MDR and XDR TB region: a retrospective data analysis

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Manuscripts

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3 **Evolving rifampicin and isoniazid mono-resistance in a high MDR and XDR TB region:**
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5 **a retrospective data analysis**
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46 Running Title: Drug resistant TB in KwaZulu-Natal
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Abstract

Objectives: South Africa ranks among the highest drug resistant tuberculosis burdened countries in the world. This study assessed the changes in resistance levels in culture confirmed *Mycobacterium tuberculosis* (MTB) in the highest burdened province of South Africa during a period where major changes in diagnostic algorithm were implemented.

Setting: This study was conducted at the central academic laboratory of the KwaZulu-Natal province of South Africa.

Participants: We analysed data for all MTB cultures performed in the KwaZulu-Natal province between 2011 and 2014. The data were collected from the laboratory information system.

Results: Out of 88 559 drug susceptibility results analysed, 18352 (20.7%) were resistant to rifampicin and 19190 (21.7%) showed resistance to isoniazid. The proportion of rifampicin resistant cases that were mono-resistant increased from 15.3% in 2011 to 21.4% in 2014 while INH mono-resistance showed a range between 13.8% and 21.1%. The MDR TB rates increased from 18.8% to 23.9% and the proportion of MDR TB cases that had XDR TB remained between 10.2% and 11.1%. Most drug resistance was found in females between the ages of 15 to 44 years and the northern districts bordering high MDR TB regions had the highest MDR TB rates.

Conclusion: Our findings show increasing rifampicin mono-resistance and a substantial amount of INH mono-resistance. This highlights a need for an initial test that detects resistance to both these drugs so as to avoid using rifampicin monotherapy during continuous phase of treatment in patients with INH mono-resistance. Furthermore, addition of isoniazid will benefit patients with rifampicin mono-resistance. Although DR TB is widespread, HIV

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3 24 and migration influence its distribution; therefore, TB control strategies should include
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5 25 interventions that target these aspects.
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Strengths and limitations of this study

- The study was performed in the country with the highest TB incidence rate and a largest HIV epidemic in the world.
- The analysed data involves a period of major shift in TB diagnostic algorithm.
- The patient level Xpert MTB/RIF data was not available in order to compare with the TB culture results.
- The absence of unique patient identifiers also affects the accuracy of the data as the removal of duplicates was imperfect.

Background

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29 The World Health Organisation (WHO) has declared multidrug resistant tuberculosis (MDR
30 TB) a global crisis. Despite the decline in the global incidence rates of tuberculosis (TB), drug
31 resistant TB cases are on the rise with 600 000 estimated incident cases of MDR plus rifampicin
32 resistant (RR) TB and more than 240 000 deaths in 2016 [1]. South Africa has the highest
33 incidence of TB in the world which WHO estimated to be 781 per 100 000 in 2016 [1]. In 2016
34 alone, South Africa had an estimated number of 19 000 rifampicin resistant cases, the second
35 highest number in Africa after Nigeria which has more than three times the South African
36 population [1]. Moreover, the first reported outbreak of extensively drug-resistant tuberculosis
37 (XDR TB) which caused global concern in 2005 was from the province of KwaZulu-Natal
38 (KZN) in South Africa [2]. While the incidence of TB in KZN is proportional to other provinces

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3 39 in the country, it remains the highest drug resistant (DR) TB burdened province with almost a
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5 40 third of the country's cases of drug resistant TB [3].
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10 42 Compounding the problem of TB in South Africa, is the high rate of co-infection with HIV
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12 43 (about 60%) [1]. While it is well known that HIV is associated with smear negative TB, smear
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14 44 microscopy was traditionally used in the initial diagnosis of TB because of its quick time to
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16 45 results and low cost [4]. On the other hand, conventional TB culture is much more sensitive
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18 46 than smear microscopy, but its high cost, complexity and long delays in getting the results
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20 47 made it impractical for routine diagnosis of TB. Therefore, when the WHO endorsed the Xpert
21
22 48 MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was subsequently introduced
23
24 49 in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated nucleic acid
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26 50 amplification test that offers better detection of TB compared to smear microscopy with an
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28 51 added advantage of the ability to detect rifampicin resistance in less than two hours in clinical
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30 52 specimens [5-6].
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37 54 The implementation of Xpert in South Africa completely changed the testing algorithm for the
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39 55 diagnosis of TB [7]. Xpert replaced smear microscopy in the initial diagnosis of TB and all
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41 56 patients that do not demonstrate rifampicin resistance are assumed to have drug susceptible TB
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43 57 and therefore initiated on standard first line TB therapy. Thus, Xpert rifampicin susceptible
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45 58 cases do not get a culture, so isoniazid mono-resistance is not routinely investigated. TB culture
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47 59 and drug susceptibility testing (DST) is only indicated for patients that demonstrate rifampicin
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49 60 resistance on the Xpert, paucibacillary TB cases missed by Xpert (HIV infected, children and
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51 61 extra-pulmonary TB) and patients that fail TB treatment.
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3 63 Despite the recent changes in the diagnosis and management of TB, there are no studies that
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5 64 have assessed their impact on culture confirmed TB. The level of isoniazid mono-resistance
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8 65 that is not routinely investigated with current diagnostic methods is unknown, but instead these
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10 66 patients are getting rifampicin monotherapy during the continuous phase of their first line TB
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12 67 therapy which could potentially fuel drug resistance. On the other hand, patients with
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14 68 rifampicin mono-resistance can benefit from the addition of isoniazid in their treatment. We
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17 69 therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-
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19 70 resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the
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21 71 drug resistance patterns and distribution among different age groups, genders and districts in
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23
24 72 KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will
25
26 73 inform targeted intervention in TB control in this high TB endemic region.
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32 75 **Methods**

33 34 35 76 **Study design**

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38 77 The study is a retrospective observational study using laboratory data for 2011 till 2014.
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42 43 79 **Study setting**

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45 80 The KZN province is one of nine provinces in South Africa and its population of just over 10
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47 81 million ranks second in the country. There are 77 public health hospitals (including 8 MDR TB
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49 82 initiation sites) within 11 health districts. Provincial *Mycobacterium tuberculosis* culture and
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51 83 drug susceptibility testing are performed in one central academic laboratory.
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56 57 85 **Laboratory Procedures**

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3 86 MTB isolation from clinical samples was routinely done using the automated BACTEC
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5 87 mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton
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8 88 Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain
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10 89 Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard
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12 90 methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin,
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14 91 streptomycin, kanamycin was performed for all cases with resistance to rifampicin or isoniazid
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16 92 on the LPA using 1% agar proportion method (APM) on Middlebrook 7H10.
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23 94 **Patient and public involvement**

24 95 The data used for this study was the routine TB diagnostic data, therefore there was no direct
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26 96 patient and public involvement.
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31 98 **Data collection and analysis**

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33 99 The TB culture and DST data was collected from the National Health Laboratory Service
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35 100 laboratory information system (LIS) which contains all electronic laboratory results. In the
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37 101 absence of a unique identifier, duplicates were removed using demographic data. The results
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39 102 were stratified according to the health districts, age and gender. For the analysis of age, cases
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41 103 without recorded age or date of birth were excluded.
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47 105 Data was described using frequencies and proportions. Continuous data was described using
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49 106 means and standard deviations (sd). Categorical outcomes were tested using the chi-squared
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51 107 test. Log binomial regression of MDR and XDR TB was performed using sex, age group,
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53 108 district and year as predictors to estimate the adjusted relative risk ratios. Data was analysed
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55 109 using Stata 14 (StataCorp., College Station, TX, USA).
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111 **Ethical consideration**

112 The data used for the study is routine data for management of TB patients; therefore, no
113 individual patient consent was required. The ethical approval to perform the retrospective
114 analysis was obtained from the Biomedical Research Ethics Committee of the University of
115 KwaZulu-Natal (REF: BE085/12).

117 **Results**

118 Between 2011 and 2014, a total of 951 209 cultures were performed for MTB in KwaZulu-
119 Natal. The total number of specimens for which culture was requested, decreased annually with
120 the average percentage decline of 14.2% (sd 11.3%) per year. Similarly, the MTB positivity
121 rate decreased by 6.0% (from 15.6% to 9.6%) (Figure 1). After removing duplicates, there were
122 36644, 30208, 22568, 14672 culture confirmed cases of TB in 2011, 2012, 2013 and 2014
123 respectively. The average percentage decline in total positives TB cases was 27.1% (sd 9.5%)
124 per year.

125 About 85% (88559) cases of culture positive TB had an LPA done to test for drug susceptibility
126 against RIF and INH (Table 1). Of these, 19190 (21.7%) were resistant to INH and 18352
127 (20.7%) were resistant to RIF. There were 953 RIF mono-resistant (RMR) cases in 2011, 767
128 in 2012, 676 in 2013 and 667 in 2014. The proportion of RMR out of all RIF resistant cases
129 increased from 15.3% in 2011 to 21.4% in 2014. Over the same four-year period, there were
130 3396 (17.7%) INH mono-resistance (IMR) cases.

131 A steady decline of both MDR and XDR TB cases was noted, with an overall decline of 49.2%
132 (from 6901 in 2011 to 3506 in 2014) and 44.9% (from 706 in 2011 to 389 in 2014) respectively
133 (Table 2). The proportion of TB cases that had MDR TB ranged from 18.8% in 2011 to 23.9%
134 in 2014, with an overall average of 21%. The overall rate of XDR TB among MDR TB cases
135 was 11% (2336 XDR TB cases out of 21221 MDR TB).

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3 136 The majority of the TB cases were males; however, females constituted highest prevalence of
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5 137 the DR TB across all age groups (Fig 2 and 3). The number of MDR TB cases was higher
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7 138 among females than males until the age of 34, thereafter males had a higher number than
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9 139 females. Similarly, for XDR TB, females constituted the most number of XDR TB. More than
10
11 140 60% of both MDR and XDR TB cases were patients between the ages of 25 and 44 years. It
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13 141 was observed that children less than 5 years of age showed the lowest rates of MDR TB whilst
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15 142 that of XDR TB was lowest between the ages of 6-14 years.

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20 143 Over the 4-year period, eThekweni district had the highest number of TB cases with 47.5% of
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22 144 all cases in KZN coming from this district (Table 2). However, the districts with the highest
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24 145 yearly proportion of MDR TB cases each year were Umkhanyakude (mean 33.2%, sd 2.3%),
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26 146 followed by Zululand (mean 28.1%, sd 2.3) and Harry Gwala (mean 26.2%, sd 9.4). The yearly
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28 147 proportion of MDR TB cases that had XDR TB were highest at Umzinyathi (mean 36.4%, sd
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30 148 5.8%) followed by eThekweni (mean 13.7%, sd 1.1%) and Uthukela (mean 13.2%, sd 5.7%)
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32 149 districts. Umkhanyakude district had the lowest proportion of XDR with a yearly mean of
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34 150 3.4% (sd 2.4%) over the study period (Figure 4).

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152 Discussion

153 In this study we observe a decline in the number of samples processed for MTB culture and
154 culture positivity rate which coincided, with the roll out of the Xpert. This is in keeping with
155 the Xpert roll out which started in March 2011 and was completed in September 2013 when
156 all health facilities in the provinces were using the Xpert for TB diagnosis. According to the
157 South African guidelines, MTB culture is not recommended for Xpert rifampicin susceptible
158 patients, which constitutes the majority of patients infected with TB, hence the decline in the
159 number of MTB cultures from 277 963 in 2011 to 172 671 in 2014. Nevertheless, the sheer

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3 160 volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR
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5 161 TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only
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7 162 performed on patients that were considered to be at risk of DR TB, but the use of Xpert for
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9 163 initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised
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11 164 indications for culture selects for cases that are more likely to have drug resistant (Xpert
12
13 165 rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert
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15 166 negative), which explains the high rates of DR TB and declining culture positivity rate observed
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17 167 in this study.
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24 169 Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In
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26 170 addition, spontaneously occurring mutations are rare compared to other TB drugs [8].
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28 171 Consequently, the development of RMR is expected to be uncommon. The finding of
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30 172 increasing mono-resistance in this context is therefore concerning. In a previous study done by
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32 173 Coovadia et al, at the same laboratory, RMR was 8.8% during the years 2007-2009 [9].
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34 174 Similarly, Mukinda et al reported increasing RMR in the Western Cape province of SA [10].
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36 175 These findings highlight the importance of testing for INH resistance in all patients with Xpert
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38 176 rifampicin resistance. This positively impacts patient management further as patients with
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40 177 confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment
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42 178 regimen.
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49 180 The development of rifampicin resistance has serious effects on the treatment of TB. Patients
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51 181 have to be treated with more expensive and more toxic drugs for a longer duration. Studies
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53 182 have been conducted in order to elucidate the causes of RMR with the majority reporting an
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55 183 association between HIV and RMR [11-12]. Factors contributing to this association include
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57 184 decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin
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3 185 serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to
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5 186 infection and permits proliferation of TB which favours transmission [14]. Given the high rate
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8 187 of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the
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10 188 increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in
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12 189 co-infected patients remains under investigation.

