BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031663
Article Type:	Research
Date Submitted by the Author:	14-May-2019
Complete List of Authors:	Mvelase, Nomonde; National Health Laboratory Service, Medical Microbiology Balakrishna, Yusentha Lutchminarain, Keeren Mlisana, Koleka
Keywords:	Tuberculosis < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES, Health policy < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Diagnostic microbiology < INFECTIOUS DISEASES

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

N. R. Mvelase [1, 2], Y. Balakrishna [3], K. Lutchminarain [1, 2], K. P. Mlisana [1, 2, 4]

[1]Department of Medical Microbiology, KwaZulu-Natal Academic Complex, National Health Laboratory Service, Durban, South Africa

[2]Department of Medical Microbiology, School of Laboratory Medicine & Medical Sciences, University of KwaZulu-Natal, Durban, South Africa

[3]Biostatistics Unit, South African Medical Research Council, Durban, South Africa

[4]Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa

Corresponding author: Nomonde R. Mvelase

Address: KwaZulu-Natal National Health Laboratory Service, Department of Medical Microbiology, Level 4, Laboratory Building, IALCH, Durban, South Africa

Email: dlaminin15@ukzn.ac.za

Running Title: Drug resistant TB in KwaZulu-Natal

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

and migration influence its distribution; therefore, TB control strategies should include interventions that target these aspects.

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

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

in the country, it remains the highest drug resistant (DR) TB burdened province with almost a third of the country's cases of drug resistant TB [3].

Compounding the problem of TB in South Africa, is the high rate of co-infection with HIV (about 60%) [1]. While it is well known that HIV is associated with smear negative TB, smear microscopy was traditionally used in the initial diagnosis of TB because of its quick time to results and low cost [4]. On the other hand, conventional TB culture is much more sensitive than smear microscopy, but its high cost, complexity and long delays in getting the results made it impractical for routine diagnosis of TB. Therefore, when the WHO endorsed the Xpert MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was subsequently introduced in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated nucleic acid amplification test that offers better detection of TB compared to smear microscopy with an added advantage of the ability to detect rifampicin resistance in less than two hours in clinical specimens [5-6].

The implementation of Xpert in South Africa completely changed the testing algorithm for the diagnosis of TB [7]. 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. We therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the drug resistance patterns and distribution among different age groups, genders and districts in KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will inform targeted intervention in TB control in this high TB endemic region.

Methods

Study design

The study is a retrospective observational study using laboratory data for 2011 till 2014.

Study setting

The 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.

Laboratory Procedures

MTB isolation from clinical samples was routinely done using the automated BACTEC mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin, streptomycin, kanamycin was performed for all cases with resistance to rifampicin or isoniazid on the LPA using 1% agar proportion method (APM) on Middlebrook 7H10.

Patient and public involvement

The data used for this study was the routine TB diagnostic data, therefore there was no direct patient and public involvement.

Data collection and analysis

The TB culture and DST 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 demographic data. 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.

Data was described using frequencies and proportions. Continuous data was described using means and standard deviations (sd). 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).

Ethical consideration

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

Results

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

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

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

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

Over the 4-year period, eThekwini district had the highest number of TB cases with 47.5% of all cases in KZN coming from this district (Table 2). However, the districts with the highest yearly proportion of MDR TB cases each year were Umkhanyakude (mean 33.2%, sd 2.3%), followed by Zululand (mean 28.1%, sd 2.3) and Harry Gwala (mean 26.2%, sd 9.4). The yearly proportion of MDR TB cases that had XDR TB were highest at Umzinyathi (mean 36.4%, sd 5.8%) followed by eThekwini (mean 13.7%, sd 1.1%) and Uthukela (mean 13.2%, sd 5.7%) districts. Umkhanyakude district had the lowest proportion of XDR with a yearly mean of 3.4% (sd 2.4%) over the study period (Figure 4).

Discussion

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

volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only performed on patients that were considered to be at risk of DR TB, but the use of Xpert for initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised indications for culture selects for cases that are more likely to have drug resistant (Xpert rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert negative), which explains the high rates of DR TB and declining culture positivity rate observed in this study.

Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In addition, spontaneously occurring mutations are rare compared to other TB drugs [8]. Consequently, the development of RMR is expected to be uncommon. The finding of increasing mono-resistance in this context is therefore concerning. In a previous study done by Coovadia et al, at the same laboratory, RMR was 8.8% during the years 2007-2009 [9]. Similarly, Mukinda et al reported increasing RMR in the Western Cape province of SA [10]. These findings highlight the importance of testing for INH resistance in all patients with Xpert rifampicin resistance. This positively impacts patient management further as patients with confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment regimen.

The development of rifampicin resistance has serious effects on the treatment of TB. Patients have to be treated with more expensive and more toxic drugs for a longer duration. Studies have been conducted in order to elucidate the causes of RMR with the majority reporting an association between HIV and RMR [11-12]. Factors contributing to this association include decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin

serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to infection and permits proliferation of TB which favours transmission [14]. Given the high rate of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in co-infected patients remains under investigation.

Rifampicin and INH are core drugs that form the backbone for first line short course therapy for the treatment of drug susceptible TB. Given the high burden of disease in this region coupled with the use of Xpert as a screening tool for DR TB, mono-resistance to INH may inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is only made using TB culture and DST following a negative Xpert result or treatment failure. The use of standard first line TB therapy in patients with undetected INH resistance equates to using rifampicin monotherapy during the continuation phase. This may subsequently lead to the development of MDR TB. This was described in a study done by Jacobson et al where treatment of patients with IMR using standard first line therapy was associated with poor outcomes and progression to MDR TB [15]. Several studies have reported previous TB therapy as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients who may require TB culture and DST in order to exclude INH resistance.

There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and XDR TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the national guidelines, a significant number of patients where Xpert detected rifampicin resistance did not get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO

TB report, that reported the percentage of MDR TB among MDR/RR-TB as 62% in South Africa, which suggests that a substantial number of Xpert RR cases are not confirmed by culture as this discrepancy cannot be explained by RMR cases [1]. Hence this indicates a change in the testing method used to diagnose tuberculosis rather than a successful TB control program, which led to underestimation of MDR and XDR TB cases in this study. Other possible reasons may include patient loss to follow up and loss of MTB viability (no growth on culture) which could be caused by long transport duration in specimens from remote areas. According to the WHO, only 41% of notified MDR/RR-TB cases from South Africa were enrolled for MDR TB treatment in 2013[18]. Although this figure improved to 62% in 2014 [20], the gap remains substantial especially given the considerable improvement in rapidity of diagnosing DR TB with Xpert. The proportion of MDR TB cases that have XDR TB also remained constant at about 11% which is comparable to the global trends at that time [18-20].

Our study found higher rates of DR TB in women compared to men which supports findings from other studies showing higher proportions of DR TB in women [21-22]. Even though reasons behind the higher DR TB predisposition among women are unknown, HIV could be a contributing factor. 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 [2]. It is well recognised that HIV is a major risk factor for development of TB and HAART reduces its incidence [23-25]. Indeed, Nanoo et al showed an inverse relationship between antiretroviral therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with the greatest decline demonstrated in the 25-44 year age group [26].

The diagnosis of DR TB in children is generally difficult due to their inability to expectorate and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB

in children is largely as a result of primary transmission from adults, the proportion of DR TB is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR TB (Figure 2) particularly MDR TB in children less than five years compared to adults which could be a reflection of the under diagnosis of DR TB in this age group. Although these rates are lower in children, they are still unacceptable as they reflect transmission of untreated adult TB.

Similar to the overall burden of TB in South Africa, DR TB is also concentrated in urban areas of KZN with eThekwini district harbouring most of the cases due to high population density. However, the rate of MDR TB cases was highest among the northern districts of the province of Umkhanyakude and Zululand. These are rural districts which share borders with Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance patterns. Mpumalanga province is known to have the highest DR TB rate in the country while Swaziland has the highest MDR TB prevalence in Africa [29-30]. In 2007, Wallengren et al reported Umzinyathi and Umkhanyakude as the districts with the highest MDR TB rates [31]. The intervention given to the Umzinyathi district following the outbreak of XDR TB in 2005 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and treatment of HIV, TB infection control and intergration of TB and HIV care) may be responsible for these decreasing rates [32]. Despite declining XDR TB rates at the Umzinyathi district (where the XDR TB outbreak was identified in 2005), it still remains the district with the highest XDR TB rates at about three times higher than the rest of KZN [33].

Limitations

Our study is limited by the retrospective design; the accuracy of the data is dependent on available information on the LIS. The absence of unique patient identifiers also affects the accuracy of the data as the removal of duplicates is imperfect. Although duplicates were

removed, we could not differentiate between new and known MDR TB patients. The patient level Xpert data was not available in order to match with the TB culture results. Nevertheless, the high burden of DR TB and the fact that all cultures are performed in one laboratory for the whole province provide an important insight to the distribution of TB in this region and may inform targeted intervention.

Conclusions

Our findings highlight the importance of DR TB diagnostic 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.

Acknowledgments

We thank the staff at the Inkosi Albert Luthuli Central Hospital TB laboratory for their dedication and hard work. We also thank Thandi Kapwata from the Environment and Health Research Unit within the South African Medical Research Council, for designing the provincial maps.

Author contributions

NRM contributed in the development of the concept, study design, data analysis and writing of the manuscript. YB performed data analysis and assisted with the writing of the manuscript. KL contributed in the interpretation of data and writing of the manuscript. KPM supervised the development of the study concept, study design, data analysis and manuscript writing.

Data sharing statement: No additional data available

Competing interests: None

Funding: None

References

1. World Health Organization. Global Tuberculosis Report 2017. http://www.who.int/tb/publications/global_report/gtbr2017_main_text.pdf

2. Gandhi NR, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet* 2006; 368: 1575-1580.

3. Ndjeka N. National Department of Health. Multi-drug resistant tuberculosis: strategic overview on MDR-TB care in South Africa. 2014. https://www.health-e.org.za/wp-content/uploads/2014/03/Strategic_overview_of_MDR_TB_RSA.pdf.

4. Hassim S, et al. Detection of a substantial rate of multidrug-resistant tuberculosis in an HIV-infected population in South Africa by active monitoring of sputum samples. *Clinical Infectious Disease* 2010; 50: 1053-1059.