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17 191 Rifampicin and INH are core drugs that form the backbone for first line short course therapy
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19 192 for the treatment of drug susceptible TB. Given the high burden of disease in this region
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21 193 coupled with the use of Xpert as a screening tool for DR TB, mono-resistance to INH may
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23 194 inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is
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25 195 only made using TB culture and DST following a negative Xpert result or treatment failure.
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27 196 The use of standard first line TB therapy in patients with undetected INH resistance equates to
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29 197 using rifampicin monotherapy during the continuation phase. This may subsequently lead to
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31 198 the development of MDR TB. This was described in a study done by Jacobson et al where
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33 199 treatment of patients with IMR using standard first line therapy was associated with poor
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35 200 outcomes and progression to MDR TB [15]. Several studies have reported previous TB therapy
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37 201 as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients
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39 202 who may require TB culture and DST in order to exclude INH resistance.
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47 204 There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and
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49 205 XDR TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast
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51 206 to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in
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53 207 South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the
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55 208 national guidelines, a significant number of patients where Xpert detected rifampicin resistance
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57 209 did not get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO
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3 210 TB report, that reported the percentage of MDR TB among MDR/RR-TB as 62% in South
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5 211 Africa, which suggests that a substantial number of Xpert RR cases are not confirmed by
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7 212 culture as this discrepancy cannot be explained by RMR cases [1]. Hence this indicates a
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9 213 change in the testing method used to diagnose tuberculosis rather than a successful TB control
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11 214 program, which led to underestimation of MDR and XDR TB cases in this study. Other possible
12
13 215 reasons may include patient loss to follow up and loss of MTB viability (no growth on culture)
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15 216 which could be caused by long transport duration in specimens from remote areas. According
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17 217 to the WHO, only 41% of notified MDR/RR-TB cases from South Africa were enrolled for
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19 218 MDR TB treatment in 2013[18]. Although this figure improved to 62% in 2014 [20], the gap
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21 219 remains substantial especially given the considerable improvement in rapidity of diagnosing
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23 220 DR TB with Xpert. The proportion of MDR TB cases that have XDR TB also remained
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25 221 constant at about 11% which is comparable to the global trends at that time [18-20].
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33 223 Our study found higher rates of DR TB in women compared to men which supports findings
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35 224 from other studies showing higher proportions of DR TB in women [21-22]. Even though
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37 225 reasons behind the higher DR TB predisposition among women are unknown, HIV could be a
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39 226 contributing factor. The majority of DR TB cases were found between the ages of 15 and 44
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41 227 years, which is the same age group that is known to have the highest HIV prevalence [2]. It is
42
43 228 well recognised that HIV is a major risk factor for development of TB and HAART reduces its
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45 229 incidence [23-25]. Indeed, Nanoo et al showed an inverse relationship between antiretroviral
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47 230 therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with
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49 231 the greatest decline demonstrated in the 25-44 year age group [26].
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56 233 The diagnosis of DR TB in children is generally difficult due to their inability to expectorate
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58 234 and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB
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3 235 in children is largely as a result of primary transmission from adults, the proportion of DR TB
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5 236 is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR TB
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7 237 (Figure 2) particularly MDR TB in children less than five years compared to adults which could
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9 238 be a reflection of the under diagnosis of DR TB in this age group. Although these rates are
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11 239 lower in children, they are still unacceptable as they reflect transmission of untreated adult TB.
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17 241 Similar to the overall burden of TB in South Africa, DR TB is also concentrated in urban areas
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19 242 of KZN with eThekweni district harbouring most of the cases due to high population density.
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21 243 However, the rate of MDR TB cases was highest among the northern districts of the province
22
23 244 of Umkhanyakude and Zululand. These are rural districts which share borders with
24
25 245 Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance
26
27 246 patterns. Mpumalanga province is known to have the highest DR TB rate in the country while
28
29 247 Swaziland has the highest MDR TB prevalence in Africa [29-30]. In 2007, Wallengren et al
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31 248 reported Umzinyathi and Umkhanyakude as the districts with the highest MDR TB rates [31].
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33 249 The intervention given to the Umzinyathi district following the outbreak of XDR TB in 2005
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35 250 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and
36
37 251 treatment of HIV, TB infection control and intergration of TB and HIV care) may be
38
39 252 responsible for these decreasing rates [32]. Despite declining XDR TB rates at the Umzinyathi
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41 253 district (where the XDR TB outbreak was identified in 2005), it still remains the district with
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43 254 the highest XDR TB rates at about three times higher than the rest of KZN [33].
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256 **Limitations**

257 Our study is limited by the retrospective design; the accuracy of the data is dependent on
258 available information on the LIS. The absence of unique patient identifiers also affects the
259 accuracy of the data as the removal of duplicates is imperfect. Although duplicates were
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3 260 removed, we could not differentiate between new and known MDR TB patients. The patient
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5 261 level Xpert data was not available in order to match with the TB culture results. Nevertheless,
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7
8 262 the high burden of DR TB and the fact that all cultures are performed in one laboratory for the
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10 263 whole province provide an important insight to the distribution of TB in this region and may
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12 264 inform targeted intervention.
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17 266 **Conclusions**

19 267 Our findings highlight the importance of DR TB diagnostic algorithms that include both
20
21 268 rifampicin and isoniazid DST in the initial testing. Early detection of RMR will allow addition
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23
24 269 of isoniazid in the treatment regimen, while detection of IMR will prevent rifampicin
25
26 270 monotherapy later on during the continuation phase of treatment which has been associated
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28 271 with development of rifampicin resistance. This will also allow us to have a clearer estimate of
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30 272 MDR TB cases. HIV and migration play a significant role in the distribution of DR TB in this
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32
33 273 region, therefore TB control measures that address these factors may have impact on DR TB
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35 274 level.
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44
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46
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48
49 279 Research Unit within the South African Medical Research Council, for designing the provincial
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51 280 maps.
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58 282 **Author contributions**

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2
3 283 NRM contributed in the development of the concept, study design, data analysis and writing
4
5 284 of the manuscript. YB performed data analysis and assisted with the writing of the manuscript.
6
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8 285 KL contributed in the interpretation of data and writing of the manuscript. KPM supervised the
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10 286 development of the study concept, study design, data analysis and manuscript writing.
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16 288 **Data sharing statement:** No additional data available

19 289 **Competing interests:** None

22 290 **Funding:** None

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Table 1. LPA results between 2011 and 2014: RIF and INH mono-resistance*				
	2011	2012	2013	2014
Total culture positives	36644	30208	22568	14672
Total cases LPA	31368	26513	18399	12279
% of LPA done	85.6	87.8	81.5	83.7
LPA Any INH Resistance	6430	5548	4167	3045
LPA Any INH Resistance (%)	20.5	20.9	22.7	24.8
LPA INH MR	845	1167	879	505
LPA INH MR (% of All INH Resistant)	13.8	21.0	21.1	17.1
LPA Any RIF Resistance	6293	5013	3912	3134
LPA Any RIF Resistance (%)	20.1	18.9	21.3	25.5
LPA RIF MR	953	767	676	667
LPA RIF MR (% of All RIF Resistant)	15.1	15.3	17.3	21.3

418 *RIF, Rifampicin; INH, isoniazid; LPA, line probe assay; MR, mono-resistance

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Table 2. Total TB positive, MDR and XDR cases per district for each year.

District	2011					2012					2013					2014				
	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%
Amajuba	597	96	16.1	8	8.3	523	86	16.4	4	4.7	353	40	11.3	4	10.0	272	35	12.9	0	0.0
Ethekwini	17519	2837	16.2	353	12.4	13453	2306	17.1	338	14.7	11118	2212	19.9	321	14.5	7404	1516	20.5	203	13.4
Harry Gwala	776	124	16.0	5	4.0	752	191	25.4	13	6.8	526	186	35.4	4	2.2	396	142	35.9	12	8.5
Ilembe	1704	269	15.8	9	3.3	1219	206	16.9	16	7.8	978	180	18.4	7	3.9	405	76	18.8	6	7.9
Ugu	2172	542	25.0	37	6.8	2443	465	19.0	47	10.1	1655	388	23.4	34	8.8	1123	268	23.9	12	4.5
Umgungundlovu	3514	520	14.8	55	10.6	2858	532	18.6	57	10.7	1486	305	20.5	27	8.9	703	159	22.6	22	13.8
Umkhanyakude	2230	732	32.8	5	0.7	1619	501	30.9	29	5.8	1214	422	34.8	15	3.6	945	341	36.1	19	5.6
Umzinyathi	1478	334	22.6	134	40.1	1295	257	19.8	102	39.7	894	192	21.5	66	34.4	674	196	29.1	54	27.6
Uthukela	1100	120	10.9	16	13.3	1023	111	10.9	13	11.7	545	71	13.0	6	8.5	261	55	21.1	12	21.8
Uthungulu	2640	589	22.3	30	5.1	2737	590	21.6	41	6.9	2315	477	20.6	26	5.5	1178	294	25.0	12	4.1
Zululand	2888	735	25.5	54	7.3	2259	662	29.3	37	5.6	1468	428	29.2	34	7.9	1168	362	31.0	28	7.7

Unknown	26	3	11.5	0	0.0	27	5	18.5	0	0.0	16	1	6.3	0	0.0	143	62	43.4	9	14.5
Total	36644	6901	18.8	706	10.2	30208	5912	19.6	697	11.8	22568	4902	21.7	544	11.1	14672	3506	23.9	389	11.1

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* MDR, multidrug-resistant; XDR, extensively drug-resistant; TB, tuberculosis

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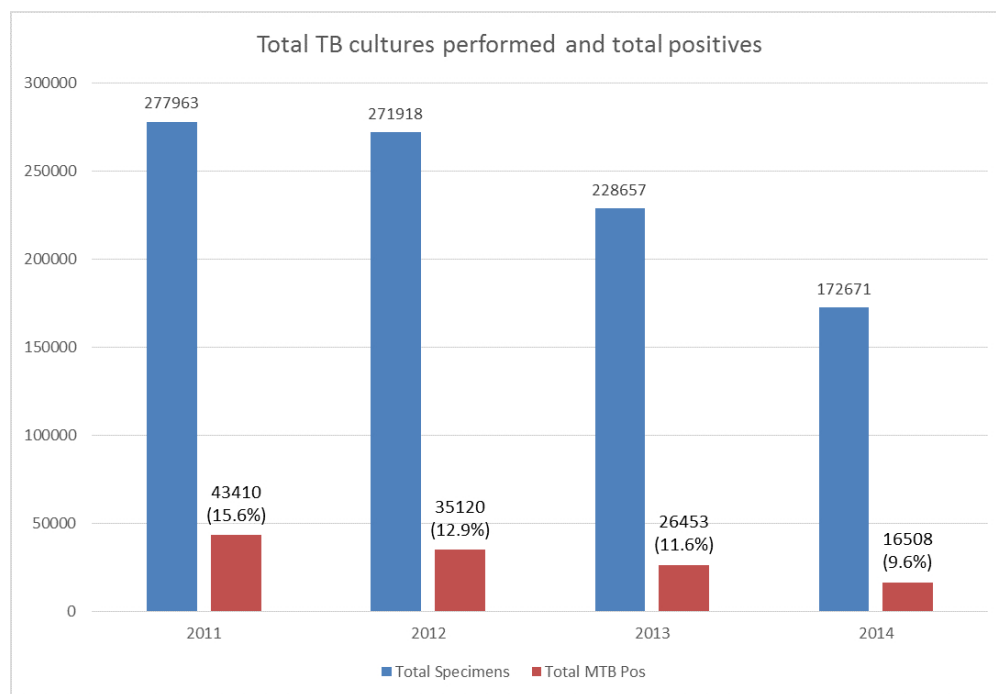


Figure 1: TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens received and the total number (and percentage) thereof that were positive.

184x127mm (150 x 150 DPI)

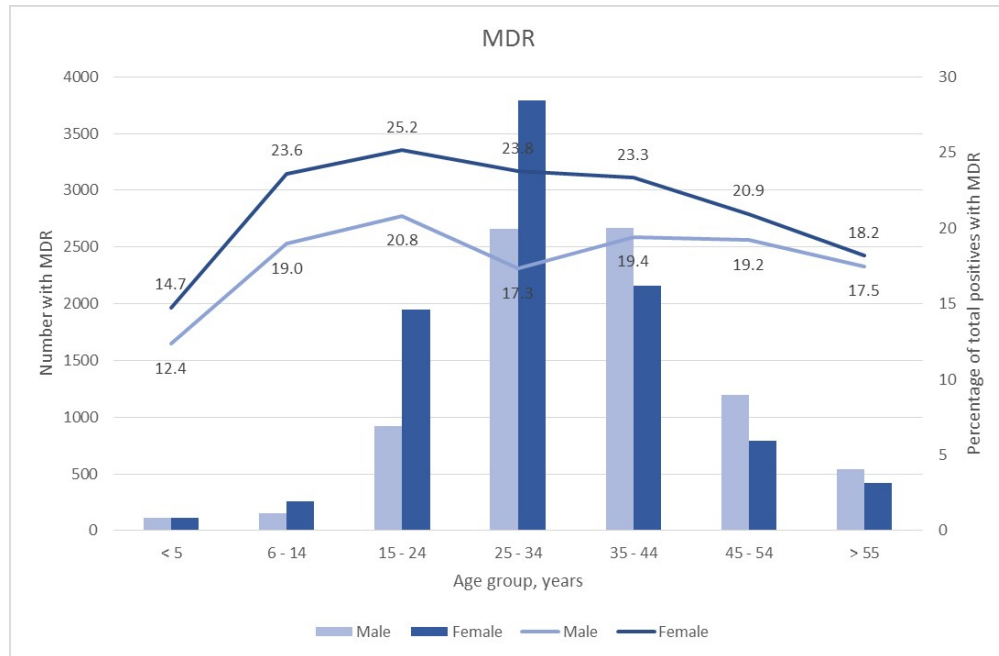


Figure 2: Distribution of MDR TB cases and proportions by gender. The number of MDR TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR TB of total positive cases by gender

184x120mm (150 x 150 DPI)

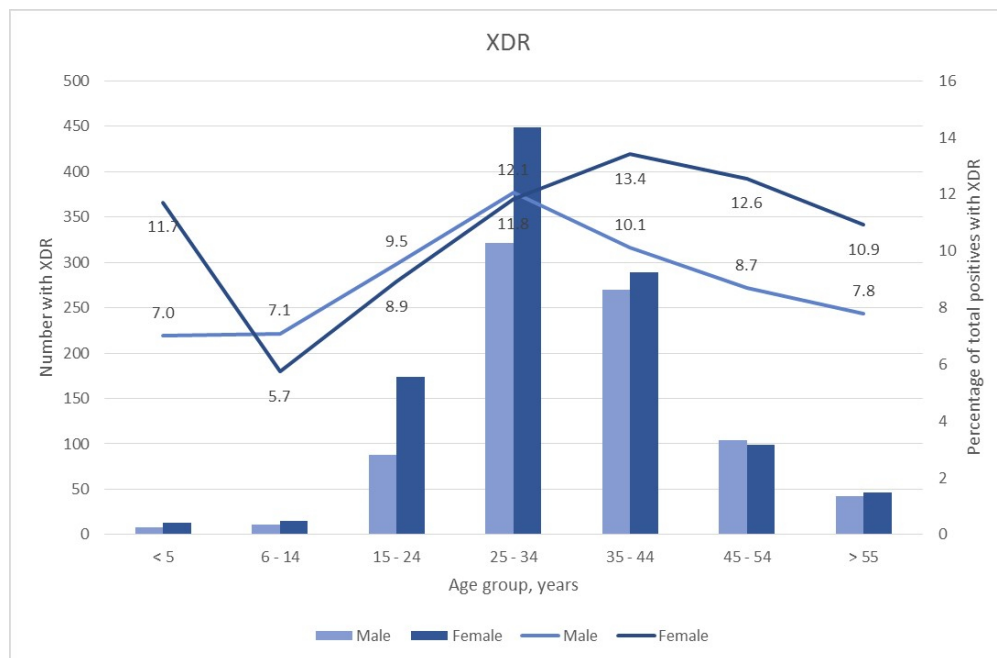


Figure 3: Distribution of XDR TB cases and proportions by gender. The number of XDR TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR of total positive cases by gender