5. Helb D, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology* 2010; 48: 229-237.

6. Boehme CC, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine* 2010; 363: 1005-1015.

7. National Department of Health, South Africa. National tuberculosis management guidelines. 2014.

https://www.health-e.org.za/wp-content/uploads/2014/06/NTCP_Adult_TB-Guidelines-27.5.2014.pdf

8. David HL. Probability distribution of drug-resistant mutants in unselected populations of Mycobacterium tuberculosis. *Journal of Applied Microbiology* 1970; 20: 810–814.

9. Coovadia YM, et al. Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. *PLOS ONE*. 2013, 8: e77712.

10. Mukinda FK, et al. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. *International Journal of Tuberculosis and Lung Disease* 2012; 16: 196–202.

11. Sandman L, et al. Risk factors for rifampin-monoresistant tuberculosis: a case-control study. *American Journal of Respiratory and Critical Care Medicine* 1999; 159:468–472.

12. Villegas L, et al. Prevalence, risk factors, and treatment outcomes of isoniazid and rifampicin mono-resistant pulmonary tuberculosis in Lima, Peru. *PLOS ONE* 2016; 11: e0152933.

13. Gurumurthy P, et al. Malabsorption of rifampicin and isoniazid in HIV infected patients with and without tuberculosis. *Clinical Infectious Disease* 2004; 38: 280–283.

14. Bifani P, et al. The evolution of drug resistance in *Mycobacterium tuberculosis*: from a mono-rifampin-resistant cluster into increasingly multidrug-resistant variants in an HIV-seropositive population. *Journal of Infectious Disease* 2008; 198: 90–94.

15. Jacobson KR, et al. Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape province, South Africa. *Clinical Infectious Disease* 2011; 53: 369–372.

16. Cattamanchi A, et al. Clinical characteristics and treatment outcomes of isoniazid mono-resistant tuberculosis. *Clinical Infectious Disease* 2009; 48: 179–185.

17. Fox L, et al. Comparison of isoniazid monoresistant tuberculosis with drug-susceptible tuberculosis and multidrug-resistant tuberculosis. *European Journal of Clinical Microbiology & Infectious Diseases* 2011; 30: 863-867.

18. World Health Organization. Global Tuberculosis Report. 2013 http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf

19. World Health Organization. Global Tuberculosis Report 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf

20. World Health Organization. Global Tuberculosis Report. 2015 http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf

21. O'Donnell MR, et al. Extensively drug-resistant tuberculosis in women, KwaZulu-Natal, South Africa. *Emerging Infectious Disease* 2011; 17: 1942–1945.

22. Cox HS, et al. Multidrug-resistant tuberculosis in Central Asia. *Emerging Infectious Disease* 2004; 10: 865–872.

23. Sonnenberg P, et al. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *Journal of Infectious Disease* 2005 15; 191: 150-158.

24. Badri M, et al. Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area. *International Journal of Tuberculosis and Lung Disease* 2001; 5: 225–232.

25. Badri M, et al. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet* 2002; 359: 2059–2064.

26. Nanoo A, et al. Nationwide and regional incidence of microbiologically confirmed pulmonary tuberculosis in South Africa, 2004–12: a time series analysis. *Lancet Infectious Disease* 2015; 15: 1066–1076.

27. Schaaf HS, et al. Surveillance of anti-tuberculosis drug resistance among children from the Western Cape Province of South Africa—an upward trend. *American Journal of Public Health* 2009; 99: 1486-1490.

28. Zignol M, et al. Multidrug-resistant tuberculosis in children: evidence from global surveillance. *European Respiratory Journal* 2013; 42: 701–707.

29. Andrews JR, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *Journal of Infectious Disease* 2007; 196: S482-S490.

30. Sanchez-Padilla E, et al. High prevalence of multidrug-resistant tuberculosis, Swaziland, 2009–2010. *Emerging Infectious Diseases* 2012; 18: 29–37.

31. Wallengren K, et al. Drug-resistant tuberculosis, KwaZulu-Natal, South Africa, 2001–2007. *Emerging Infectious Diseases* 2011; 17: 1913-1916.

32. Bateman C. Tugela Ferry's extensively drug-resistant tuberculosis – 10 years on. *South African Medical Journal* 2015;105: 517-520.

33. Lim JR, et al. Incidence and geographic distribution of extensively drug resistant tuberculosis in KwaZulu-Natal province, South Africa. *PLOS ONE* 2015; 10: e0132076.

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

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

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

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

422

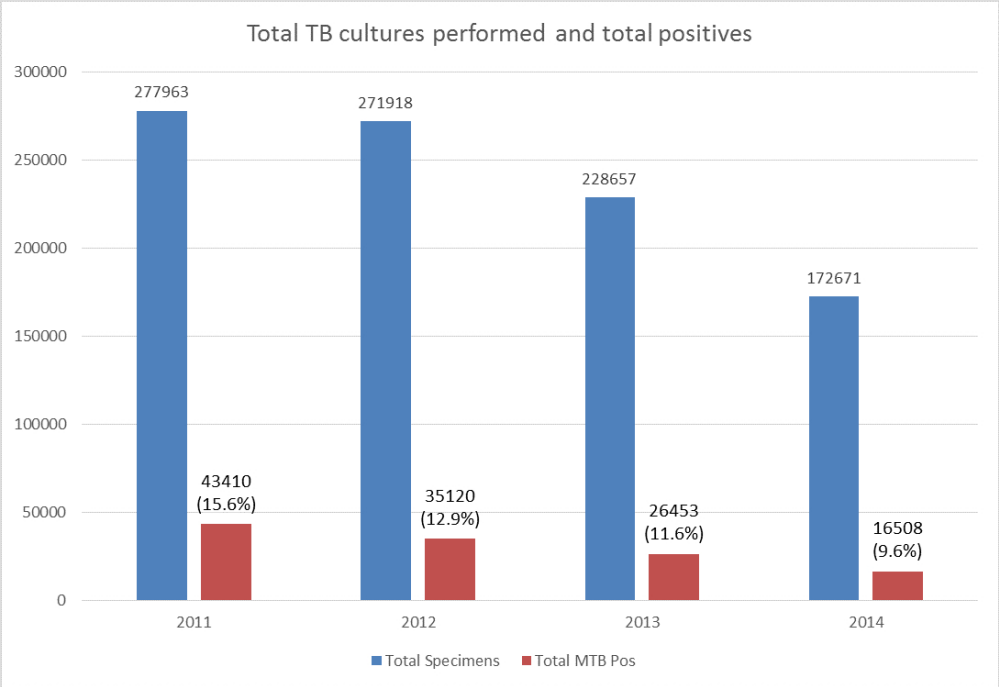


Figure 1: TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens received and the total number (and percentage) thereof that were positive.

184x127mm (150 x 150 DPI)

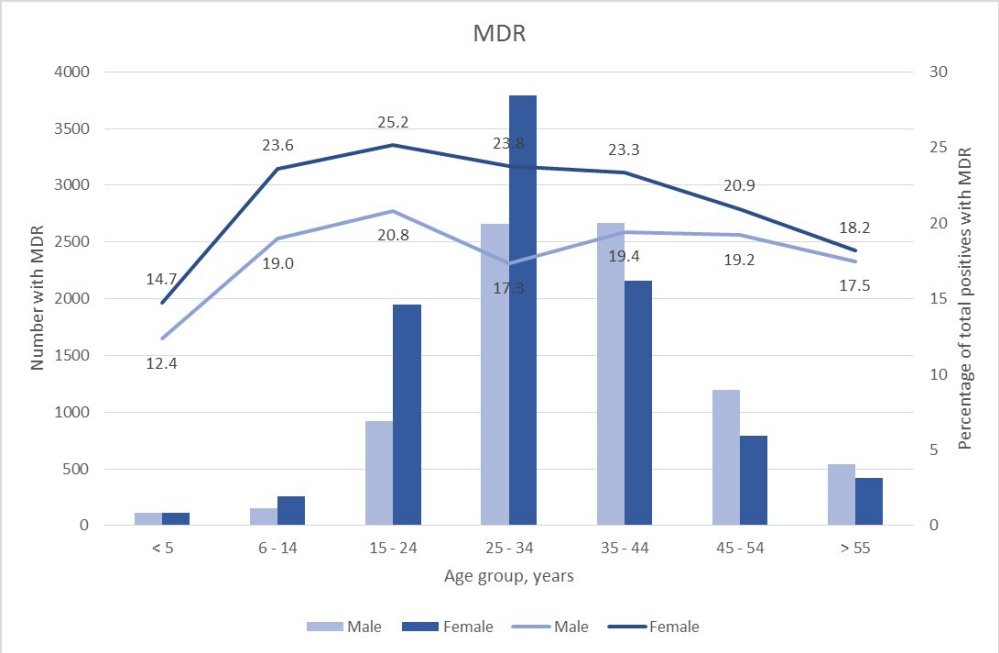


Figure 2: Distribution of MDR TB cases and proportions by gender. The number of MDR TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR TB of total positive cases by gender

184x120mm (150 x 150 DPI)

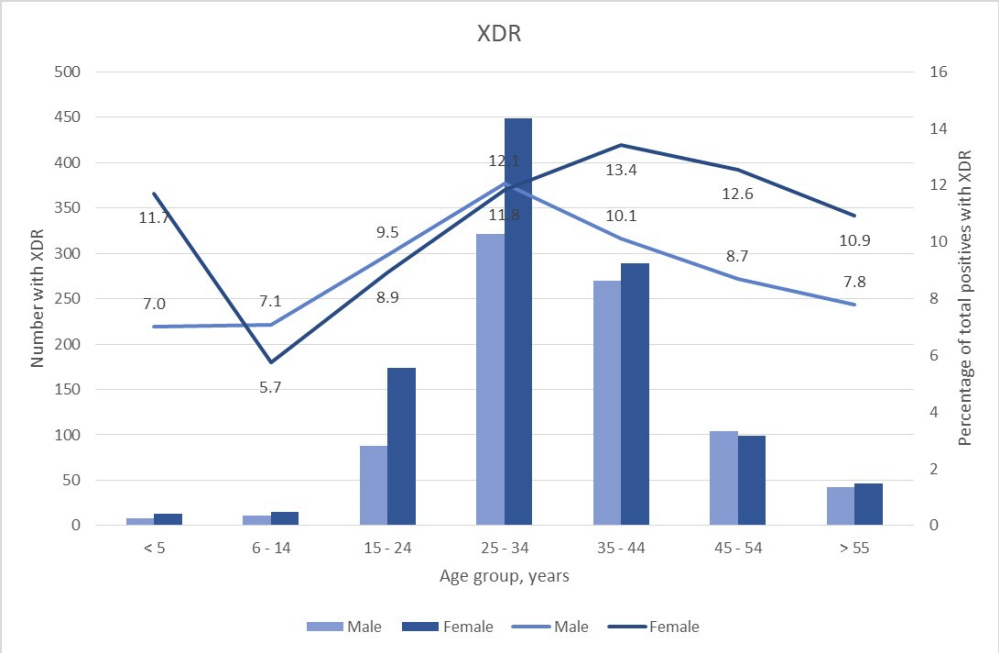


Figure 3: Distribution of XDR TB cases and proportions by gender. The number of XDR TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR of total positive cases by gender