184x120mm (150 x 150 DPI)

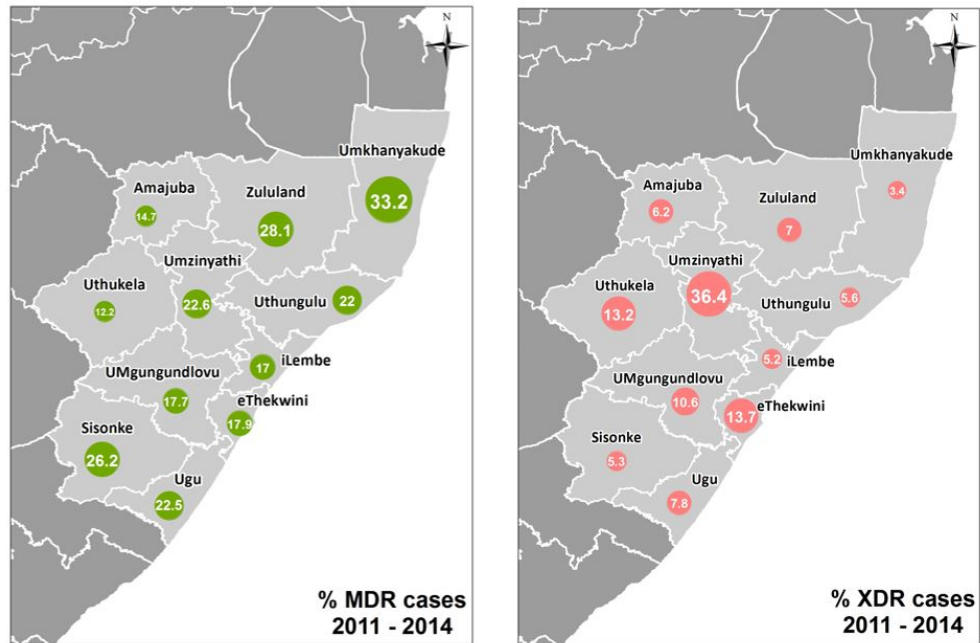


Figure 4: The first panel depicts the percentage of MDR TB cases per district for the period 2011 – 2014. The percentage of MDR TB cases among TB cases diagnosed by culture between 2011 and 2014. The size of the circle represents the percentage

252x168mm (96 x 96 DPI)

BMJ Open

Evolving rifampicin and isoniazid mono-resistance in a high MDR and XDR TB region: a retrospective data analysis

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Manuscripts

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3 **Evolving rifampicin and isoniazid mono-resistance in a high MDR and XDR-TB region:**
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5 **a retrospective data analysis**
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46 Running Title: Drug resistant TB in KwaZulu-Natal
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Abstract

Objectives: South Africa ranks among the highest drug resistant tuberculosis burdened countries in the world. This study assessed the changes in resistance levels in culture confirmed *Mycobacterium tuberculosis* (MTB) in the highest burdened province of South Africa during a period where major changes in diagnostic algorithm were implemented.

Setting: This retrospective observational study was conducted at the central academic laboratory of the KwaZulu-Natal province of South Africa.

Participants: We analysed data for all MTB cultures performed in the KwaZulu-Natal province between 2011 and 2014. The data were collected from the laboratory information system.

Results: Out of 88 559 drug susceptibility results analysed, 18352 (20.7%) were resistant to rifampicin and 19190 (21.7%) showed resistance to isoniazid. The proportion of rifampicin resistant cases that were mono-resistant increased from 15.3% in 2011 to 21.4% in 2014 while INH mono-resistance showed a range between 13.8% and 21.1%. The MDR-TB rates increased from 18.8% to 23.9% and the proportion of MDR-TB cases that had XDR-TB remained between 10.2% and 11.1%. Most drug resistance was found in females between the ages of 15 to 44 years and the northern districts bordering high MDR-TB regions had the highest MDR-TB rates.

Conclusion: Our findings show increasing rifampicin mono-resistance and a substantial amount of INH mono-resistance. This highlights a need for an initial test that detects resistance to both these drugs so as to avoid using rifampicin monotherapy during continuous phase of treatment in patients with INH mono-resistance. Furthermore, addition of isoniazid will benefit patients with rifampicin mono-resistance. Although DR-TB is widespread, HIV

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3 24 and migration influence its distribution; therefore, TB control strategies should include
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5 25 interventions that target these aspects.
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Strengths and limitations of this study

- The study was performed in the country with one of the highest TB incidence rate and a largest HIV epidemic in the world.
- The analysed data involves a period of major shift in TB diagnostic algorithm.
- The patient level Xpert MTB/RIF data was not available in order to compare with the TB culture results.
- The absence of unique patient identifiers also affects the accuracy of the data as the removal of duplicates was imperfect.

Background

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29 The World Health Organisation (WHO) has declared multidrug resistant tuberculosis a global
30 crisis. Multidrug-resistant tuberculosis (MDR TB) is defined as resistance to isoniazid and
31 rifampicin. Despite the decline in the global incidence rates of tuberculosis (TB), drug resistant
32 TB cases are on the rise with 558 000 estimated incident cases of MDR plus rifampicin resistant
33 (RR) TB and more than 230 000 deaths in 2017 [1]. South Africa has one of the highest
34 incidence of TB in the world which WHO estimated to be 567 per 100 000 in 2017 [1]. In 2017
35 alone, South Africa had an estimated number of 14 000 rifampicin resistant cases, the second
36 highest number in Africa after Nigeria which has more than three times the South African
37 population [1]. Moreover, the first reported outbreak of extensively drug-resistant (XDR)
38 tuberculosis (defined as MDR-TB plus resistance to any second line injectable and a
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3 39 fluoroquinolone) which caused global concern in 2005 was from the province of KwaZulu-
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5 40 Natal (KZN) in South Africa [2]. While the incidence of TB in KZN is proportional to other
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8 41 provinces in the country, it remains the highest drug resistant (DR) TB burdened province with
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10 42 almost a third of the country's cases of drug resistant TB [3].
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14 44 Compounding the problem of TB in South Africa, is the high rate of co-infection with Human
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16 45 Immunodeficiency Virus (HIV) (about 60%) [1]. While it is well known that HIV is associated
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18 46 with smear negative TB, smear microscopy was traditionally used in the initial diagnosis of TB
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20 47 because of its quick time to results and low cost [4]. On the other hand, conventional TB culture
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22 48 is much more sensitive than smear microscopy, but its high cost, complexity and long delays
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24 49 in getting the results made it impractical for routine diagnosis of TB. Therefore, when the WHO
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26 50 endorsed the Xpert MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was
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28 51 subsequently introduced in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated
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30 52 nucleic acid amplification test that offers better detection of TB compared to smear microscopy
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32 53 with an added advantage of the ability to detect rifampicin resistance in less than two hours in
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34 54 clinical specimens [5-6].
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42 56 The implementation of Xpert in South Africa completely changed the testing algorithm for the
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44 57 diagnosis of TB [7]. Xpert replaced smear microscopy in the initial diagnosis of TB and all
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46 58 patients that do not demonstrate rifampicin resistance are assumed to have drug susceptible TB
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48 59 and therefore initiated on standard first line TB therapy. Thus, Xpert rifampicin susceptible
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50 60 cases do not get a culture, so isoniazid mono-resistance is not routinely investigated. TB culture
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52 61 and drug susceptibility testing (DST) is only indicated for patients that demonstrate rifampicin
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54 62 resistance on the Xpert, paucibacillary TB cases missed by Xpert (HIV infected, children and
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56 63 extra-pulmonary TB) and patients that fail TB treatment.
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6 65 Despite the recent changes in the diagnosis and management of TB, there are no studies that
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8 66 have assessed their impact on culture confirmed TB. The level of isoniazid mono-resistance
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10 67 that is not routinely investigated with current diagnostic methods is unknown, but instead these
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12 68 patients are getting rifampicin monotherapy during the continuous phase of their first line TB
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14 69 therapy which could potentially fuel drug resistance. On the other hand, patients with
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17 70 rifampicin mono-resistance can benefit from the addition of isoniazid in their treatment. We
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19 71 therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-
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21 72 resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the
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23 73 drug resistance patterns and distribution among different age groups, genders and districts in
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26 74 KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will
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28 75 inform targeted intervention in TB control in this high TB endemic region.
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33 34 35 77 **Methods**

36 37 38 78 **Study design**

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41 79 The study is a retrospective observational study using laboratory data for 2011 till 2014.
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45 46 81 **Study setting**

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48 82 The KZN province is one of nine provinces in South Africa and its population of just over 10
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50 83 million ranks second in the country. There are 77 public health hospitals (including 8 MDR-
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52 84 TB initiation sites) within 11 health districts. Provincial *Mycobacterium tuberculosis* culture
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54 85 and drug susceptibility testing are performed in one central academic laboratory.
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58 59 87 **Laboratory Procedures**

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3 88 MTB isolation from clinical samples was routinely done using the automated BACTEC
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5 89 mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton
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7 90 Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain
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9 91 Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard
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11 92 methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin,
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13 93 streptomycin, kanamycin was performed for all TB culture positive cases using 1% agar
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15 94 proportion method (APM) on Middlebrook 7H10.
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23 96 **Patient and public involvement**

24 97 The data used for this study was the routine TB diagnostic data, therefore there was no direct
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26 98 patient and public involvement.
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31 100 **Data collection and analysis**

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33 101 The TB culture and DST data was collected from the National Health Laboratory Service
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35 102 laboratory information system (LIS) which contains all electronic laboratory results. In the
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37 103 absence of a unique identifier, duplicates were removed using MRN number (number given by
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39 104 the laboratory to specimens from the same patient) and demographic data (name, surname and
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41 105 date of birth). The results were stratified according to the health districts, age and gender. For
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43 106 the analysis of age, cases without recorded age or date of birth were excluded.
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49 108 Data was described using frequencies and proportions. Continuous data was described using
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51 109 means, standard deviations (sd) and 95% confidence intervals (95% CI). Categorical outcomes
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53 110 were tested using the chi-squared test. Log binomial regression of MDR and XDR-TB was
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55 111 performed using sex, age group, district and year as predictors to estimate the adjusted relative
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57 112 risk ratios. Data was analysed using Stata 14 (StataCorp., College Station, TX, USA).
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114 Ethical consideration

115 The data used for the study is routine data for management of TB patients; therefore, no
116 individual patient consent was required. The ethical approval to perform the retrospective
117 analysis was obtained from the Biomedical Research Ethics Committee of the University of
118 KwaZulu-Natal (REF: BE085/12).

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Results

121 Between 2011 and 2014, a total of 951 209 specimens were cultured for MTB in KwaZulu-
122 Natal (Figure 1). The total number of specimens for which culture was requested, decreased
123 annually with the average percentage difference (decline) of -14.2% [(95% CI -42.3% to
124 13.9%) and (sd 11.3%)] per year. Similarly, the MTB positivity rate decreased by 6.0% (from
125 15.6% to 9.6%) (Figure 2). After removing duplicates, there were 36644, 30208, 22568, 14672
126 culture confirmed cases of TB in 2011, 2012, 2013 and 2014 respectively. The average
127 percentage decline in total positive TB cases was 27.1% [(95% CI 3.5% to 50.7%) and (sd
128 9.5%)] per year.

129 About 85% (88559) cases of culture positive TB had an LPA done to test for drug susceptibility
130 against RIF and INH (Table 1). Of these, 19190 (21.7%) were resistant to INH and 18352
131 (20.7%) were resistant to RIF. There were 953 RIF mono-resistant (RMR) cases in 2011, 767
132 in 2012, 676 in 2013 and 667 in 2014. RMR refers to the proportion rifampicin resistant cases
133 that are susceptible to INH. The RMR increased from 15.3% in 2011 to 21.4% in 2014. Over
134 the same four-year period, there were 3396 (17.7%) INH mono-resistance (IMR) cases. IMR
135 refers to the proportion INH resistant cases that are susceptible to rifampicin.

136 A steady decline of both MDR-TB and XDR-TB cases was noted, with an overall decline of
137 49.2% (from 6901 in 2011 to 3506 in 2014) and 44.9% (from 706 in 2011 to 389 in 2014)

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3 138 respectively (Table 2). The proportion of TB cases that had MDR-TB ranged from 18.8% in
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5 139 2011 to 23.9% in 2014, with an overall average of 21%. The overall rate of XDR-TB among
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7 140 MDR-TB cases was 11% (2336 XDR-TB cases out of 21221 MDR-TB).
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11 141 The majority of the TB cases were males; however, females constituted highest prevalence of
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13 142 the DR-TB across all age groups (Figure 3 and 4). The number of MDR-TB cases was higher
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15 143 among females than males until the age of 34, thereafter males had a higher number than
16
17 144 females. Similarly, for XDR-TB, females constituted the most number of XDR-TB. More than
18
19 145 60% of both MDR-TB and XDR-TB cases were patients between the ages of 25 and 44 years.
20
21 146 It was observed that children less than 5 years of age showed the lowest rates of MDR-TB
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23 147 whilst that of XDR-TB was lowest between the ages of 6-14 years.
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28 148 Over the 4-year period, eThekweni district had the highest number of TB cases with 47.5% of
29
30 149 all cases in KZN coming from this district (Table 2). However, the districts with the highest
31
32 150 yearly proportion of MDR-TB cases each year were Umkhanyakude [(mean 33.2%, sd 2.3%),
33
34 151 (95% CI 29.5% to 36.9%)] followed by Zululand [(mean 28.1%, sd 2.3), (95% CI 24.4% to
35
36 152 31.8%)] and Harry Gwala [(mean 26.2%, sd 9.4), (95% CI 11.2% to 41.2%)]. The yearly
37
38 153 proportion of MDR-TB cases that had XDR-TB were highest at Umzinyathi [(mean 36.4%, sd
39
40 154 5.8%), (95% CI 27.2% to 45.6%)] followed by eThekweni [(mean 13.7%, sd 1.1%), (95% CI
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42 155 11.9% to 15.5%)] and Uthukela [(mean 13.2%, sd 5.7%), (95% CI 4.1% to 22.3%)] districts.
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44 156 Umkhanyakude district had the lowest proportion of XDR-TB with a yearly mean of 3.4% [(sd
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46 157 2.4%), (95% CI -0.4% to 7.2%)] over the study period (Figure 5).
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Discussion

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3 160 In this study we observe a decline in the number of samples processed for MTB culture and
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5 161 culture positivity rate which coincided, with the roll out of the Xpert. This is in keeping with
6
7 162 the Xpert roll out which started in March 2011 and was completed in September 2013 when
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9 163 all health facilities in the provinces were using the Xpert for TB diagnosis. According to the
10
11 164 South African guidelines, MTB culture is not recommended for Xpert rifampicin susceptible
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13 165 patients, which constitutes the majority of patients infected with TB, hence the decline in the
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15 166 number of MTB cultures from 277 963 in 2011 to 172 671 in 2014. Nevertheless, the sheer
16
17 167 volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR
18
19 168 TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only
20
21 169 performed on patients that were considered to be at risk of DR TB, but the use of Xpert for
22
23 170 initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised
24
25 171 indications for culture selects for cases that are more likely to have drug resistant (Xpert
26
27 172 rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert
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29 173 negative), which explains the high rates of DR TB and declining culture positivity rate observed
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31 174 in this study.
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40 176 Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In
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42 177 addition, spontaneously occurring mutations are rare compared to other TB drugs [8].
43
44 178 Consequently, the development of RMR is expected to be uncommon. The finding of
45
46 179 increasing mono-resistance in this context is therefore concerning. In a previous study done by
47
48 180 Coovadia *et al*, at the same laboratory, RMR was 8.8% during the years 2007-2009 [9].
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50 181 Similarly, Mukinda *et al* reported increasing RMR in the Western Cape province of SA [10].
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52 182 These findings highlight the importance of testing for INH resistance in all patients with Xpert
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54 183 rifampicin resistance. This positively impacts patient management further as patients with
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3 184 confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment
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5 185 regimen.
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10 187 The development of rifampicin resistance has serious effects on the treatment of TB. Patients
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12 188 have to be treated with more expensive and more toxic drugs for a longer duration. Studies
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14 189 have been conducted in order to elucidate the causes of RMR with the majority reporting an
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16 190 association between HIV and RMR [11-12]. Factors contributing to this association include
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18 191 decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin
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20 192 serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to
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22 193 infection and permits proliferation of TB which favours transmission [14]. Given the high rate
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24 194 of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the
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26 195 increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in
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28 196 co-infected patients remains under investigation.
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34
35 198 Rifampicin and INH are core drugs that form the backbone for first line short course therapy
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37 199 for the treatment of drug susceptible TB. Given the high burden of disease in this region
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39 200 coupled with the use of Xpert as a screening tool for DR-TB, mono-resistance to INH may
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41 201 inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is
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43 202 only made using TB culture and DST following a negative Xpert result or treatment failure.
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45 203 The use of standard first line TB therapy in patients with undetected INH resistance equates to
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47 204 using rifampicin monotherapy during the continuation phase. This may subsequently lead to
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49 205 the development of MDR-TB. This was described in a study done by Jacobson *et al* where
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51 206 treatment of patients with IMR using standard first line therapy was associated with poor
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53 207 outcomes and progression to MDR-TB [15]. Several studies have reported previous TB therapy
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3 208 as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients
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5 209 who may require TB culture and DST in order to exclude INH resistance.
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10 211 There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and
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12 212 XDR-TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast
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14 213 to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in
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16 214 South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the
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18 215 national guidelines, a significant number of patients with Xpert rifampicin resistant TB did not
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20 216 get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO TB
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22 217 report, which reported the percentage of MDR-TB among MDR/RR-TB as 62% in South
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24 218 Africa. This suggests that a substantial number of Xpert rifampicin resistant TB cases are not
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26 219 confirmed by culture because this discrepancy cannot be explained by RMR cases [1]. Another
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28 220 possible reason may be due to patients that are lost to follow up. In KZN province, the specimen
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30 221 for TB culture is only collected when the patient comes back for Xpert results. Consequently,
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32 222 if patients did not return for the results, then specimens for TB culture would not have been
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34 223 collected. According to the WHO, only 41% of notified MDR/RR-TB cases from South Africa
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36 224 were enrolled for MDR-TB treatment in 2013[18]. Although this figure improved to 62% in
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38 225 2014 [20], the gap remains substantial especially given the considerable improvement in
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40 226 rapidity of diagnosing DR-TB with Xpert. Therefore, this decline in culture confirmed DR-
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42 227 TB indicates a change in the testing method used to diagnose tuberculosis rather than a
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44 228 successful TB control program, which led to underestimation of MDR-TB and XDR-TB cases
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46 229 in this study. The proportion of MDR-TB cases that have XDR-TB remained constant at about
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48 230 11% which is comparable to the global trends at that time [18-20].
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3 232 Our study found higher rates of DR-TB in women compared to men which supports findings
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5 233 from other studies showing higher proportions of DR-TB in women [21-22]. Even though
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8 234 reasons behind the higher DR-TB predisposition among women are unknown, HIV could be a
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10 235 contributing factor. The majority of DR-TB cases were found between the ages of 15 and 44
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12 236 years, which is the same age group that is known to have the highest HIV prevalence [2]. It is
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15 237 well recognised that HIV is a major risk factor for development of TB and HAART reduces its
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17 238 incidence [23-25]. Indeed, Nanoo *et al* showed an inverse relationship between antiretroviral
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19 239 therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with
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21 240 the greatest decline demonstrated in the 25-44 year age group [26].
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26 242 The diagnosis of DR-TB in children is generally difficult due to their inability to expectorate
27
28 243 and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB
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30 244 in children is largely as a result of primary transmission from adults, the proportion of DR-TB
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32 245 is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR-TB
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34 246 particularly MDR-TB in children less than five years compared to adults which could be a
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36 247 reflection of the under diagnosis of DR-TB in this age group. Although these rates are lower in
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38 248 children, they are still unacceptable as they reflect transmission of untreated adult TB.
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44 250 Similar to the overall burden of TB in South Africa, DR-TB is also concentrated in urban areas
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46 251 of KZN with eThekweni district harbouring most of the cases due to high population density.
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48 252 However, the rate of MDR-TB cases was highest among the northern districts of the province
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50 253 of Umkhanyakude and Zululand. These are rural districts which share borders with
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52 254 Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance
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54 255 patterns. Mpumalanga province is known to have the highest DR-TB rate in the country while
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56 256 Swaziland has the highest MDR-TB prevalence in Africa [29-30]. In 2007, Wallengren *et al*
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3 257 reported Umzinyathi and Umkhanyakude as the districts with the highest MDR-TB rates [31].
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5 258 The intervention given to the Umzinyathi district following the outbreak of XDR-TB in 2005
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8 259 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and
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10 260 treatment of HIV, TB infection control and intergration of TB and HIV care) may be
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12 261 responsible for these decreasing rates [32]. Despite declining XDR-TB rates at the Umzinyathi
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14 262 district (where the XDR-TB outbreak was identified in 2005), it still remains the district with
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16
17 263 the highest XDR-TB rates at about three times higher than the rest of KZN [33].
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22 265 **Limitations**

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24 266 Our study is limited by the retrospective design; the accuracy of the data is dependent on
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26 267 available information on the LIS. The absence of unique patient identifiers also affects the
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28 268 accuracy of the data as the removal of duplicates is imperfect. Although duplicates were
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30 269 removed, we could not differentiate between new and known MDR-TB patients. The patient
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33 270 level Xpert data was not available in order to match with the TB culture results. Nevertheless,
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35 271 the high burden of DR-TB and the fact that all cultures are performed in one laboratory for the
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37 272 whole province provide an important insight to the distribution of TB in this region and may
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40 273 inform targeted intervention.
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42 274 Although the data used for this study is relatively old, it represents a critical time of drastic
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44 275 changes in the diagnosis of DR-TB. There have been no subsequent changes in the TB
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46 276 diagnostic algorithm, therefore the findings highlighted in this study should still be relevant to
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49 277 the current setting.
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54 279 **Conclusions**

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56 280 Our findings highlight the importance of DR-TB diagnostic algorithms that include both
57
58 281 rifampicin and isoniazid DST in the initial testing. Early detection of RMR will allow addition
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3 282 of isoniazid in the treatment regimen, while detection of IMR will prevent rifampicin
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5 283 monotherapy later on during the continuation phase of treatment which has been associated
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8 284 with development of rifampicin resistance. This will also allow us to have a clearer estimate of
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10 285 MDR-TB cases. HIV and migration play a significant role in the distribution of DR-TB in this
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12 286 region, therefore TB control measures that address these factors may have impact on DR-TB
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31 293 maps.
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Author contributions

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40 296 NRM contributed in the development of the concept, study design, data analysis and writing
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42 297 of the manuscript. YB performed data analysis and assisted with the writing of the manuscript.
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44 298 KL contributed in the interpretation of data and writing of the manuscript. KM supervised the
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46 299 development of the study concept, study design, data analysis and manuscript writing.
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33 **Table 1. LPA results between 2011 and 2014: RIF and INH mono-resistance***

	2011	2012	2013	2014
Total culture positives	36644	30208	22568	14672
Total cases LPA	31368	26513	18399	12279
% of LPA done	85.6	87.8	81.5	83.7
LPA Any INH Resistance	6430	5548	4167	3045
LPA Any INH Resistance (%)	20.5	20.9	22.7	24.8
LPA INH MR	845	1167	879	505
LPA INH MR (% of All INH Resistant)	13.8	21.0	21.1	17.1
LPA Any RIF Resistance	6293	5013	3912	3134
LPA Any RIF Resistance (%)	20.1	18.9	21.3	25.5
LPA RIF MR	953	767	676	667
LPA RIF MR (% of All RIF Resistant)	15.1	15.3	17.3	21.3

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53 428 *RIF, Rifampicin; INH, isoniazid; LPA, line probe assay; MR, mono-resistance
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Table 2. Total TB positive, MDR and XDR cases per district for each year.

District	2011					2012					2013					2014				
	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%
Amajuba	597	96	16.1	8	8.3	523	86	16.4	4	4.7	353	40	11.3	4	10.0	272	35	12.9	0	0.0
Ethekwini	17519	2837	16.2	353	12.4	13453	2306	17.1	338	14.7	11118	2212	19.9	321	14.5	7404	1516	20.5	203	13.4
Harry Gwala	776	124	16.0	5	4.0	752	191	25.4	13	6.8	526	186	35.4	4	2.2	396	142	35.9	12	8.5
Ilembe	1704	269	15.8	9	3.3	1219	206	16.9	16	7.8	978	180	18.4	7	3.9	405	76	18.8	6	7.9
Ugu	2172	542	25.0	37	6.8	2443	465	19.0	47	10.1	1655	388	23.4	34	8.8	1123	268	23.9	12	4.5
Umgungundlovu	3514	520	14.8	55	10.6	2858	532	18.6	57	10.7	1486	305	20.5	27	8.9	703	159	22.6	22	13.8
Umkhanyakude	2230	732	32.8	5	0.7	1619	501	30.9	29	5.8	1214	422	34.8	15	3.6	945	341	36.1	19	5.6
Umzinyathi	1478	334	22.6	134	40.1	1295	257	19.8	102	39.7	894	192	21.5	66	34.4	674	196	29.1	54	27.6
Uthukela	1100	120	10.9	16	13.3	1023	111	10.9	13	11.7	545	71	13.0	6	8.5	261	55	21.1	12	21.8
Uthungulu	2640	589	22.3	30	5.1	2737	590	21.6	41	6.9	2315	477	20.6	26	5.5	1178	294	25.0	12	4.1
Zululand	2888	735	25.5	54	7.3	2259	662	29.3	37	5.6	1468	428	29.2	34	7.9	1168	362	31.0	28	7.7

Unknown	26	3	11.5	0	0.0	27	5	18.5	0	0.0	16	1	6.3	0	0.0	143	62	43.4	9	14.5
Total	36644	6901	18.8	706	10.2	30208	5912	19.6	697	11.8	22568	4902	21.7	544	11.1	14672	3506	23.9	389	11.1

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* MDR, multidrug-resistant; XDR, extensively drug-resistant; TB, tuberculosis