184x120mm (150 x 150 DPI)

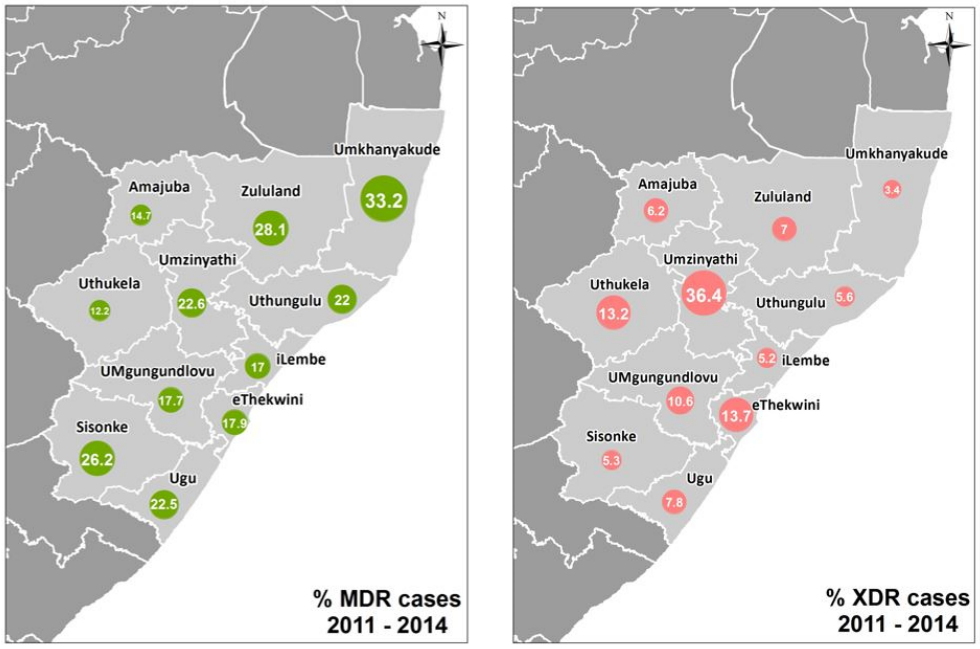


Figure 4: The first panel depicts the percentage of MDR TB cases per district for the period 2011 – 2014. The percentage of MDR TB cases among TB cases diagnosed by culture between 2011 and 2014. The size of the circle represents the percentage

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-031663.R1
Article Type:	Original research
Date Submitted by the Author:	10-Sep-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

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

N. R. Mvelase [1, 2], Y. Balakrishna [3], K. Lutchminarain [1, 2], K. Mlisana [1, 2, 4]

[1]Department of Medical Microbiology, KwaZulu-Natal Academic Complex, National Health Laboratory Service, Durban, South Africa

[2]Department of Medical Microbiology, School of Laboratory Medicine & Medical Sciences, University of KwaZulu-Natal, Durban, South Africa

[3]Biostatistics Unit, South African Medical Research Council, Durban, South Africa

[4]Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa

Corresponding author: Nomonde R. Mvelase

Address: KwaZulu-Natal National Health Laboratory Service, Department of Medical Microbiology, Level 4, Laboratory Building, IALCH, Durban, South Africa

Email: dlaminin15@ukzn.ac.za

Running Title: Drug resistant TB in KwaZulu-Natal

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

and migration influence its distribution; therefore, TB control strategies should include interventions that target these aspects.

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

The World Health Organisation (WHO) has declared multidrug resistant tuberculosis a global crisis. Multidrug-resistant tuberculosis (MDR TB) is defined as resistance to isoniazid and rifampicin. Despite the decline in the global incidence rates of tuberculosis (TB), drug resistant TB cases are on the rise with 558 000 estimated incident cases of MDR plus rifampicin resistant (RR) TB and more than 230 000 deaths in 2017 [1]. South Africa has one of the highest incidence of TB in the world which WHO estimated to be 567 per 100 000 in 2017 [1]. In 2017 alone, South Africa had an estimated number of 14 000 rifampicin resistant cases, the second highest number in Africa after Nigeria which has more than three times the South African population [1]. Moreover, the first reported outbreak of extensively drug-resistant (XDR) tuberculosis (defined as MDR-TB plus resistance to any second line injectable and a

fluoroquinolone) which caused global concern in 2005 was from the province of KwaZulu-Natal (KZN) in South Africa [2]. While the incidence of TB in KZN is proportional to other provinces in the country, it remains the highest drug resistant (DR) TB burdened province with almost a third of the country's cases of drug resistant TB [3].

Compounding the problem of TB in South Africa, is the high rate of co-infection with Human Immunodeficiency Virus (HIV) (about 60%) [1]. While it is well known that HIV is associated with smear negative TB, smear microscopy was traditionally used in the initial diagnosis of TB because of its quick time to results and low cost [4]. On the other hand, conventional TB culture is much more sensitive than smear microscopy, but its high cost, complexity and long delays in getting the results made it impractical for routine diagnosis of TB. Therefore, when the WHO endorsed the Xpert MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was subsequently introduced in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated nucleic acid amplification test that offers better detection of TB compared to smear microscopy with an added advantage of the ability to detect rifampicin resistance in less than two hours in clinical specimens [5-6].

The implementation of Xpert in South Africa completely changed the testing algorithm for the diagnosis of TB [7]. 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. We therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the drug resistance patterns and distribution among different age groups, genders and districts in KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will inform targeted intervention in TB control in this high TB endemic region.

Methods

Study design

The study is a retrospective observational study using laboratory data for 2011 till 2014.

Study setting

The 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.

Laboratory Procedures

MTB isolation from clinical samples was routinely done using the automated BACTEC mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin, streptomycin, kanamycin was performed for all TB culture positive cases using 1% agar proportion method (APM) on Middlebrook 7H10.

Patient and public involvement

The data used for this study was the routine TB diagnostic data, therefore there was no direct patient and public involvement.

Data collection and analysis

The TB culture and DST 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.

Data was described using frequencies and proportions. 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).

Ethical consideration

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

Results

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

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

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

respectively (Table 2). The proportion of TB cases that had MDR-TB ranged from 18.8% in 2011 to 23.9% in 2014, with an overall average of 21%. The overall rate of XDR-TB among MDR-TB cases was 11% (2336 XDR-TB cases out of 21221 MDR-TB).

The majority of the TB cases were males; however, females constituted highest prevalence of the DR-TB across all age groups (Figure 3 and 4). The number of MDR-TB cases was higher among females than males until the age of 34, thereafter males had a higher number than females. Similarly, for XDR-TB, females constituted the most number of XDR-TB. More than 60% of both MDR-TB and XDR-TB cases were patients between the ages of 25 and 44 years. It was observed that children less than 5 years of age showed the lowest rates of MDR-TB whilst that of XDR-TB was lowest between the ages of 6-14 years.

Over the 4-year period, eThekwini district had the highest number of TB cases with 47.5% of all cases in KZN coming from this district (Table 2). However, the districts with the highest yearly proportion of MDR-TB cases each year were Umkhanyakude [(mean 33.2%, sd 2.3%), (95% CI 29.5% to 36.9%)]; followed by Zululand [(mean 28.1%, sd 2.3), (95% CI 24.4% to 31.8%)] and Harry Gwala [(mean 26.2%, sd 9.4), (95% CI 11.2% to 41.2%)]. The yearly proportion of MDR-TB cases that had XDR-TB were highest at Umzinyathi [(mean 36.4%, sd 5.8%), (95% CI 27.2% to 45.6%)] followed by eThekwini [(mean 13.7%, sd 1.1%), (95% CI 11.9% to 15.5%)] and Uthukela [(mean 13.2%, sd 5.7%), (95% CI 4.1% to 22.3%)] districts. Umkhanyakude district had the lowest proportion of XDR-TB with a yearly mean of 3.4% [(sd 2.4%), (95% CI -0.4% to 7.2%)] over the study period (Figure 5).

Discussion

In this study we observe a decline in the number of samples processed for MTB culture and culture positivity rate which coincided, with the roll out of the Xpert. This is in keeping with the Xpert roll out which started in March 2011 and was completed in September 2013 when all health facilities in the provinces were using the Xpert for TB diagnosis. According to the South African guidelines, MTB culture is not recommended for Xpert rifampicin susceptible patients, which constitutes the majority of patients infected with TB, hence the decline in the number of MTB cultures from 277 963 in 2011 to 172 671 in 2014. Nevertheless, the sheer volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only performed on patients that were considered to be at risk of DR TB, but the use of Xpert for initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised indications for culture selects for cases that are more likely to have drug resistant (Xpert rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert negative), which explains the high rates of DR TB and declining culture positivity rate observed in this study.

Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In addition, spontaneously occurring mutations are rare compared to other TB drugs [8]. Consequently, the development of RMR is expected to be uncommon. The finding of increasing mono-resistance in this context is therefore concerning. In a previous study done by Coovadia *et al,* at the same laboratory, RMR was 8.8% during the years 2007-2009 [9]. Similarly, Mukinda *et al* reported increasing RMR in the Western Cape province of SA [10]. These findings highlight the importance of testing for INH resistance in all patients with Xpert rifampicin resistance. This positively impacts patient management further as patients with

confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment regimen.

The development of rifampicin resistance has serious effects on the treatment of TB. Patients have to be treated with more expensive and more toxic drugs for a longer duration. Studies have been conducted in order to elucidate the causes of RMR with the majority reporting an association between HIV and RMR [11-12]. Factors contributing to this association include decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to infection and permits proliferation of TB which favours transmission [14]. Given the high rate of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in co-infected patients remains under investigation.

Rifampicin and INH are core drugs that form the backbone for first line short course therapy for the treatment of drug susceptible TB. Given the high burden of disease in this region coupled with the use of Xpert as a screening tool for DR-TB, mono-resistance to INH may inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is only made using TB culture and DST following a negative Xpert result or treatment failure. The use of standard first line TB therapy in patients with undetected INH resistance equates to using rifampicin monotherapy during the continuation phase. This may subsequently lead to the development of MDR-TB. This was described in a study done by Jacobson *et al* where treatment of patients with IMR using standard first line therapy was associated with poor outcomes and progression to MDR-TB [15]. Several studies have reported previous TB therapy

as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients who may require TB culture and DST in order to exclude INH resistance.

There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and XDR-TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the national guidelines, a significant number of patients with Xpert rifampicin resistant TB did not get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO TB report, which reported the percentage of MDR-TB among MDR/RR-TB as 62% in South Africa. This suggests that a substantial number of Xpert rifampicin resistant TB cases are not confirmed by culture because this discrepancy cannot be explained by RMR cases [1]. Another possible reason may be due to patients that are lost to follow up. In KZN province, the specimen for TB culture is only collected when the patient comes back for Xpert results. Consequently, if patients did not return for the results, then specimens for TB culture would not have been collected. According to the WHO, only 41% of notified MDR/RR-TB cases from South Africa were enrolled for MDR-TB treatment in 2013[18]. Although this figure improved to 62% in 2014 [20], the gap remains substantial especially given the considerable improvement in rapidity of diagnosing DR-TB with Xpert. Therefore, this decline in culture confirmed DR-TB indicates a change in the testing method used to diagnose tuberculosis rather than a successful TB control program, which led to underestimation of MDR-TB and XDR-TB cases in this study. The proportion of MDR-TB cases that have XDR-TB remained constant at about 11% which is comparable to the global trends at that time [18-20].

Our study found higher rates of DR-TB in women compared to men which supports findings from other studies showing higher proportions of DR-TB in women [21-22]. Even though reasons behind the higher DR-TB predisposition among women are unknown, HIV could be a contributing factor. 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 [2]. It is well recognised that HIV is a major risk factor for development of TB and HAART reduces its incidence [23-25]. Indeed, Nanoo *et al* showed an inverse relationship between antiretroviral therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with the greatest decline demonstrated in the 25-44 year age group [26].

The diagnosis of DR-TB in children is generally difficult due to their inability to expectorate and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB in children is largely as a result of primary transmission from adults, the proportion of DR-TB is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR-TB particularly MDR-TB in children less than five years compared to adults which could be a reflection of the under diagnosis of DR-TB in this age group. Although these rates are lower in children, they are still unacceptable as they reflect transmission of untreated adult TB.

Similar to the overall burden of TB in South Africa, DR-TB is also concentrated in urban areas of KZN with eThekwini district harbouring most of the cases due to high population density. However, the rate of MDR-TB cases was highest among the northern districts of the province of Umkhanyakude and Zululand. These are rural districts which share borders with Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance patterns. Mpumalanga province is known to have the highest DR-TB rate in the country while Swaziland has the highest MDR-TB prevalence in Africa [29-30]. In 2007, Wallengren *et al*

reported Umzinyathi and Umkhanyakude as the districts with the highest MDR-TB rates [31]. The intervention given to the Umzinyathi district following the outbreak of XDR-TB in 2005 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and treatment of HIV, TB infection control and intergration of TB and HIV care) may be responsible for these decreasing rates [32]. Despite declining XDR-TB rates at the Umzinyathi district (where the XDR-TB outbreak was identified in 2005), it still remains the district with the highest XDR-TB rates at about three times higher than the rest of KZN [33].

Limitations

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

Although the data used for this study is relatively old, it represents a critical time of drastic changes in the diagnosis of DR-TB. There have been no subsequent changes in the TB diagnostic algorithm, therefore the findings highlighted in this study should still be relevant to the current setting.

Conclusions

Our findings highlight the importance of DR-TB diagnostic 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.

Acknowledgments

We thank the staff at the Inkosi Albert Luthuli Central Hospital TB laboratory for their dedication and hard work. We also thank Thandi Kapwata from the Environment and Health Research Unit within the South African Medical Research Council, for designing the provincial maps.

Author contributions

NRM contributed in the development of the concept, study design, data analysis and writing of the manuscript. YB performed data analysis and assisted with the writing of the manuscript. KL contributed in the interpretation of data and writing of the manuscript. KM supervised the development of the study concept, study design, data analysis and manuscript writing.

Data sharing statement: No data are available.

Competing interests: None

Funding: None

References

1. World Health Organization. Global Tuberculosis Report 2018. https://www.who.int/tb/publications/global_report/gtbr2018_main_text_28Feb2019.pdf?ua=1

2. Gandhi NR, *et al*. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet* 2006; 368: 1575-1580.

3. Ndjeka N. National Department of Health. Multi-drug resistant tuberculosis: strategic overview on MDR-TB care in South Africa. 2014. https://www.health-e.org.za/wp-content/uploads/2014/03/Strategic_overview_of_MDR_TB_RSA.pdf.

4. Hassim S, *et al*. Detection of a substantial rate of multidrug-resistant tuberculosis in an HIV-infected population in South Africa by active monitoring of sputum samples. *Clinical Infectious Disease* 2010; 50: 1053-1059.

5. Helb D, *et al*. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology* 2010; 48: 229-237.

6. Boehme CC, *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine* 2010; 363: 1005-1015.

7. National Department of Health, South Africa. National tuberculosis management guidelines. 2014.

https://www.health-e.org.za/wp-content/uploads/2014/06/NTCP_Adult_TB-Guidelines-27.5.2014.pdf

8. McGrath M, *et al*. Mutation rate and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Journal of Antimicrobial Chemotherapy* 2014; 69: 292–302

9. Coovadia YM, *et al*. Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. *PLOS ONE*. 2013, 8: e77712.

10. Mukinda FK, *et al*. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. *International Journal of Tuberculosis and Lung Disease* 2012; 16: 196–202.

11. Sharling L, *et al*. Rifampin-Resistant Tuberculosis in The United States, 1998-2014. *Clinical Infectious Diseases* 2019; ciz491. https://doi.org/10.1093/cid/ciz491

12. Villegas L, *et al*. Prevalence, risk factors, and treatment outcomes of isoniazid and rifampicin mono-resistant pulmonary tuberculosis in Lima, Peru. *PLOS ONE* 2016; 11: e0152933.

13. Gurumurthy P, *et al*. Malabsorption of rifampicin and isoniazid in HIV infected patients with and without tuberculosis. *Clinical Infectious Disease* 2004; 38: 280–283.

14. Bifani P, *et al*. The evolution of drug resistance in *Mycobacterium tuberculosis*: from a mono-rifampin-resistant cluster into increasingly multidrug-resistant variants in an HIV-seropositive population. *Journal of Infectious Disease* 2008; 198: 90–94.

15. Jacobson KR, *et al*. Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape province, South Africa. *Clinical Infectious Disease* 2011; 53: 369–372.

16. Cattamanchi A, *et al*. Clinical characteristics and treatment outcomes of isoniazid mono-resistant tuberculosis. *Clinical Infectious Disease* 2009; 48: 179–185.

17. Fox L, *et al*. Comparison of isoniazid monoresistant tuberculosis with drug-susceptible tuberculosis and multidrug-resistant tuberculosis. *European Journal of Clinical Microbiology & Infectious Diseases* 2011; 30: 863-867.

18. World Health Organization. Global Tuberculosis Report. 2013
    a. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf

19. World Health Organization. Global Tuberculosis Report 2014.

a. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf

20. World Health Organization. Global Tuberculosis Report. 2015

a. http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf

21. O'Donnell MR, *et al*. Extensively drug-resistant tuberculosis in women, KwaZulu-Natal, South Africa. *Emerging Infectious Disease* 2011; 17: 1942–1945.

22. Cox HS, *et al*. Multidrug-resistant tuberculosis in Central Asia. *Emerging Infectious Disease* 2004; 10: 865–872.

23. Sonnenberg P, *et al*. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *Journal of Infectious Disease* 2005 15; 191: 150-158.

24. Badri M, *et al*. Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area. *International Journal of Tuberculosis and Lung Disease* 2001; 5: 225–232.

25. Badri M, *et al*. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet* 2002; 359: 2059–2064.

26. Nanoo A, *et al*. Nationwide and regional incidence of microbiologically confirmed pulmonary tuberculosis in South Africa, 2004–12: a time series analysis. *Lancet Infectious Disease* 2015; 15: 1066–1076.

27. Schaaf HS, *et al.* Surveillance of anti-tuberculosis drug resistance among children from the Western Cape Province of South Africa—an upward trend. *American Journal of Public Health* 2009; 99: 1486-1490.

28. Zignol M, *et al*. Multidrug-resistant tuberculosis in children: evidence from global surveillance. *European Respiratory Journal* 2013; 42: 701–707.

29. Andrews JR, *et al*. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *Journal of Infectious Disease* 2007; 196: S482-S490.

30. Sanchez-Padilla E, *et al*. High prevalence of multidrug-resistant tuberculosis, Swaziland, 2009–2010. *Emerging Infectious Diseases* 2012; 18: 29–37.

31. Wallengren K, *et al*. Drug-resistant tuberculosis, KwaZulu-Natal, South Africa, 2001–2007. *Emerging Infectious Diseases* 2011; 17: 1913-1916.

32. Bateman C. Tugela Ferry's extensively drug-resistant tuberculosis – 10 years on. *South African Medical Journal* 2015;105: 517-520.

33. Lim JR, *et al*. Incidence and geographic distribution of extensively drug resistant tuberculosis in KwaZulu-Natal province, South Africa. *PLOS ONE* 2015; 10: e0132076.