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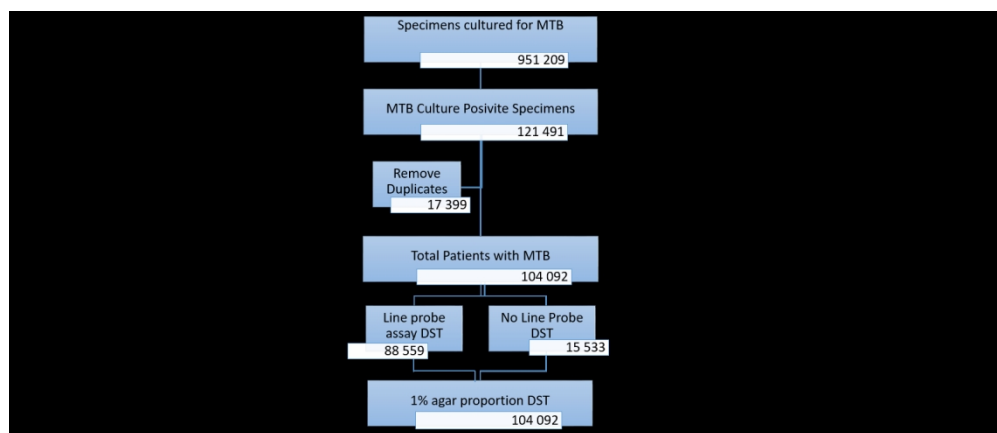
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3 432 Figure 1: Flow diagram showing the number of specimens received and the laboratory
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5 433 procedures performed at the TB culture laboratory.
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9 434 Figure 2: TB culture specimens processed between 2011 and 2014. The figure shows the total
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11 435 number of specimens received and the total number (and percentage) thereof that were positive.
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14 436 Figure 3: Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB
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16 437 cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-
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18 438 TB of total positive cases by gender
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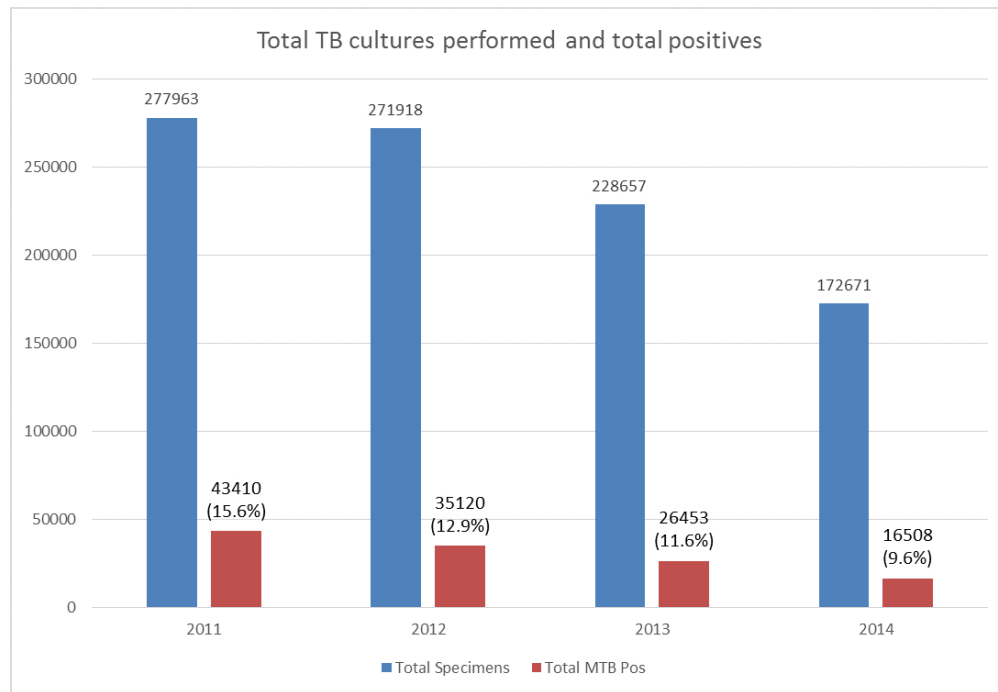
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22 439 Figure 4: Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB
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24 440 cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-
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26 441 TB of total positive cases by gender.
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30 442 Figure 5: The first panel depicts the percentage of MDR-TB cases per district for the period
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32 443 2011 – 2014. The percentage of MDR-TB cases among TB cases diagnosed by culture between
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34 444 2011 and 2014. The size of the circle represents the percentage. The second panel depicts the
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36 445 percentage of XDR-TB cases per district for the period 2011 – 2014. The percentage of XDR-
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38 446 TB cases among MDR-TB cases diagnosed by culture between 2011 and 2014. The map was
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41 447 produced specifically for the purposes of this study, it is therefore not under copyright.
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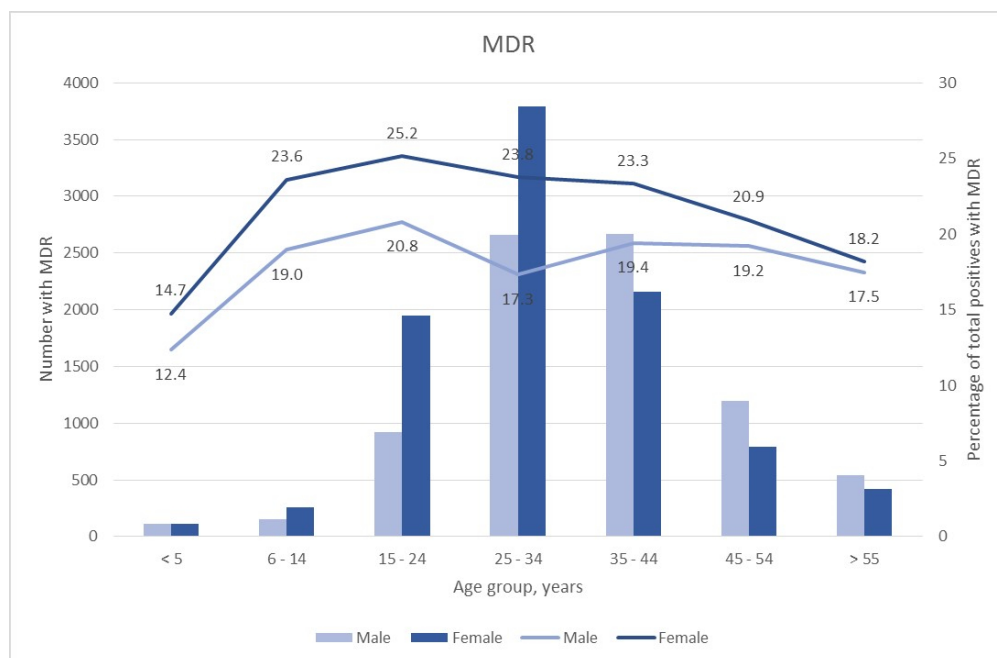
Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

292x124mm (150 x 150 DPI)



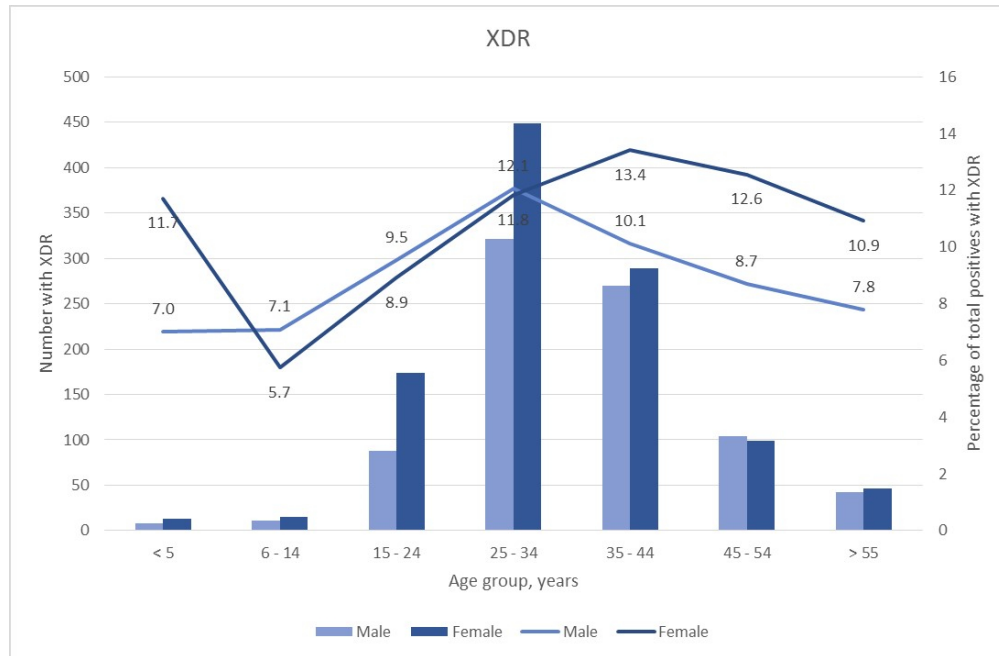
29 TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens
30 received and the total number (and percentage) thereof that were positive.

31 184x127mm (150 x 150 DPI)



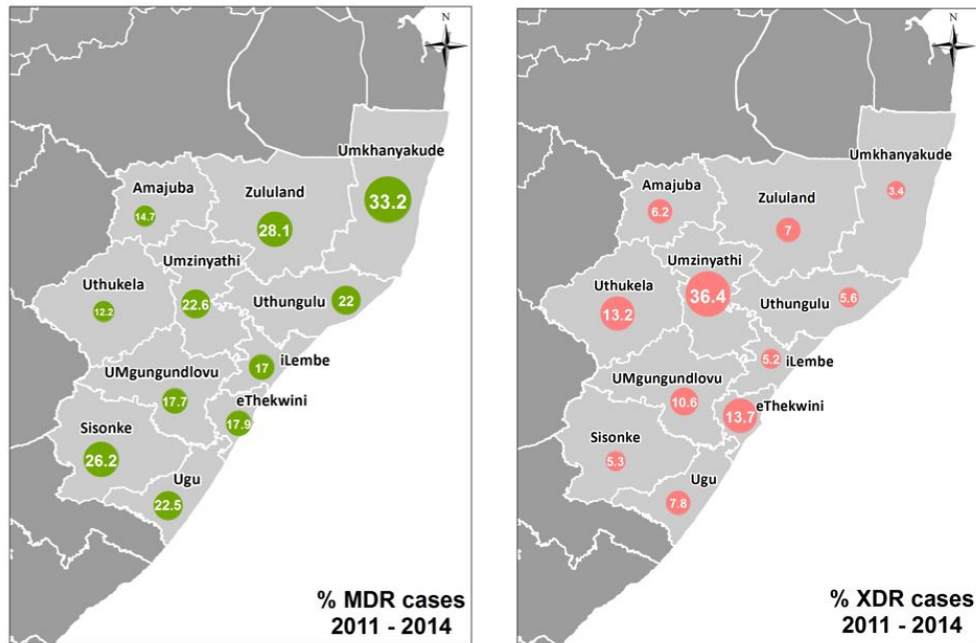
Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-TB of total positive cases by gender

184x120mm (150 x 150 DPI)



Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-TB of total positive cases by gender.

184x120mm (150 x 150 DPI)



The first panel depicts the percentage of MDR-TB cases per district for the period 2011 – 2014. The percentage of MDR-TB cases among TB cases diagnosed by culture between 2011 and 2014. The size of the circle represents the percentage. The second panel depicts the percentage of XDR-TB cases per district for the period 2011 – 2014. The percentage of XDR-TB cases among MDR-TB cases diagnosed by culture between 2011 and 2014. The map was produced specifically for the purposes of this study, it is therefore not under copyright.

252x168mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	2 2	Retrospective observational study We analysed data for all MTB cultures performed in the KwaZulu-Natal province between 2011 and 2014. Our findings show increasing rifampicin mono-resistance and a substantial amount of INH mono-resistance. Although DR-TB is widespread, HIV and migration influence its distribution.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5	Xpert replaced smear microscopy in the initial diagnosis of TB and all patients that do not demonstrate rifampicin resistance are assumed to have drug susceptible TB and therefore initiated on standard first line TB therapy. Thus, Xpert rifampicin susceptible cases do not get a culture, so isoniazid mono-resistance is not routinely investigated. TB culture and drug susceptibility testing (DST) is only indicated for patients that demonstrate rifampicin resistance on the Xpert, paucibacillary TB cases missed by Xpert (HIV infected, children and extra-pulmonary TB) and patients that fail TB treatment. Despite the recent changes in the diagnosis and management of TB, there are no studies that have assessed their impact on culture confirmed TB. The level of isoniazid mono-resistance that is not routinely investigated

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					with current diagnostic methods is unknown, but instead these patients are getting rifampicin monotherapy during the continuous phase of their first line TB therapy which could potentially fuel drug resistance. On the other hand, patients with rifampicin mono-resistance can benefit from the addition of isoniazid in their treatment.
Objectives	3	State specific objectives, including any prespecified hypotheses	5	Evaluate the amount of rifampicin and isoniazid mono-resistance. Describe the drug resistance patterns and distribution among different age groups, genders and districts in KZN, South Africa.	
Methods					
Study design	4	Present key elements of study design early in the paper	5	The study is a retrospective observational study using laboratory data.	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5	This retrospective observational study was conducted at the central academic laboratory of the KwaZulu-Natal province of South Africa using TB culture data for 2011-2014. KZN province is one of nine provinces in South Africa and its population of just over 10 million ranks second in the country. There are 77 public health hospitals (including 8 MDR-TB initiation sites) within 11 health districts. Provincial Mycobacterium tuberculosis culture and drug susceptibility testing are performed in one central academic laboratory.	
.Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	6	The TB culture and drug susceptibility data was collected from the National Health Laboratory Service laboratory information system (LIS) which contains all electronic laboratory results. TB All	

		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants		cultures performed in the provincial TB laboratory from 2011 till 2014 were included
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed		
		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable		Not applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6	The data was collected from the National Health Laboratory Service laboratory information system (LIS) which contains all electronic laboratory results. In the absence of a unique identifier, duplicates were removed using MRN number (number given by the laboratory to specimens from the same patient) and demographic data (name, surname and date of birth). The results were stratified according to the health districts, age and gender. For the analysis of age, cases without recorded age or date of birth were excluded.
Bias	9	Describe any efforts to address potential sources of bias		Not Applicable
Study size	10	Explain how the study size was arrived at		Not Applicable: All results included

Continued on next page

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2	Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6	Data was described using frequencies and proportions.
3					
4	Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6	Continuous data was described using means, standard deviations (sd) and 95% confidence intervals (95% CI). Categorical outcomes were tested using the chi-squared test. Log binomial regression of MDR and XDR-TB was performed using sex, age group, district and year as predictors to estimate the adjusted relative risk ratios. Data was analysed using Stata 14 (StataCorp., College Station, TX, USA).
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20			(b) Describe any methods used to examine subgroups and interactions		
21			(c) Explain how missing data were addressed		
22			(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed		
23			<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed		
24			<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy		
25			(e) Describe any sensitivity analyses		
26					
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29	Results				
30	Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1	A flow diagram is used to report number of specimens and individuals at each stage
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34			(b) Give reasons for non-participation at each stage		
35			(c) Consider use of a flow diagram		
36	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7	-Between 2011 and 2014, a total of 951 209 specimens were cultured for MTB in KwaZulu-Natal
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-After removing duplicates, there were 36644, 30208, 22568, 14672 culture confirmed cases of TB in 2011, 2012, 2013 and 2014 respectively

(b) Indicate number of participants with missing data for each variable of interest

(c) Cohort study—Summarise follow-up time (eg, average and total amount)

Outcome data 15* Cohort study—Report numbers of outcome events or summary measures over time Not Applicable

Case-control study—Report numbers in each exposure category, or summary measures of exposure

Cross-sectional study—Report numbers of outcome events or summary measures

Main results 16 (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included 7-8 Standard deviations and 95% confidence intervals are given

(b) Report category boundaries when continuous variables were categorized

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		
Discussion				
Key results	18	Summarise key results with reference to study objectives	8-12	<p>Key findings:</p> <ul style="list-style-type: none"> -Increasing mono-resistance highlight the importance of testing for INH resistance in all patients with Xpert rifampicin resistance -Significant INH mono-resistance that is currently missed in the initial diagnosis of TB. - The majority of DR-TB cases were found between the ages of 15 and 44 years, which is the same age group that is known to have the highest HIV prevalence -DR TB is high in areas bordering high DR TB regions, thus migration may influence resistance patterns.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13	<ul style="list-style-type: none"> -Retrospective design; the accuracy of the data is dependent on available information. - Absence of unique patient identifiers: affects the accuracy of the data as the removal of duplicates is imperfect - Could not differentiate between new and known MDR-TB patients - Xpert MTB/RIF data was not available for comparison
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13	Our findings highlight the importance of DR-TB diagnostic

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algorithms that include both rifampicin and isoniazid DST in the initial testing. Early detection of RMR will allow addition of isoniazid in the treatment regimen, while detection of IMR will prevent rifampicin monotherapy later on during the continuation phase of treatment which has been associated with development of rifampicin resistance. This will also allow us to have a clearer estimate of MDR-TB cases. HIV and migration play a significant role in the distribution of DR-TB in this region, therefore TB control measures that address these factors may have impact on DR-TB level.