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

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

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

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

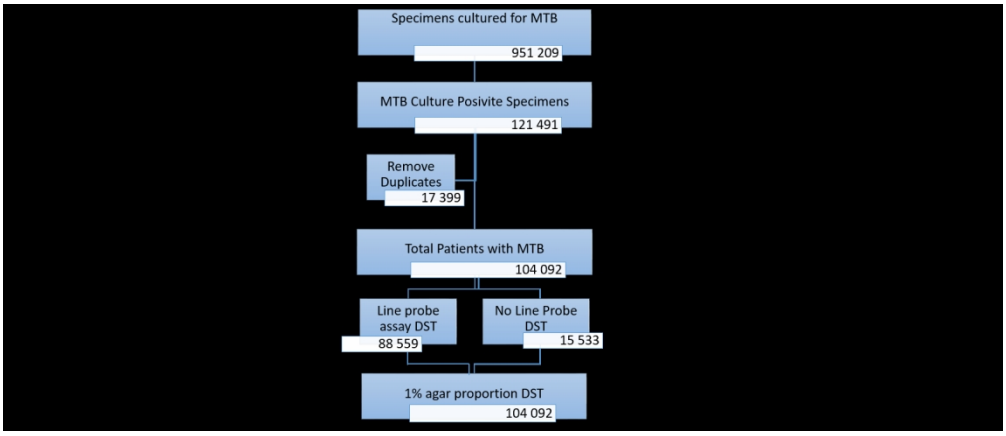
Figure 1: Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

Figure 2: 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.

Figure 3: 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

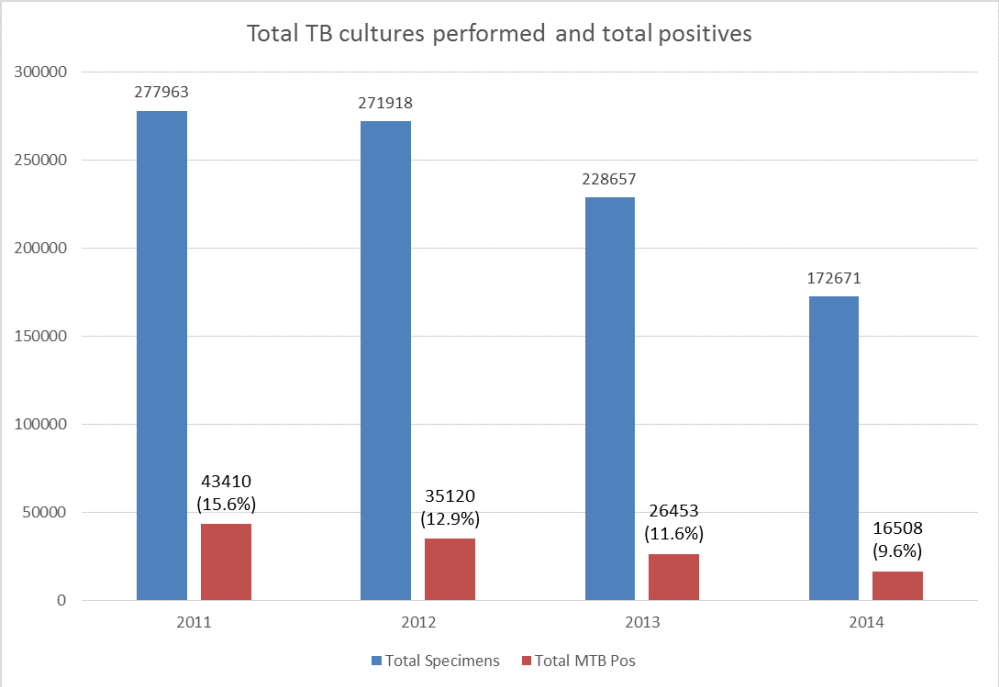
Figure 4: Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-TB of total positive cases by gender.

Figure 5: 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.



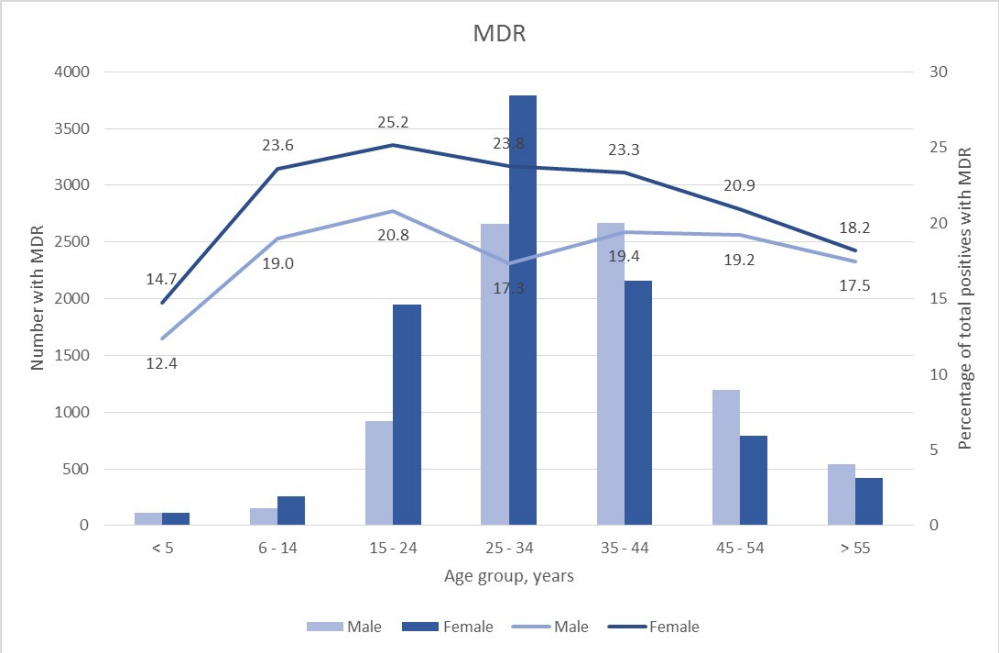
Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

292x124mm (150 x 150 DPI)



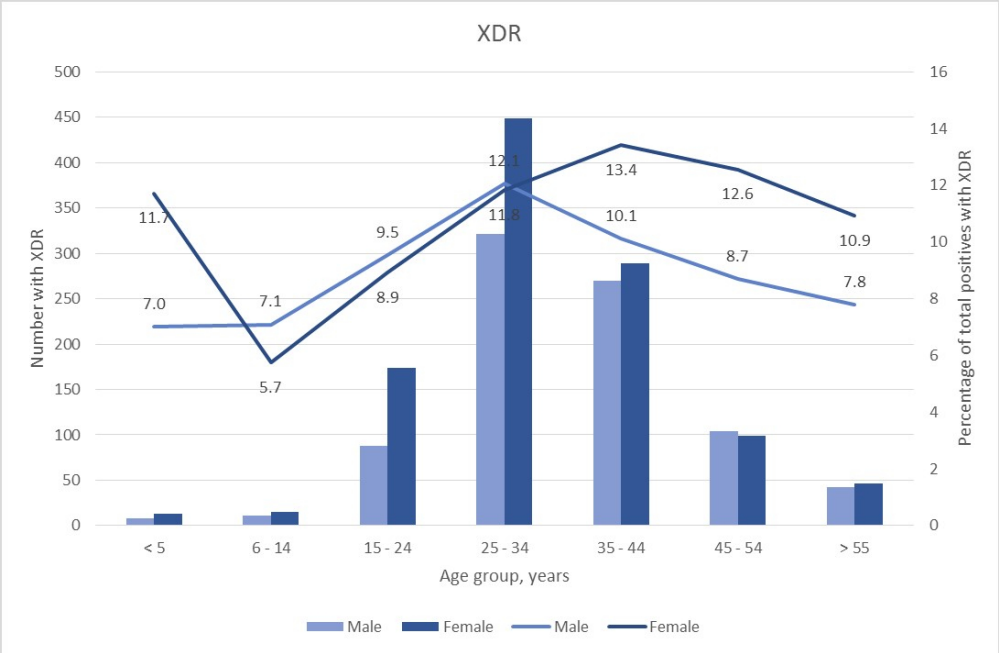
TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens received and the total number (and percentage) thereof that were positive.

184x127mm (150 x 150 DPI)



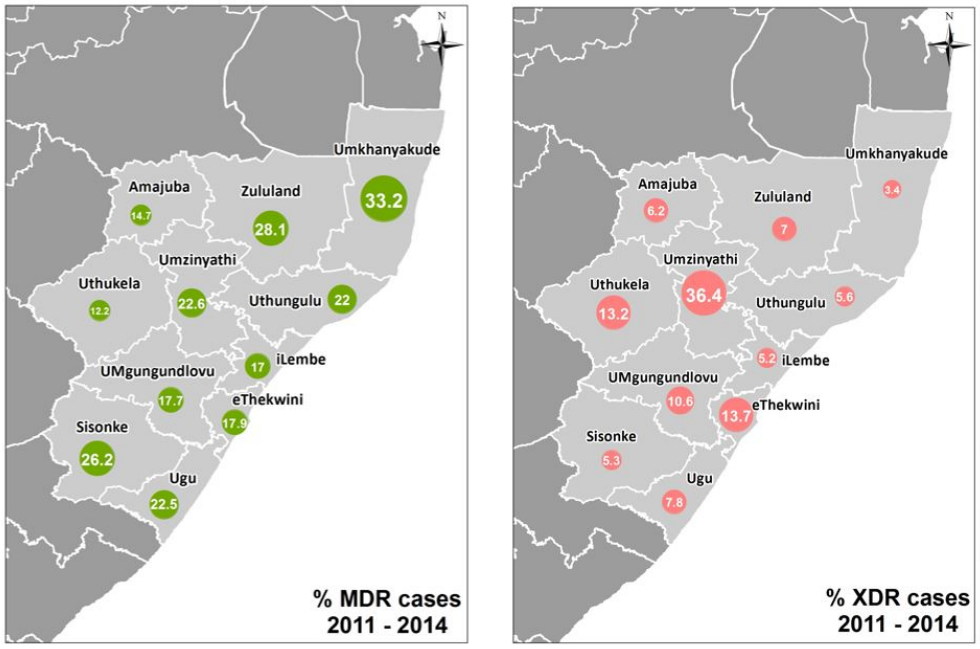
Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-TB of total positive cases by gender

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	2	Retrospective observational study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	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.
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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

		Cross-sectional study—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*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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

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.
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).
		(*b*) Describe any methods used to examine subgroups and interactions		
		(*c*) Explain how missing data were addressed		
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		
		(*e*) Describe any sensitivity analyses		
Results				
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
		(b) Give reasons for non-participation at each stage		
		(c) Consider use of a flow diagram		
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

				-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	Key findings: -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	-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

				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:	*BMJ Open*
Manuscript ID	bmjopen-2019-031663.R2
Article Type:	Original research
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

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

N. R. Mvelase [1, 2], Y. Balakrishna [3], K. Lutchminarain [1, 2], K. Mlisana [1, 2, 4]

[1]Department of Medical Microbiology, KwaZulu-Natal Academic Complex, National Health Laboratory Service, Durban, South Africa

[2]Department of Medical Microbiology, School of Laboratory Medicine & Medical Sciences, University of KwaZulu-Natal, Durban, South Africa

[3]Biostatistics Unit, South African Medical Research Council, Durban, South Africa

[4]Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa

Corresponding author: Nomonde R. Mvelase

Address: KwaZulu-Natal National Health Laboratory Service, Department of Medical Microbiology, Level 4, Laboratory Building, IALCH, Durban, South Africa

Email: dlaminin15@ukzn.ac.za

Running Title: Drug resistant TB in KwaZulu-Natal

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

and migration influence its distribution; therefore, TB control strategies should include interventions that target these aspects.