Generalisability	21	Discuss the generalisability (external validity) of the study results	13	Conclusion applies to a wide variety of settings
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based		None

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Evolving rifampicin and isoniazid mono-resistance in a high multidrug-resistant and extensively drug-resistant tuberculosis region: a retrospective data analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031663.R2
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Date Submitted by the Author:	08-Oct-2019
Complete List of Authors:	Mvelase, Nomonde; National Health Laboratory Service, Medical Microbiology Balakrishna, Yusentha; South African Medical Research Council, Biostatistics Unit Lutchminarain, Keeren; National Health Laboratory Service, Medical Microbiology ; University of KwaZulu-Natal, Medical Microbiology Mlisana, Koleka; National Health Laboratory Service, Medical Microbiology; University of KwaZulu-Natal, Medical Microbiology
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Diagnostics, Epidemiology, Public health, Infectious diseases, Health policy
Keywords:	Tuberculosis < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Diagnostic microbiology < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES

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Manuscripts

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3 **Evolving rifampicin and isoniazid mono-resistance in a high multidrug-resistant and**
4 **extensively drug-resistant tuberculosis region: a retrospective data analysis**
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46 Running Title: Drug resistant TB in KwaZulu-Natal
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Abstract

Objectives: South Africa ranks among the highest drug resistant tuberculosis burdened countries in the world. This study assessed the changes in resistance levels in culture confirmed *Mycobacterium tuberculosis* (MTB) in the highest burdened province of South Africa during a period where major changes in diagnostic algorithm were implemented.

Setting: This study was conducted at the central academic laboratory of the KwaZulu-Natal province of South Africa.

Participants: We analysed data for all MTB cultures performed in the KwaZulu-Natal province between 2011 and 2014. The data were collected from the laboratory information system.

Results: Out of 88 559 drug susceptibility results analysed, 18352 (20.7%) were resistant to rifampicin and 19190 (21.7%) showed resistance to isoniazid. The proportion of rifampicin resistant cases that were mono-resistant increased from 15.3% in 2011 to 21.4% in 2014 while INH mono-resistance showed a range between 13.8% and 21.1%. The MDR-TB rates increased from 18.8% to 23.9% and the proportion of MDR-TB cases that had XDR-TB remained between 10.2% and 11.1%. Most drug resistance was found in females between the ages of 15 to 44 years and the northern districts bordering high MDR-TB regions had the highest MDR-TB rates.

Conclusion: Our findings show increasing rifampicin mono-resistance and a substantial amount of INH mono-resistance. This highlights a need for an initial test that detects resistance to both these drugs so as to avoid using rifampicin monotherapy during continuous phase of treatment in patients with INH mono-resistance. Furthermore, addition of isoniazid will benefit patients with rifampicin mono-resistance. Although DR-TB is widespread, HIV

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3 24 and migration influence its distribution; therefore, TB control strategies should include
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5 25 interventions that target these aspects.
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Strengths and limitations of this study

- The study was performed in the country with one of the highest TB incidence rate and a largest HIV epidemic in the world.
- The analysed data involves a period of major shift in TB diagnostic algorithm.
- The patient level Xpert MTB/RIF data was not available in order to compare with the TB culture results.
- The absence of unique patient identifiers also affects the accuracy of the data as the removal of duplicates was imperfect.

Background

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29 The World Health Organisation (WHO) has declared multidrug resistant tuberculosis a global
30 crisis. Multidrug-resistant tuberculosis (MDR TB) is defined as resistance to isoniazid and
31 rifampicin. Despite the decline in the global incidence rates of tuberculosis (TB), drug resistant
32 TB cases are on the rise with 558 000 estimated incident cases of MDR plus rifampicin resistant
33 (RR) TB and more than 230 000 deaths in 2017 [1]. South Africa has one of the highest
34 incidence of TB in the world which WHO estimated to be 567 per 100 000 in 2017 [1]. In 2017
35 alone, South Africa had an estimated number of 14 000 rifampicin resistant cases, the second
36 highest number in Africa after Nigeria which has more than three times the South African
37 population [1]. Moreover, the first reported outbreak of extensively drug-resistant (XDR)
38 tuberculosis (defined as MDR-TB plus resistance to any second line injectable and a
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3 39 fluoroquinolone) which caused global concern in 2005 was from the province of KwaZulu-
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5 40 Natal (KZN) in South Africa [2]. While the incidence of TB in KZN is proportional to other
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7 41 provinces in the country, it remains the highest drug resistant (DR) TB burdened province with
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9 42 almost a third of the country's cases of drug resistant TB [3].
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14 44 Compounding the problem of TB in South Africa, is the high rate of co-infection with Human
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16 45 Immunodeficiency Virus (HIV) (about 60%) [1]. While it is well known that HIV is associated
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18 46 with smear negative TB, smear microscopy was traditionally used in the initial diagnosis of TB
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20 47 because of its quick time to results and low cost [4]. On the other hand, conventional TB culture
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22 48 is much more sensitive than smear microscopy, but its high cost, complexity and long delays
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24 49 in getting the results made it impractical for routine diagnosis of TB. Therefore, when the WHO
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26 50 endorsed the Xpert MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was
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28 51 subsequently introduced in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated
29
30 52 nucleic acid amplification test that offers better detection of TB compared to smear microscopy
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32 53 with an added advantage of the ability to detect rifampicin resistance in less than two hours in
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34 54 clinical specimens [5-6].
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42 56 The implementation of Xpert in South Africa completely changed the testing algorithm for the
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44 57 diagnosis of TB [7]. Xpert replaced smear microscopy in the initial diagnosis of TB and all
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46 58 patients that do not demonstrate rifampicin resistance are assumed to have drug susceptible TB
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48 59 and therefore initiated on standard first line TB therapy. Thus, Xpert rifampicin susceptible
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50 60 cases do not get a culture, so isoniazid mono-resistance is not routinely investigated. TB culture
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52 61 and drug susceptibility testing (DST) is only indicated for patients that demonstrate rifampicin
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54 62 resistance on the Xpert, paucibacillary TB cases missed by Xpert (HIV infected, children and
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56 63 extra-pulmonary TB) and patients that fail TB treatment.
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6 65 Despite the recent changes in the diagnosis and management of TB, there are no studies that
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8 66 have assessed their impact on culture confirmed TB. The level of isoniazid mono-resistance
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10 67 that is not routinely investigated with current diagnostic methods is unknown, but instead these
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12 68 patients are getting rifampicin monotherapy during the continuous phase of their first line TB
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14 69 therapy which could potentially fuel drug resistance. On the other hand, patients with
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17 70 rifampicin mono-resistance can benefit from the addition of isoniazid in their treatment. We
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19 71 therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-
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21 72 resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the
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23 73 drug resistance patterns and distribution among different age groups, genders and districts in
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26 74 KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will
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28 75 inform targeted intervention in TB control in this high TB endemic region.
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33 34 35 77 **Methods**

36 37 38 78 **Study design**

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41 79 The study is a retrospective observational study using laboratory data for 2011 till 2014.
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45 46 81 **Study setting**

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48 82 The KZN province is one of nine provinces in South Africa and its population of just over 10
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50 83 million ranks second in the country. There are 77 public health hospitals (including 8 MDR-
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52 84 TB initiation sites) within 11 health districts. Provincial *Mycobacterium tuberculosis* culture
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54 85 and drug susceptibility testing are performed in one central academic laboratory.
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58 59 87 **Laboratory Procedures** 60

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3 88 MTB isolation from clinical samples was routinely done using the automated BACTEC
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5 89 mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton
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7 90 Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain
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9 91 Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard
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11 92 methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin,
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13 93 streptomycin, kanamycin was performed for all TB culture positive cases using 1% agar
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15 94 proportion method (APM) on Middlebrook 7H10.
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23 96 **Patient and public involvement**

24 97 The data used for this study was the routine TB diagnostic data, therefore there was no direct
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26 98 patient and public involvement.
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31 100 **Data collection and analysis**

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33 101 The TB culture and DST data was collected from the National Health Laboratory Service
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35 102 laboratory information system (LIS) which contains all electronic laboratory results. In the
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37 103 absence of a unique identifier, duplicates were removed using MRN number (number given by
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39 104 the laboratory to specimens from the same patient) and demographic data (name, surname and
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41 105 date of birth). The results were stratified according to the health districts, age and gender. For
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43 106 the analysis of age, cases without recorded age or date of birth were excluded.
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49 108 Data was described using frequencies and proportions. Continuous data was described using
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51 109 means, standard deviations (sd) and 95% confidence intervals (95% CI). Categorical outcomes
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53 110 were tested using the chi-squared test. Log binomial regression of MDR and XDR-TB was
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55 111 performed using sex, age group, district and year as predictors to estimate the adjusted relative
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57 112 risk ratios. Data was analysed using Stata 14 (StataCorp., College Station, TX, USA).
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114 Ethical consideration

115 The data used for the study is routine data for management of TB patients; therefore, no
116 individual patient consent was required. The ethical approval to perform the retrospective
117 analysis was obtained from the Biomedical Research Ethics Committee of the University of
118 KwaZulu-Natal (REF: BE085/12).

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Results

121 Between 2011 and 2014, a total of 951 209 specimens were cultured for MTB in KwaZulu-
122 Natal (Figure 1). The total number of specimens for which culture was requested, decreased
123 annually with the average percentage difference (decline) of -14.2% [(95% CI -42.3% to
124 13.9%) and (sd 11.3%)] per year. Similarly, the MTB positivity rate decreased by 6.0% (from
125 15.6% to 9.6%) (Figure 2). After removing duplicates, there were 36644, 30208, 22568, 14672
126 culture confirmed cases of TB in 2011, 2012, 2013 and 2014 respectively. The average
127 percentage decline in total positive TB cases was 27.1% [(95% CI 3.5% to 50.7%) and (sd
128 9.5%)] per year.

129 About 85% (88559) cases of culture positive TB had an LPA done to test for drug susceptibility
130 against RIF and INH (Table 1). Of these, 19190 (21.7%) were resistant to INH and 18352
131 (20.7%) were resistant to RIF. There were 953 RIF mono-resistant (RMR) cases in 2011, 767
132 in 2012, 676 in 2013 and 667 in 2014. RMR refers to the proportion rifampicin resistant cases
133 that are susceptible to INH. The RMR increased from 15.3% in 2011 to 21.4% in 2014. Over
134 the same four-year period, there were 3396 (17.7%) INH mono-resistance (IMR) cases. IMR
135 refers to the proportion INH resistant cases that are susceptible to rifampicin.

136 A steady decline of both MDR-TB and XDR-TB cases was noted, with an overall decline of
137 49.2% (from 6901 in 2011 to 3506 in 2014) and 44.9% (from 706 in 2011 to 389 in 2014)

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3 138 respectively (Table 2). The proportion of TB cases that had MDR-TB ranged from 18.8% in
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5 139 2011 to 23.9% in 2014, with an overall average of 21%. The overall rate of XDR-TB among
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7 140 MDR-TB cases was 11% (2336 XDR-TB cases out of 21221 MDR-TB).
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11 141 The majority of the TB cases were males; however, females constituted highest prevalence of
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13 142 the DR-TB across all age groups (Figure 3 and 4). The number of MDR-TB cases was higher
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15 143 among females than males until the age of 34, thereafter males had a higher number than
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17 144 females. Similarly, for XDR-TB, females constituted the most number of XDR-TB. More than
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19 145 60% of both MDR-TB and XDR-TB cases were patients between the ages of 25 and 44 years.
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21 146 It was observed that children less than 5 years of age showed the lowest rates of MDR-TB
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23 147 whilst that of XDR-TB was lowest between the ages of 6-14 years.
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28 148 Over the 4-year period, eThekweni district had the highest number of TB cases with 47.5% of
29
30 149 all cases in KZN coming from this district (Table 2). However, the districts with the highest
31
32 150 yearly proportion of MDR-TB cases each year were Umkhanyakude [(mean 33.2%, sd 2.3%),
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34 151 (95% CI 29.5% to 36.9%)] followed by Zululand [(mean 28.1%, sd 2.3), (95% CI 24.4% to
35
36 152 31.8%)] and Harry Gwala [(mean 26.2%, sd 9.4), (95% CI 11.2% to 41.2%)]. The yearly
37
38 153 proportion of MDR-TB cases that had XDR-TB were highest at Umzinyathi [(mean 36.4%, sd
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40 154 5.8%), (95% CI 27.2% to 45.6%)] followed by eThekweni [(mean 13.7%, sd 1.1%), (95% CI
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42 155 11.9% to 15.5%)] and Uthukela [(mean 13.2%, sd 5.7%), (95% CI 4.1% to 22.3%)] districts.
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44 156 Umkhanyakude district had the lowest proportion of XDR-TB with a yearly mean of 3.4% [(sd
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46 157 2.4%), (95% CI -0.4% to 7.2%)] over the study period (Figure 5).
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Discussion