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

The World Health Organisation (WHO) has declared multidrug resistant tuberculosis a global crisis. Multidrug-resistant tuberculosis (MDR TB) is defined as resistance to isoniazid and rifampicin. Despite the decline in the global incidence rates of tuberculosis (TB), drug resistant TB cases are on the rise with 558 000 estimated incident cases of MDR plus rifampicin resistant (RR) TB and more than 230 000 deaths in 2017 [1]. South Africa has one of the highest incidence of TB in the world which WHO estimated to be 567 per 100 000 in 2017 [1]. In 2017 alone, South Africa had an estimated number of 14 000 rifampicin resistant cases, the second highest number in Africa after Nigeria which has more than three times the South African population [1]. Moreover, the first reported outbreak of extensively drug-resistant (XDR) tuberculosis (defined as MDR-TB plus resistance to any second line injectable and a

fluoroquinolone) which caused global concern in 2005 was from the province of KwaZulu-Natal (KZN) in South Africa [2]. While the incidence of TB in KZN is proportional to other provinces in the country, it remains the highest drug resistant (DR) TB burdened province with almost a third of the country's cases of drug resistant TB [3].

Compounding the problem of TB in South Africa, is the high rate of co-infection with Human Immunodeficiency Virus (HIV) (about 60%) [1]. While it is well known that HIV is associated with smear negative TB, smear microscopy was traditionally used in the initial diagnosis of TB because of its quick time to results and low cost [4]. On the other hand, conventional TB culture is much more sensitive than smear microscopy, but its high cost, complexity and long delays in getting the results made it impractical for routine diagnosis of TB. Therefore, when the WHO endorsed the Xpert MTB/RIF (Cepheid GeneXpert, Sunnyvale, Ca, USA) in 2010, it was subsequently introduced in South Africa in 2011. The Xpert MTB/RIF (Xpert) is an automated nucleic acid amplification test that offers better detection of TB compared to smear microscopy with an added advantage of the ability to detect rifampicin resistance in less than two hours in clinical specimens [5-6].

The implementation of Xpert in South Africa completely changed the testing algorithm for the diagnosis of TB [7]. 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. We therefore undertook this study to evaluate the amount of rifampicin and isoniazid mono-resistance, so as to ensure optimal and appropriate diagnostic algorithms. We also describe the drug resistance patterns and distribution among different age groups, genders and districts in KZN, South Africa. Understanding the patterns and distributions of drug resistant TB will inform targeted intervention in TB control in this high TB endemic region.

Methods

Study design

The study is a retrospective observational study using laboratory data for 2011 till 2014.

Study setting

The 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.

Laboratory Procedures

MTB isolation from clinical samples was routinely done using the automated BACTEC mycobacteria growth indication tubes (MGIT) 960 system (BACTEC MGIT Becton Dickinson, USA). Indirect line probe assay (LPA) [GenoType MTBDRplus assay, Hain Lifescience, Nehren, Germany] was performed on all positive MGIT cultures using standard methods. Thereafter, additional DST for isoniazid (INH), rifampicin (RIF), ofloxacin, streptomycin, kanamycin was performed for all TB culture positive cases using 1% agar proportion method (APM) on Middlebrook 7H10.

Patient and public involvement

The data used for this study was the routine TB diagnostic data, therefore there was no direct patient and public involvement.

Data collection and analysis

The TB culture and DST 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.

Data was described using frequencies and proportions. 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).

Ethical consideration

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

Results

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

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

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

respectively (Table 2). The proportion of TB cases that had MDR-TB ranged from 18.8% in 2011 to 23.9% in 2014, with an overall average of 21%. The overall rate of XDR-TB among MDR-TB cases was 11% (2336 XDR-TB cases out of 21221 MDR-TB).

The majority of the TB cases were males; however, females constituted highest prevalence of the DR-TB across all age groups (Figure 3 and 4). The number of MDR-TB cases was higher among females than males until the age of 34, thereafter males had a higher number than females. Similarly, for XDR-TB, females constituted the most number of XDR-TB. More than 60% of both MDR-TB and XDR-TB cases were patients between the ages of 25 and 44 years. It was observed that children less than 5 years of age showed the lowest rates of MDR-TB whilst that of XDR-TB was lowest between the ages of 6-14 years.

Over the 4-year period, eThekwini district had the highest number of TB cases with 47.5% of all cases in KZN coming from this district (Table 2). However, the districts with the highest yearly proportion of MDR-TB cases each year were Umkhanyakude [(mean 33.2%, sd 2.3%), (95% CI 29.5% to 36.9%)]; followed by Zululand [(mean 28.1%, sd 2.3), (95% CI 24.4% to 31.8%)] and Harry Gwala [(mean 26.2%, sd 9.4), (95% CI 11.2% to 41.2%)]. The yearly proportion of MDR-TB cases that had XDR-TB were highest at Umzinyathi [(mean 36.4%, sd 5.8%), (95% CI 27.2% to 45.6%)] followed by eThekwini [(mean 13.7%, sd 1.1%), (95% CI 11.9% to 15.5%)] and Uthukela [(mean 13.2%, sd 5.7%), (95% CI 4.1% to 22.3%)] districts. Umkhanyakude district had the lowest proportion of XDR-TB with a yearly mean of 3.4% [(sd 2.4%), (95% CI -0.4% to 7.2%)] over the study period (Figure 5).

Discussion

In this study we observe a decline in the number of samples processed for MTB culture and culture positivity rate which coincided, with the roll out of the Xpert. This is in keeping with the Xpert roll out which started in March 2011 and was completed in September 2013 when all health facilities in the provinces were using the Xpert for TB diagnosis. According to the South African guidelines, MTB culture is not recommended for Xpert rifampicin susceptible patients, which constitutes the majority of patients infected with TB, hence the decline in the number of MTB cultures from 277 963 in 2011 to 172 671 in 2014. Nevertheless, the sheer volumes of MTB cultures are still enormous which reflects the overwhelming burden of DR TB in this region. Prior to Xpert introduction, drug susceptibility testing for MTB was only performed on patients that were considered to be at risk of DR TB, but the use of Xpert for initial diagnosis of TB enables screening for rifampicin resistance in all patients. The revised indications for culture selects for cases that are more likely to have drug resistant (Xpert rifampicin resistant TB) and paucibacillary TB (extra-pulmonary TB and HIV positive Xpert negative), which explains the high rates of DR TB and declining culture positivity rate observed in this study.

Rifampicin is always used in combination with other drugs in the treatment of TB in SA. In addition, spontaneously occurring mutations are rare compared to other TB drugs [8]. Consequently, the development of RMR is expected to be uncommon. The finding of increasing mono-resistance in this context is therefore concerning. In a previous study done by Coovadia *et al,* at the same laboratory, RMR was 8.8% during the years 2007-2009 [9]. Similarly, Mukinda *et al* reported increasing RMR in the Western Cape province of SA [10]. These findings highlight the importance of testing for INH resistance in all patients with Xpert rifampicin resistance. This positively impacts patient management further as patients with

confirmed rifampicin mono-resistance could benefit by using isoniazid in their treatment regimen.

The development of rifampicin resistance has serious effects on the treatment of TB. Patients have to be treated with more expensive and more toxic drugs for a longer duration. Studies have been conducted in order to elucidate the causes of RMR with the majority reporting an association between HIV and RMR [11-12]. Factors contributing to this association include decreased drug bioavailability, and drug- drug interactions which lead to decreased rifampicin serum levels [13]. Furthermore, advanced immunosuppression increases susceptibility to infection and permits proliferation of TB which favours transmission [14]. Given the high rate of TB/HIV co-infection in our setting, it is possible that HIV may be contributing to the increasing rate of RMR. Whether using a higher dose of rifampicin proves to be beneficial in co-infected patients remains under investigation.

Rifampicin and INH are core drugs that form the backbone for first line short course therapy for the treatment of drug susceptible TB. Given the high burden of disease in this region coupled with the use of Xpert as a screening tool for DR-TB, mono-resistance to INH may inevitably be overlooked. According to the national TB algorithm, a diagnosis of IMR TB is only made using TB culture and DST following a negative Xpert result or treatment failure. The use of standard first line TB therapy in patients with undetected INH resistance equates to using rifampicin monotherapy during the continuation phase. This may subsequently lead to the development of MDR-TB. This was described in a study done by Jacobson *et al* where treatment of patients with IMR using standard first line therapy was associated with poor outcomes and progression to MDR-TB [15]. Several studies have reported previous TB therapy

as a risk factor for IMR [16-17]. Identifying risk factors for IMR could help to select patients who may require TB culture and DST in order to exclude INH resistance.