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3 160 In this study we observe a decline in the number of samples processed for MTB culture and
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5 161 culture positivity rate which coincided, with the roll out of the Xpert. This is in keeping with
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7 162 the Xpert roll out which started in March 2011 and was completed in September 2013 when
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9 163 all health facilities in the provinces were using the Xpert for TB diagnosis. According to the
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11 164 South African guidelines, MTB culture is not recommended for Xpert rifampicin susceptible
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13 165 patients, which constitutes the majority of patients infected with TB, hence the decline in the
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15 166 number of MTB cultures from 277 963 in 2011 to 172 671 in 2014. Nevertheless, the sheer
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17 167 volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR
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19 168 TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only
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21 169 performed on patients that were considered to be at risk of DR TB, but the use of Xpert for
22
23 170 initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised
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25 171 indications for culture selects for cases that are more likely to have drug resistant (Xpert
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27 172 rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert
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29 173 negative), which explains the high rates of DR TB and declining culture positivity rate observed
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31 174 in this study.
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40 176 Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In
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42 177 addition, spontaneously occurring mutations are rare compared to other TB drugs [8].
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44 178 Consequently, the development of RMR is expected to be uncommon. The finding of
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46 179 increasing mono-resistance in this context is therefore concerning. In a previous study done by
47
48 180 Coovadia *et al*, at the same laboratory, RMR was 8.8% during the years 2007-2009 [9].
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50 181 Similarly, Mukinda *et al* reported increasing RMR in the Western Cape province of SA [10].
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52 182 These findings highlight the importance of testing for INH resistance in all patients with Xpert
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54 183 rifampicin resistance. This positively impacts patient management further as patients with
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3 184 confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment
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5 185 regimen.
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10 187 The development of rifampicin resistance has serious effects on the treatment of TB. Patients
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12 188 have to be treated with more expensive and more toxic drugs for a longer duration. Studies
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14 189 have been conducted in order to elucidate the causes of RMR with the majority reporting an
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16 190 association between HIV and RMR [11-12]. Factors contributing to this association include
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18 191 decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin
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20 192 serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to
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22 193 infection and permits proliferation of TB which favours transmission [14]. Given the high rate
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24 194 of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the
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26 195 increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in
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28 196 co-infected patients remains under investigation.
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35 198 Rifampicin and INH are core drugs that form the backbone for first line short course therapy
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37 199 for the treatment of drug susceptible TB. Given the high burden of disease in this region
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39 200 coupled with the use of Xpert as a screening tool for DR-TB, mono-resistance to INH may
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41 201 inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is
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43 202 only made using TB culture and DST following a negative Xpert result or treatment failure.
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45 203 The use of standard first line TB therapy in patients with undetected INH resistance equates to
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47 204 using rifampicin monotherapy during the continuation phase. This may subsequently lead to
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49 205 the development of MDR-TB. This was described in a study done by Jacobson *et al* where
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51 206 treatment of patients with IMR using standard first line therapy was associated with poor
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53 207 outcomes and progression to MDR-TB [15]. Several studies have reported previous TB therapy
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3 208 as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients
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5 209 who may require TB culture and DST in order to exclude INH resistance.
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10 211 There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and
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12 212 XDR-TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast
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14 213 to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in
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16 214 South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the
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18 215 national guidelines, a significant number of patients with Xpert rifampicin resistant TB did not
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20 216 get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO TB
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22 217 report, which reported the percentage of MDR-TB among MDR/RR-TB as 62% in South
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24 218 Africa. This suggests that a substantial number of Xpert rifampicin resistant TB cases are not
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26 219 confirmed by culture because this discrepancy cannot be explained by RMR cases [1]. Another
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28 220 possible reason may be due to patients that are lost to follow up. In KZN province, the specimen
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30 221 for TB culture is only collected when the patient comes back for Xpert results. Consequently,
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32 222 if patients did not return for the results, then specimens for TB culture would not have been
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34 223 collected. According to the WHO, only 41% of notified MDR/RR-TB cases from South Africa
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36 224 were enrolled for MDR-TB treatment in 2013[18]. Although this figure improved to 62% in
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38 225 2014 [20], the gap remains substantial especially given the considerable improvement in
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40 226 rapidity of diagnosing DR-TB with Xpert. Therefore, this decline in culture confirmed DR-
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42 227 TB indicates a change in the testing method used to diagnose tuberculosis rather than a
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44 228 successful TB control program, which led to underestimation of MDR-TB and XDR-TB cases
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46 229 in this study. The proportion of MDR-TB cases that have XDR-TB remained constant at about
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48 230 11% which is comparable to the global trends at that time [18-20].
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3 232 Our study found higher rates of DR-TB in women compared to men which supports findings
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5 233 from other studies showing higher proportions of DR-TB in women [21-22]. Even though
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7 234 reasons behind the higher DR-TB predisposition among women are unknown, HIV could be a
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9 235 contributing factor. The majority of DR-TB cases were found between the ages of 15 and 44
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11 236 years, which is the same age group that is known to have the highest HIV prevalence [2]. It is
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13 237 well recognised that HIV is a major risk factor for development of TB and HAART reduces its
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15 238 incidence [23-25]. Indeed, Nanoo *et al* showed an inverse relationship between antiretroviral
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17 239 therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with
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19 240 the greatest decline demonstrated in the 25-44 year age group [26].
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26 242 The diagnosis of DR-TB in children is generally difficult due to their inability to expectorate
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28 243 and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB
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30 244 in children is largely as a result of primary transmission from adults, the proportion of DR-TB
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32 245 is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR-TB
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34 246 particularly MDR-TB in children less than five years compared to adults which could be a
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36 247 reflection of the under diagnosis of DR-TB in this age group. Although these rates are lower in
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38 248 children, they are still unacceptable as they reflect transmission of untreated adult TB.
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45 250 Similar to the overall burden of TB in South Africa, DR-TB is also concentrated in urban areas
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47 251 of KZN with eThekweni district harbouring most of the cases due to high population density.
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49 252 However, the rate of MDR-TB cases was highest among the northern districts of the province
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51 253 of Umkhanyakude and Zululand. These are rural districts which share borders with
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53 254 Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance
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55 255 patterns. Mpumalanga province is known to have the highest DR-TB rate in the country while
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57 256 Swaziland has the highest MDR-TB prevalence in Africa [29-30]. In 2007, Wallengren *et al*
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3 257 reported Umzinyathi and Umkhanyakude as the districts with the highest MDR-TB rates [31].
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5 258 The intervention given to the Umzinyathi district following the outbreak of XDR-TB in 2005
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7 259 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and
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9 260 treatment of HIV, TB infection control and intergration of TB and HIV care) may be
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11 261 responsible for these decreasing rates [32]. Despite declining XDR-TB rates at the Umzinyathi
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13 262 district (where the XDR-TB outbreak was identified in 2005), it still remains the district with
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15 263 the highest XDR-TB rates at about three times higher than the rest of KZN [33].
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22 265 **Limitations**

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24 266 Our study is limited by the retrospective design; the accuracy of the data is dependent on
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26 267 available information on the LIS. The absence of unique patient identifiers also affects the
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28 268 accuracy of the data as the removal of duplicates is imperfect. Although duplicates were
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30 269 removed, we could not differentiate between new and known MDR-TB patients. The patient
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32 270 level Xpert data was not available in order to match with the TB culture results. Nevertheless,
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34 271 the high burden of DR-TB and the fact that all cultures are performed in one laboratory for the
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36 272 whole province provide an important insight to the distribution of TB in this region and may
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38 273 inform targeted intervention.
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44 275 Although the data used for this study is relatively old, it represents a critical time of drastic
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46 276 changes in the diagnosis of DR-TB. There have been no subsequent changes in the TB
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48 277 diagnostic algorithm, therefore the findings highlighted in this study should still be relevant to
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50 278 the current setting. The data presented is not prevalence data as only data from MTB positive
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52 279 cultures was used. Thus, the results may be an underrepresentation as patients that were lost to
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54 280 follow up and patients with contaminated/loss of viability cultures were excluded.
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3 282 **Conclusions**

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5 283 Our findings highlight the importance of DR-TB diagnostic algorithms that include both
6
7 284 rifampicin and isoniazid DST in the initial testing. Early detection of RMR will allow addition
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9 285 of isoniazid in the treatment regimen, while detection of IMR will prevent rifampicin
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11 286 monotherapy later on during the continuation phase of treatment which has been associated
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13 287 with development of rifampicin resistance. This will also allow us to have a clearer estimate of
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15 288 MDR-TB cases. HIV and migration play a significant role in the distribution of DR-TB in this
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17 289 region, therefore TB control measures that address these factors may have impact on DR-TB
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19 290 level.
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28 292 **Acknowledgments**

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30
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32
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37 296 maps.
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44 298 **Author contributions**

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47 299 NRM contributed in the development of the concept, study design, data analysis and writing
48
49 300 of the manuscript. YB performed data analysis and assisted with the writing of the manuscript.
50
51 301 KL contributed in the interpretation of data and writing of the manuscript. KM supervised the
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53 302 development of the study concept, study design, data analysis and manuscript writing.
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3 304 **Data sharing statement:** Data may be obtained from a third party and are not publicly
4
5 305 available. The data for this study will only be available upon reasonable request and provided
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7 306 approval is obtained from the it's custodians, i.e. the Department of Health and the National
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9 307 Health Laboratory Service.

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13 308 **Competing interests:** None

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16 309 **Funding:** None

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22 311 **References**

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53 **Table 1. LPA results between 2011 and 2014: RIF and INH mono-resistance***

	2011	2012	2013	2014
Total culture positives	36644	30208	22568	14672
Total cases LPA	31368	26513	18399	12279
% of LPA done	85.6	87.8	81.5	83.7

LPA Any INH Resistance	6430	5548	4167	3045
LPA Any INH Resistance (%)	20.5	20.9	22.7	24.8
LPA INH MR	845	1167	879	505
LPA INH MR (% of All INH Resistant)	13.8	21.0	21.1	17.1
LPA Any RIF Resistance	6293	5013	3912	3134
LPA Any RIF Resistance (%)	20.1	18.9	21.3	25.5
LPA RIF MR	953	767	676	667
LPA RIF MR (% of All RIF Resistant)	15.1	15.3	17.3	21.3

434 *RIF, Rifampicin; INH, isoniazid; LPA, line probe assay; MR, mono-resistance

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Table 2. Total TB positive, MDR and XDR cases per district for each year.

District	2011					2012					2013					2014				
	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%	Total positives	MDR	%	XDR	%
Amajuba	597	96	16.1	8	8.3	523	86	16.4	4	4.7	353	40	11.3	4	10.0	272	35	12.9	0	0.0
Ethekwini	17519	2837	16.2	353	12.4	13453	2306	17.1	338	14.7	11118	2212	19.9	321	14.5	7404	1516	20.5	203	13.4
Harry Gwala	776	124	16.0	5	4.0	752	191	25.4	13	6.8	526	186	35.4	4	2.2	396	142	35.9	12	8.5
Ilembe	1704	269	15.8	9	3.3	1219	206	16.9	16	7.8	978	180	18.4	7	3.9	405	76	18.8	6	7.9
Ugu	2172	542	25.0	37	6.8	2443	465	19.0	47	10.1	1655	388	23.4	34	8.8	1123	268	23.9	12	4.5
Umgungundlovu	3514	520	14.8	55	10.6	2858	532	18.6	57	10.7	1486	305	20.5	27	8.9	703	159	22.6	22	13.8
Umkhanyakude	2230	732	32.8	5	0.7	1619	501	30.9	29	5.8	1214	422	34.8	15	3.6	945	341	36.1	19	5.6
Umzinyathi	1478	334	22.6	134	40.1	1295	257	19.8	102	39.7	894	192	21.5	66	34.4	674	196	29.1	54	27.6
Uthukela	1100	120	10.9	16	13.3	1023	111	10.9	13	11.7	545	71	13.0	6	8.5	261	55	21.1	12	21.8
Uthungulu	2640	589	22.3	30	5.1	2737	590	21.6	41	6.9	2315	477	20.6	26	5.5	1178	294	25.0	12	4.1
Zululand	2888	735	25.5	54	7.3	2259	662	29.3	37	5.6	1468	428	29.2	34	7.9	1168	362	31.0	28	7.7

Unknown	26	3	11.5	0	0.0	27	5	18.5	0	0.0	16	1	6.3	0	0.0	143	62	43.4	9	14.5
Total	36644	6901	18.8	706	10.2	30208	5912	19.6	697	11.8	22568	4902	21.7	544	11.1	14672	3506	23.9	389	11.1

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437 * MDR, multidrug-resistant; XDR, extensively drug-resistant; TB, tuberculosis