There was an overall decline in the numbers of MDR (from 6901 in 2011 to 3506 in 2014) and XDR-TB (from 706 in 2011 to 389 in 2014) cases identified using culture. This was in contrast to the increasing number of MDR/RR-TB cases following the introduction of the Xpert in South Africa during this time [18-20]. Perhaps a plausible explanation is that, contrary to the national guidelines, a significant number of patients with Xpert rifampicin resistant TB did not get a subsequent MTB culture for confirmation. This was supported by the 2016 WHO TB report, which reported the percentage of MDR-TB among MDR/RR-TB as 62% in South Africa. This suggests that a substantial number of Xpert rifampicin resistant TB cases are not confirmed by culture because this discrepancy cannot be explained by RMR cases [1]. Another possible reason may be due to patients that are lost to follow up. In KZN province, the specimen for TB culture is only collected when the patient comes back for Xpert results. Consequently, if patients did not return for the results, then specimens for TB culture would not have been collected. According to the WHO, only 41% of notified MDR/RR-TB cases from South Africa were enrolled for MDR-TB treatment in 2013[18]. Although this figure improved to 62% in 2014 [20], the gap remains substantial especially given the considerable improvement in rapidity of diagnosing DR-TB with Xpert. Therefore, this decline in culture confirmed DR-TB indicates a change in the testing method used to diagnose tuberculosis rather than a successful TB control program, which led to underestimation of MDR-TB and XDR-TB cases in this study. The proportion of MDR-TB cases that have XDR-TB remained constant at about 11% which is comparable to the global trends at that time [18-20].

Our study found higher rates of DR-TB in women compared to men which supports findings from other studies showing higher proportions of DR-TB in women [21-22]. Even though reasons behind the higher DR-TB predisposition among women are unknown, HIV could be a contributing factor. 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 [2]. It is well recognised that HIV is a major risk factor for development of TB and HAART reduces its incidence [23-25]. Indeed, Nanoo *et al* showed an inverse relationship between antiretroviral therapy coverage and the incidence of microbiologically confirmed TB in South Africa, with the greatest decline demonstrated in the 25-44 year age group [26].

The diagnosis of DR-TB in children is generally difficult due to their inability to expectorate and the paucibacillary nature of childhood TB. Consequently, DR data is limited, but since TB in children is largely as a result of primary transmission from adults, the proportion of DR-TB is reported to be similar to that of adults [27-28]. Herein, we observed lower rates of DR-TB particularly MDR-TB in children less than five years compared to adults which could be a reflection of the under diagnosis of DR-TB in this age group. Although these rates are lower in children, they are still unacceptable as they reflect transmission of untreated adult TB.

Similar to the overall burden of TB in South Africa, DR-TB is also concentrated in urban areas of KZN with eThekwini district harbouring most of the cases due to high population density. However, the rate of MDR-TB cases was highest among the northern districts of the province of Umkhanyakude and Zululand. These are rural districts which share borders with Mpumalanga province, Swaziland and Mozambique, thus migration may influence resistance patterns. Mpumalanga province is known to have the highest DR-TB rate in the country while Swaziland has the highest MDR-TB prevalence in Africa [29-30]. In 2007, Wallengren *et al*

reported Umzinyathi and Umkhanyakude as the districts with the highest MDR-TB rates [31]. The intervention given to the Umzinyathi district following the outbreak of XDR-TB in 2005 (Intensive case finding, early diagnosis and initiation treatment for TB, early diagnosis and treatment of HIV, TB infection control and intergration of TB and HIV care) may be responsible for these decreasing rates [32]. Despite declining XDR-TB rates at the Umzinyathi district (where the XDR-TB outbreak was identified in 2005), it still remains the district with the highest XDR-TB rates at about three times higher than the rest of KZN [33].

Limitations

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

Although the data used for this study is relatively old, it represents a critical time of drastic changes in the diagnosis of DR-TB. There have been no subsequent changes in the TB diagnostic algorithm, therefore the findings highlighted in this study should still be relevant to the current setting. The data presented is not prevalence data as only data from MTB positive cultures was used. Thus, the results may be an underrepresentation as patients that were lost to follow up and patients with contaminated/loss of viability cultures were excluded.

Conclusions

Our findings highlight the importance of DR-TB diagnostic 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.

Acknowledgments

We thank the staff at the Inkosi Albert Luthuli Central Hospital TB laboratory for their dedication and hard work. We also thank Thandi Kapwata from the Environment and Health Research Unit within the South African Medical Research Council, for designing the provincial maps.

Author contributions

NRM contributed in the development of the concept, study design, data analysis and writing of the manuscript. YB performed data analysis and assisted with the writing of the manuscript. KL contributed in the interpretation of data and writing of the manuscript. KM supervised the development of the study concept, study design, data analysis and manuscript writing.

Data sharing statement: Data may be obtained from a third party and are not publicly available. The data for this study will only be available upon reasonable request and provided approval is obtained from the it's custodians, i.e. the Department of Health and the National Health Laboratory Service.

Competing interests: None

Funding: None

References

1. World Health Organization. Global Tuberculosis Report 2018. https://www.who.int/tb/publications/global_report/gtbr2018_main_text_28Feb2019.pdf?ua=1

2. Gandhi NR, *et al*. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet* 2006; 368: 1575-1580.

3. Ndjeka N. National Department of Health. Multi-drug resistant tuberculosis: strategic overview on MDR-TB care in South Africa. 2014. https://www.health-e.org.za/wp-content/uploads/2014/03/Strategic_overview_of_MDR_TB_RSA.pdf.

4. Hassim S, *et al*. Detection of a substantial rate of multidrug-resistant tuberculosis in an HIV-infected population in South Africa by active monitoring of sputum samples. *Clinical Infectious Disease* 2010; 50: 1053-1059.

5. Helb D, *et al*. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology* 2010; 48: 229-237.

6. Boehme CC, *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine* 2010; 363: 1005-1015.

7. National Department of Health, South Africa. National tuberculosis management guidelines. 2014.

https://www.health-e.org.za/wp-content/uploads/2014/06/NTCP_Adult_TB-Guidelines-27.5.2014.pdf

8. McGrath M, *et al*. Mutation rate and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Journal of Antimicrobial Chemotherapy* 2014; 69: 292–302

9. Coovadia YM, *et al*. Rifampicin mono-resistance in *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. *PLOS ONE*. 2013, 8: e77712.

10. Mukinda FK, *et al*. Rise in rifampicin-monoresistant tuberculosis in Western Cape, South Africa. *International Journal of Tuberculosis and Lung Disease* 2012; 16: 196–202.

11. Sharling L, *et al*. Rifampin-Resistant Tuberculosis in The United States, 1998-2014. *Clinical Infectious Diseases* 2019; ciz491. https://doi.org/10.1093/cid/ciz491

12. Villegas L, *et al*. Prevalence, risk factors, and treatment outcomes of isoniazid and rifampicin mono-resistant pulmonary tuberculosis in Lima, Peru. *PLOS ONE* 2016; 11: e0152933.

13. Gurumurthy P, *et al*. Malabsorption of rifampicin and isoniazid in HIV infected patients with and without tuberculosis. *Clinical Infectious Disease* 2004; 38: 280–283.

14. Bifani P, *et al*. The evolution of drug resistance in *Mycobacterium tuberculosis*: from a mono-rifampin-resistant cluster into increasingly multidrug-resistant variants in an HIV-seropositive population. *Journal of Infectious Disease* 2008; 198: 90–94.

15. Jacobson KR, *et al*. Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape province, South Africa. *Clinical Infectious Disease* 2011; 53: 369–372.

16. Cattamanchi A, *et al*. Clinical characteristics and treatment outcomes of isoniazid mono-resistant tuberculosis. *Clinical Infectious Disease* 2009; 48: 179–185.

17. Fox L, *et al*. Comparison of isoniazid monoresistant tuberculosis with drug-susceptible tuberculosis and multidrug-resistant tuberculosis. *European Journal of Clinical Microbiology & Infectious Diseases* 2011; 30: 863-867.

18. World Health Organization. Global Tuberculosis Report. 2013
    a. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf

19. World Health Organization. Global Tuberculosis Report 2014.
    a. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf

20. World Health Organization. Global Tuberculosis Report. 2015
    a. http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf

21. O'Donnell MR, *et al*. Extensively drug-resistant tuberculosis in women, KwaZulu-Natal, South Africa. *Emerging Infectious Disease* 2011; 17: 1942–1945.

22. Cox HS, *et al*. Multidrug-resistant tuberculosis in Central Asia. *Emerging Infectious Disease* 2004; 10: 865–872.

23. Sonnenberg P, *et al*. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *Journal of Infectious Disease* 2005 15; 191: 150-158.

24. Badri M, *et al*. Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area. *International Journal of Tuberculosis and Lung Disease* 2001; 5: 225–232.

25. Badri M, *et al*. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet* 2002; 359: 2059–2064.

26. Nanoo A, *et al*. Nationwide and regional incidence of microbiologically confirmed pulmonary tuberculosis in South Africa, 2004–12: a time series analysis. *Lancet Infectious Disease* 2015; 15: 1066–1076.

27. Schaaf HS, *et al*. Surveillance of anti-tuberculosis drug resistance among children from the Western Cape Province of South Africa—an upward trend. *American Journal of Public Health* 2009; 99: 1486-1490.

28. Zignol M, *et al*. Multidrug-resistant tuberculosis in children: evidence from global surveillance. *European Respiratory Journal* 2013; 42: 701–707.

29. Andrews JR, *et al*. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *Journal of Infectious Disease* 2007; 196: S482-S490.

30. Sanchez-Padilla E, *et al*. High prevalence of multidrug-resistant tuberculosis, Swaziland, 2009–2010. *Emerging Infectious Diseases* 2012; 18: 29–37.

31. Wallengren K, *et al*. Drug-resistant tuberculosis, KwaZulu-Natal, South Africa, 2001–2007. *Emerging Infectious Diseases* 2011; 17: 1913-1916.

32. Bateman C. Tugela Ferry's extensively drug-resistant tuberculosis – 10 years on. *South African Medical Journal* 2015;105: 517-520.

33. Lim JR, *et al*. Incidence and geographic distribution of extensively drug resistant tuberculosis in KwaZulu-Natal province, South Africa. *PLOS ONE* 2015; 10: e0132076.

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

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

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

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

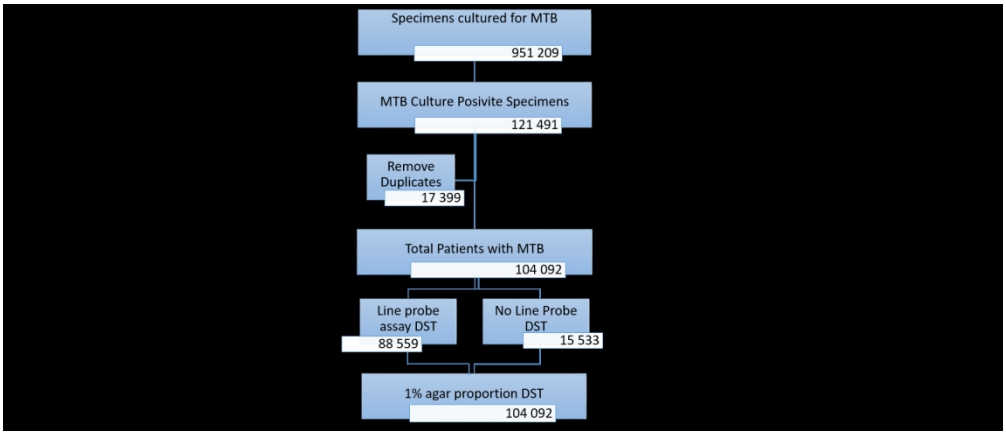
Figure 1: Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

Figure 2: 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.

Figure 3: 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

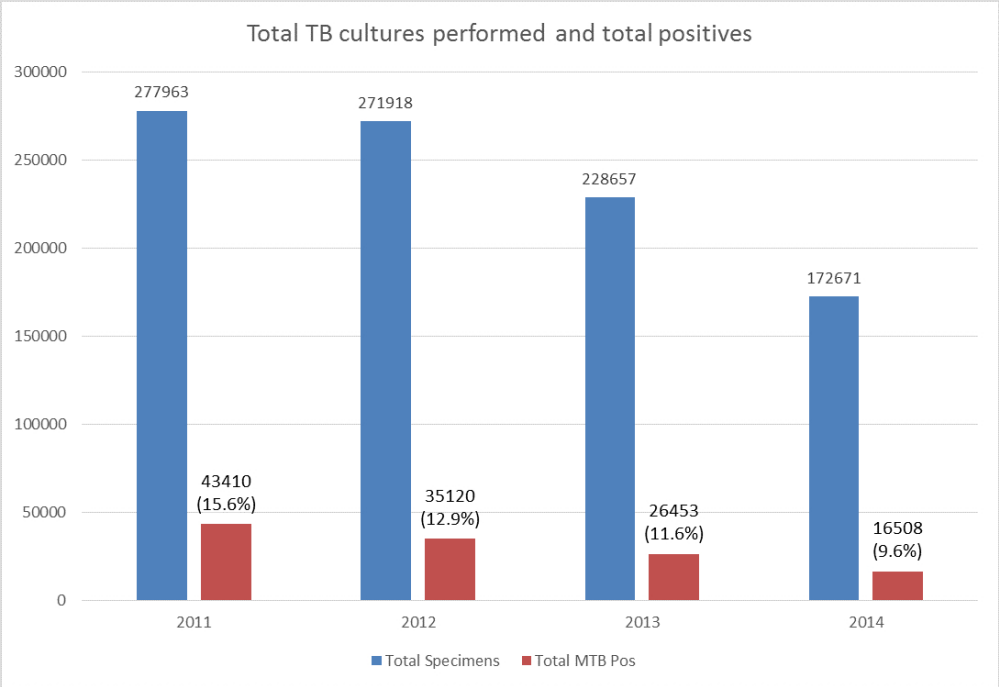
Figure 4: Distribution of XDR-TB cases and proportions by gender. The number of XDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is XDR-TB of total positive cases by gender.

Figure 5: 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



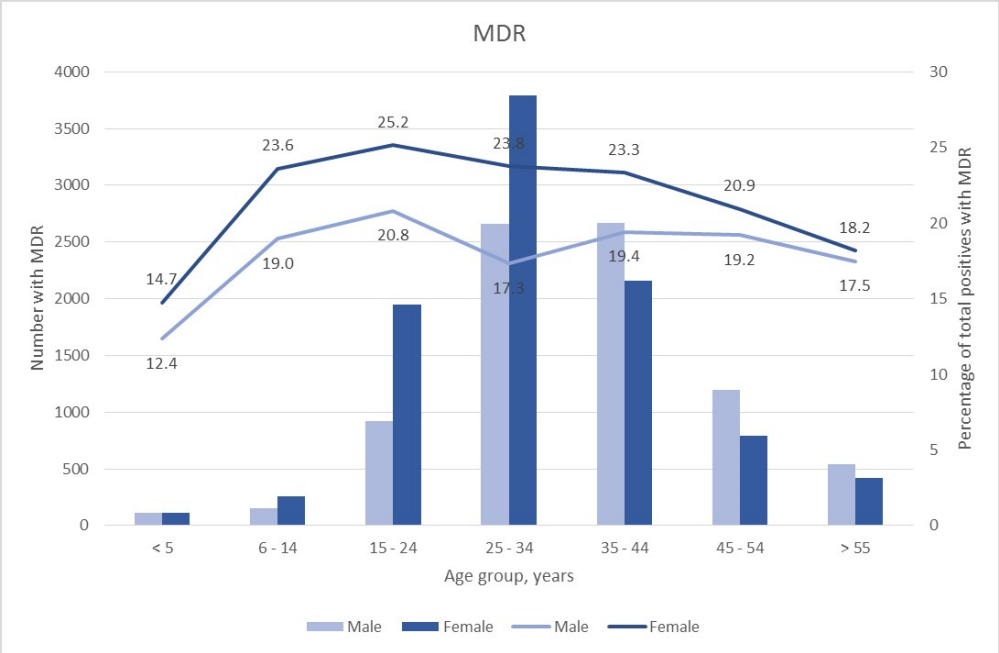
Flow diagram showing the number of specimens received and the laboratory procedures performed at the TB culture laboratory.

292x124mm (150 x 150 DPI)



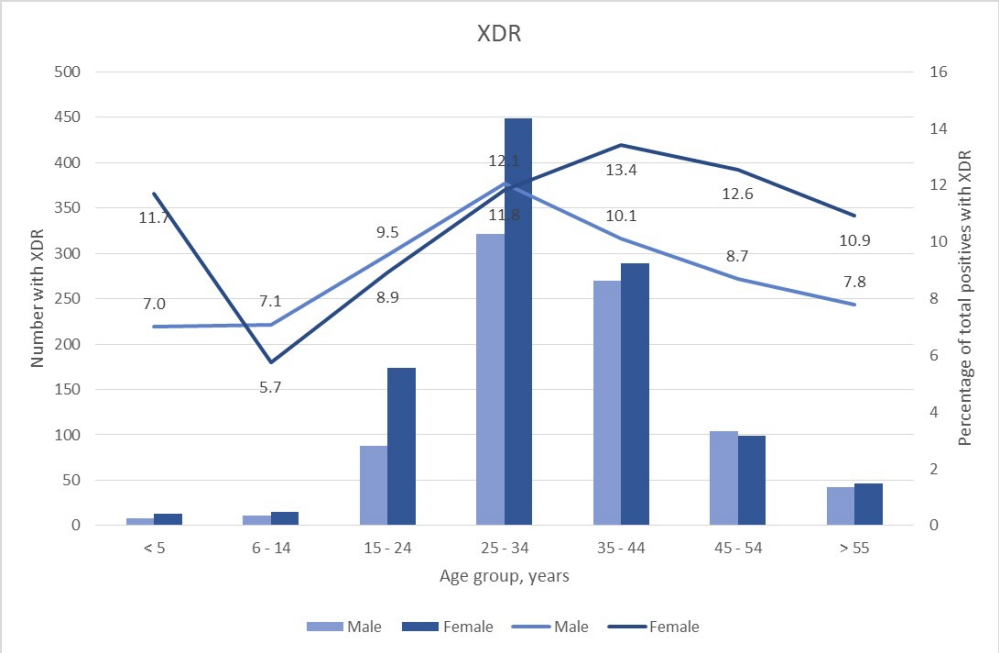
TB culture specimens processed between 2011 and 2014. The figure shows the total number of specimens received and the total number (and percentage) thereof that were positive.

184x127mm (150 x 150 DPI)



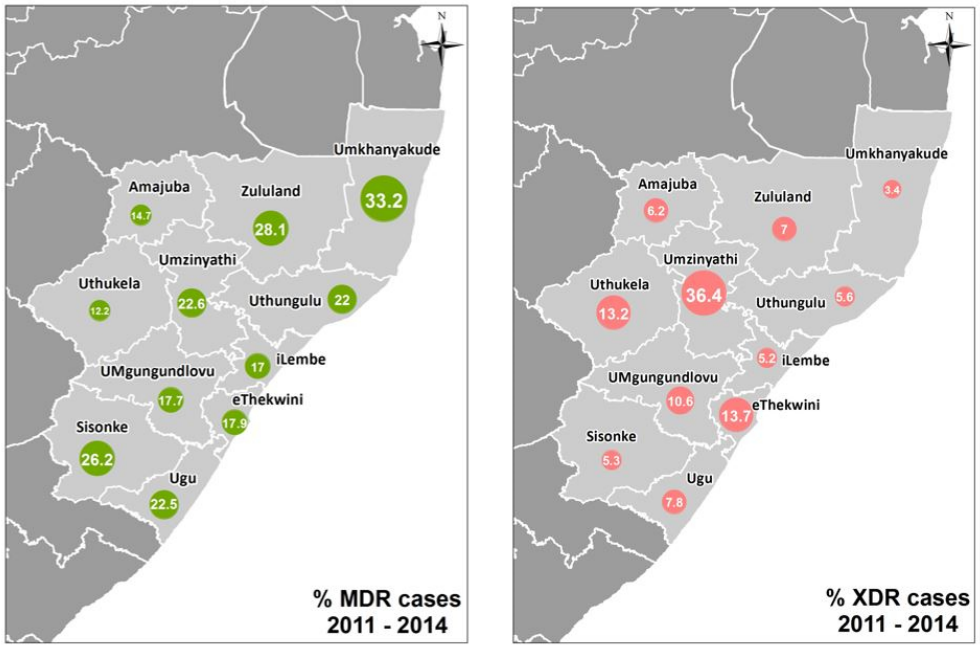
Distribution of MDR-TB cases and proportions by gender. The number of MDR-TB cases (represented by bars) by gender and the percentage (represented by lines) that is MDR-TB of total positive cases by gender

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	2	Retrospective observational study
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	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.
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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

		Cross-sectional study—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*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—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

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.
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).
		(*b*) Describe any methods used to examine subgroups and interactions		
		(*c*) Explain how missing data were addressed		
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy		
		(*e*) Describe any sensitivity analyses		
Results				
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
		(b) Give reasons for non-participation at each stage		
		(c) Consider use of a flow diagram		
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

				-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	Key findings: -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	-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

				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.