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3 438 Figure 1: Flow diagram showing the number of specimens received and the laboratory
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5 439 procedures performed at the TB culture laboratory.
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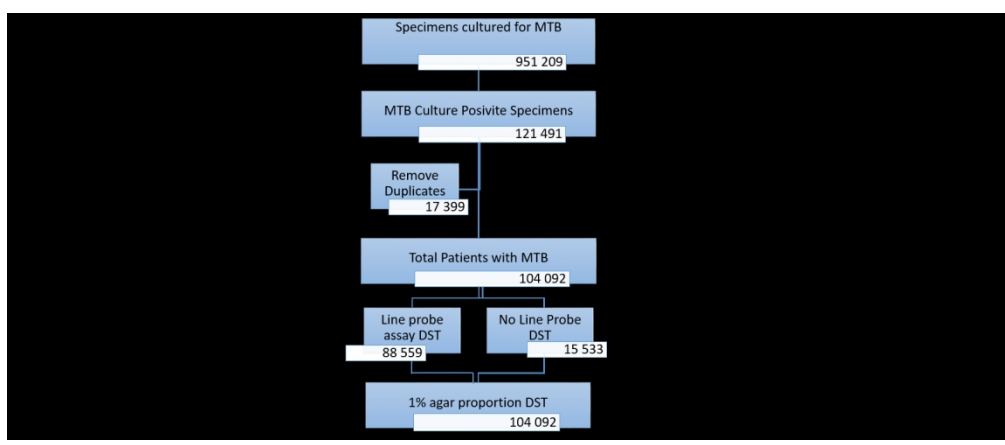
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9 440 Figure 2: TB culture specimens processed between 2011 and 2014. The figure shows the total
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11 441 number of specimens received and the total number (and percentage) thereof that were positive.
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14 442 Figure 3: Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB
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16 443 cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-
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18 444 TB of total positive cases by gender
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21 445 Figure 4: Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB
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23 446 cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-
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25 447 TB of total positive cases by gender.
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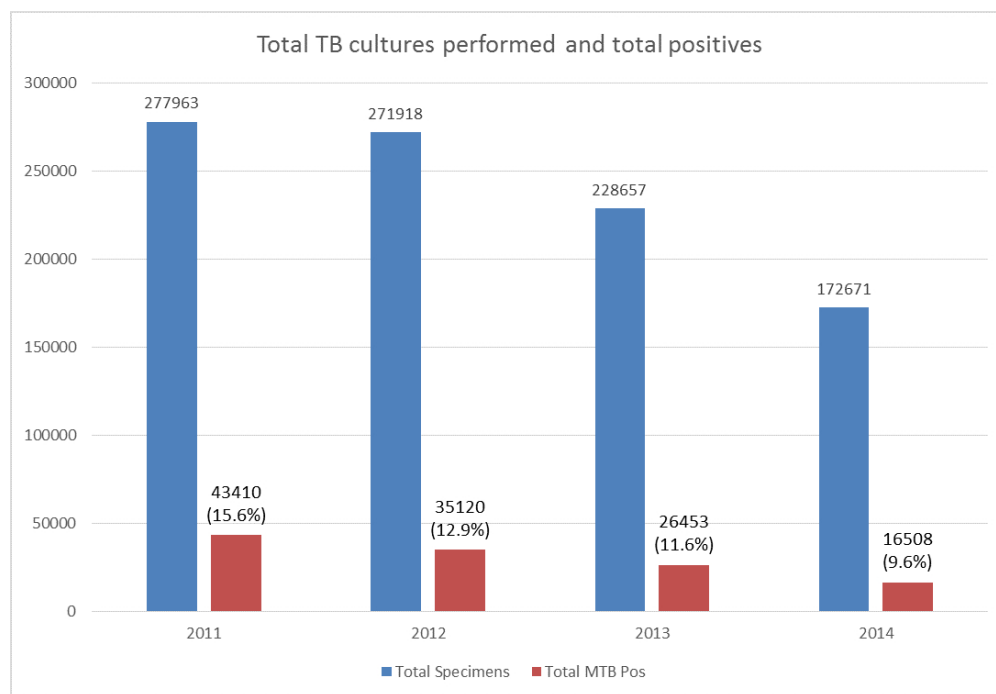
29 448 Figure 5: The first panel depicts the percentage of MDR-TB cases per district for the period
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31 449 2011 – 2014. The percentage of MDR-TB cases among TB cases diagnosed by culture between
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33 450 2011 and 2014. The size of the circle represents the percentage. The second panel depicts the
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35 451 percentage of XDR-TB cases per district for the period 2011 – 2014. The percentage of XDR-
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37 452 TB cases among MDR-TB cases diagnosed by culture between 2011 and 2014. The map was
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39 453 produced specifically for the purposes of this study, it is therefore not under copyright
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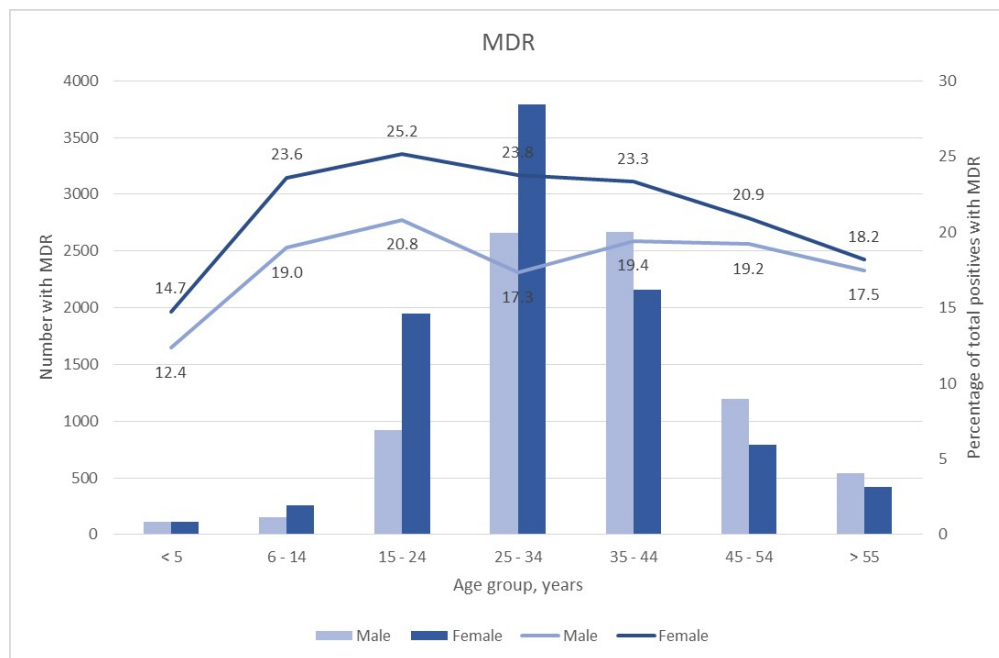
Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

292x124mm (150 x 150 DPI)



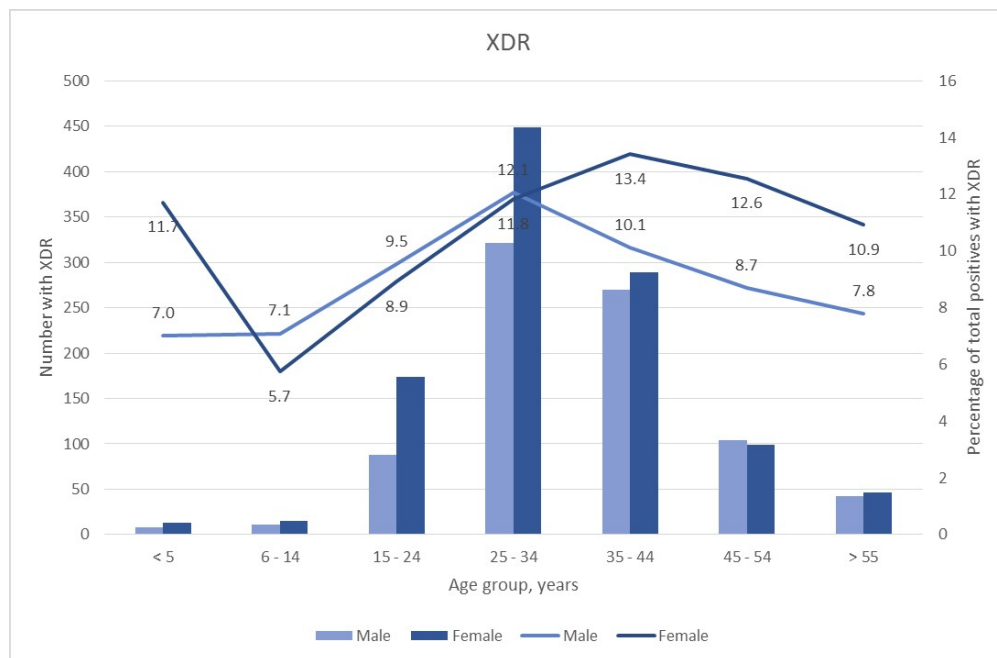
29 TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens
30 received and the total number (and percentage) thereof that were positive.

31 184x127mm (150 x 150 DPI)



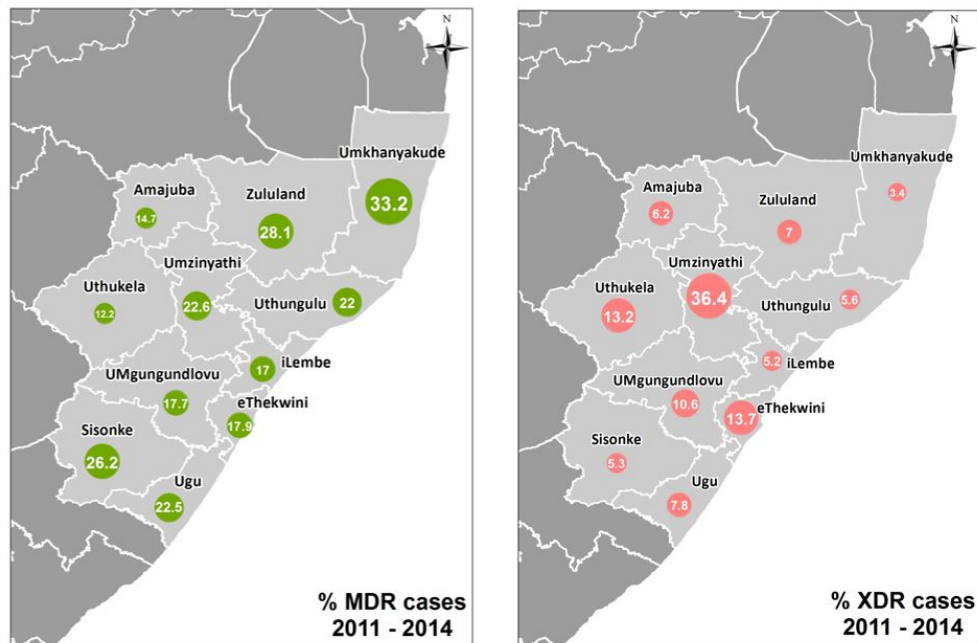
Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-TB of total positive cases by gender

184x120mm (150 x 150 DPI)



Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-TB of total positive cases by gender.

184x120mm (150 x 150 DPI)



The first panel depicts the percentage of MDR-TB cases per district for the period 2011 – 2014. The percentage of MDR-TB cases among TB cases diagnosed by culture between 2011 and 2014. The size of the circle represents the percentage. The second panel depicts the percentage of XDR-TB cases per district for the period 2011 – 2014. The percentage of XDR-TB cases among MDR-TB cases diagnosed by culture between 2011 and 2014. The map was produced specifically for the purposes of this study, it is therefore not under copyright.

252x168mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found	2 2	Retrospective observational study We analysed data for all MTB cultures performed in the KwaZulu-Natal province between 2011 and 2014. Our findings show increasing rifampicin mono-resistance and a substantial amount of INH mono-resistance. Although DR-TB is widespread, HIV and migration influence its distribution.
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5	Xpert replaced smear microscopy in the initial diagnosis of TB and all patients that do not demonstrate rifampicin resistance are assumed to have drug susceptible TB and therefore initiated on standard first line TB therapy. Thus, Xpert rifampicin susceptible cases do not get a culture, so isoniazid mono-resistance is not routinely investigated. TB culture and drug susceptibility testing (DST) is only indicated for patients that demonstrate rifampicin resistance on the Xpert, paucibacillary TB cases missed by Xpert (HIV infected, children and extra-pulmonary TB) and patients that fail TB treatment. Despite the recent changes in the diagnosis and management of TB, there are no studies that have assessed their impact on culture confirmed TB. The level of isoniazid mono-resistance that is not routinely investigated

with current diagnostic methods is unknown, but instead these patients are getting rifampicin monotherapy during the continuous phase of their first line TB therapy which could potentially fuel drug resistance. On the other hand, patients with rifampicin mono-resistance can benefit from the addition of isoniazid in their treatment.

Objectives	3	State specific objectives, including any prespecified hypotheses	5	Evaluate the amount of rifampicin and isoniazid mono-resistance. Describe the drug resistance patterns and distribution among different age groups, genders and districts in KZN, South Africa.
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Methods

Study design	4	Present key elements of study design early in the paper	5	The study is a retrospective observational study using laboratory data.
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Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5	This retrospective observational study was conducted at the central academic laboratory of the KwaZulu-Natal province of South Africa using TB culture data for 2011-2014. KZN province is one of nine provinces in South Africa and its population of just over 10 million ranks second in the country. There are 77 public health hospitals (including 8 MDR-TB initiation sites) within 11 health districts. Provincial Mycobacterium tuberculosis culture and drug susceptibility testing are performed in one central academic laboratory.
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.Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	6	The TB culture and drug susceptibility data was collected from the National Health Laboratory Service laboratory information system (LIS) which contains all electronic laboratory results. TB All
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		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants		cultures performed in the provincial TB laboratory from 2011 till 2014 were included
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed		
		<i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable		Not applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6	The data was collected from the National Health Laboratory Service laboratory information system (LIS) which contains all electronic laboratory results. In the absence of a unique identifier, duplicates were removed using MRN number (number given by the laboratory to specimens from the same patient) and demographic data (name, surname and date of birth). The results were stratified according to the health districts, age and gender. For the analysis of age, cases without recorded age or date of birth were excluded.
Bias	9	Describe any efforts to address potential sources of bias		Not Applicable
Study size	10	Explain how the study size was arrived at		Not Applicable: All results included

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2	Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6	Data was described using frequencies and proportions.
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4	Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6	Continuous data was described using means, standard deviations (sd) and 95% confidence intervals (95% CI). Categorical outcomes were tested using the chi-squared test. Log binomial regression of MDR and XDR-TB was performed using sex, age group, district and year as predictors to estimate the adjusted relative risk ratios. Data was analysed using Stata 14 (StataCorp., College Station, TX, USA).
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20			(b) Describe any methods used to examine subgroups and interactions		
21			(c) Explain how missing data were addressed		
22			(d) Cohort study—If applicable, explain how loss to follow-up was addressed		
23			Case-control study—If applicable, explain how matching of cases and controls was addressed		
24			Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy		
25			(e) Describe any sensitivity analyses		
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29	Results				
30	Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1	A flow diagram is used to report number of specimens and individuals at each stage
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34			(b) Give reasons for non-participation at each stage		
35			(c) Consider use of a flow diagram		
36	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7	-Between 2011 and 2014, a total of 951 209 specimens were cultured for MTB in KwaZulu-Natal
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				-After removing duplicates, there were 36644, 30208, 22568, 14672 culture confirmed cases of TB in 2011, 2012, 2013 and 2014 respectively
		(b) Indicate number of participants with missing data for each variable of interest		
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)		
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time		Not Applicable
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure		
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures		
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8	Standard deviations and 95% confidence intervals are given
		(b) Report category boundaries when continuous variables were categorized		
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period		

Continued on next page

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2	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses		
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4	Discussion				
5	Key results	18	Summarise key results with reference to study objectives	8-12	Key findings:
6					-Increasing mono-resistance
7					highlight the importance of testing
8					for INH resistance in all patients
9					with Xpert rifampicin resistance
10					-Significant INH mono-resistance
11					that is currently missed in the initial
12					diagnosis of TB.
13					- The majority of DR-TB cases
14					were found between the ages of 15
15					and 44 years, which is the same age
16					group that is known to have the
17					highest HIV prevalence
18					-DR TB is high in areas bordering
19					high DR TB regions, thus migration
20					may influence resistance patterns.
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25	Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13	-Retrospective design; the accuracy
26					of the data is dependent on
27					available information.
28					- Absence of unique patient
29					identifiers: affects the accuracy of
30					the data as the removal of
31					duplicates is imperfect
32					- Could not differentiate between
33					new and known MDR-TB patients
34					- Xpert MTB/RIF data was not
35					available for comparison
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39	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-13	Our findings highlight the
40					importance of DR-TB diagnostic
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algorithms that include both rifampicin and isoniazid DST in the initial testing. Early detection of RMR will allow addition of isoniazid in the treatment regimen, while detection of IMR will prevent rifampicin monotherapy later on during the continuation phase of treatment which has been associated with development of rifampicin resistance. This will also allow us to have a clearer estimate of MDR-TB cases. HIV and migration play a significant role in the distribution of DR-TB in this region, therefore TB control measures that address these factors may have impact on DR-TB level.

Generalisability	21	Discuss the generalisability (external validity) of the study results	13	Conclusion applies to a wide variety of settings
Other information				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based		None

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.