NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service

South African Tuberculosis Drug Resistance Survey 2012–14

Acknowledgments

We would like to thank all that contributed to the success of the 2012-14 Tuberculosis (TB) Drug Resistance Survey (DRS) in South Africa.

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Abbreviations

ART	Antiretroviral therapy
CC	Critical concentration
CDC	Centers for Disease Control and Prevention
CDW	Corporate Data Warehouse
CRF	Case Report Form
CTB	Centre for Tuberculosis
DoH	Department of Health
DOTS	Directly Observed Therapy Short Course
DRS	Drug Resistance Survey
DST	Drug susceptibility testing
EMA	European Medicines Agency
EPTB	Extra-pulmonary Tuberculosis
FDA	United States Food and Drug Administration
GXP	Xpert MTB/RIF
HBC	High Burden Country
HIV	Human immunodeficiency virus
IMR	Isoniazid mono-resistant
INH	Isoniazid
IPT	Isoniazid preventative therapy
IPC	Infection Prevention and Control
IUATLD	International Union against Tuberculosis and Lung Disease
LIS	Laboratory Information System
LPA	Line probe assay
LTBI	Latent TB infection
LTFU	Loss to follow up
MGIT	Mycobacterial growth indicator tube
MTB	*Mycobacterium tuberculosis*
M&E	Monitoring and evaluation
MDR-TB	Multidrug-resistant tuberculosis
mPTB	Microbiologically confirmed pulmonary TB
NDoH	National Department of Health
NHLS	National Health Laboratory Services
NICD	National Institute of Communicable Diseases
NTRL	National TB Reference Laboratory
NTM	Non-tuberculous mycobacteria
NTP	National Tuberculosis Control Programme
PAS	Para-aminosalicylic acid
PDoH	Provincial Department for Health
PHC	Primary healthcare
PLHIV	Person/People living with HIV
PTB	Pulmonary TB
RCT	Randomised control trial
RHE	Rifampicin+Isoniazid+Ethambutol
RHZE	Rifampicin+Isoniazid+Pyrazinamid+Ethambutol
RIF	Rifampicin
RMR	Rifampicin mono-resistant
RR	Rifampicin-resistant
RSE	Robust standard error
SANAS	South African National Accreditation System
SLT	Second-line testing
TAT	Turnaround time
TDR	Totally drug-resistant
TB	Tuberculosis
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis
ZN	Ziehl-Neelsen

Executive Summary

The South African Tuberculosis Drug-Resistant Survey (DRS) 2012-14 sought to determine the prevalence of multidrug-resistant TB (MDR-TB) and other TB drug resistance in South Africa, enrolling participants from 442 randomly selected facilities in all nine provinces of the country. It was the largest TB DRS conducted with over 200 000 persons screened, over 5 000 000 data elements double-captured and more than 300 000 primary survey laboratory tests completed, including 100 000 individual drug susceptibility tests (against first and second-line drugs). Compared with the previous survey, the culture positivity rate was lower and in line with current recommendations, suggesting that patients are presenting earlier than before and that they are being appropriately screened.

The prevalence of MDR-TB nationally was measured at 2.1% (95% CI: 1.5%-2.7%) in new cases and 4.6% (CI 95%: 3.2%-6.0%) in retreatment cases with an overall, MDR-TB estimate of 2.8%; (95%CI: 2.0%-3.6%). Compared to the previous survey in 2001-02, the MDR-TB prevalence has remained relatively stable over the ten-year period with the overall MDR-TB rate in the previous survey being 2.9% (95% CI: 2.4%-3.5%). Provincial MDR-TB prevalence varied with six of nine provinces showing MDR-TB rates below 2% among new cases in the current survey. The highest rate observed was in Mpumalanga province with an overall rate of 5.1% (95% CI: 3.7%-7.0%), including both new and previously treated cases, which was higher than the national rate (2.8%; 95% CI: 2.0%-3.6%). This is a particular concern requiring urgent intervention.

Contrasted to the MDR-TB prevalence nationally, the rate of any rifampicin-resistance prevalence has increased since the previous survey, with the overall prevalence being 4.6% (95% CI: 3.5%-5.7%) nationally in the current survey, compared with 3.4% (95% CI: 2.8%-3.9%) in the previous survey. The increase was primarily seen among new cases, almost doubling from 1.8% (95% CI: 1.3%-2.3%) to 3.4% (95% CI: 2.5%-4.3%), highlighting the likely role of transmission. The use of Xpert MTB/RIF as the primary diagnostic tool will be important to detect these cases with any rifampicin resistance early, together with rapid initiation of therapy to halt further transmission. Rifampicin mono-resistance (RMR) which showed a low prevalence in the previous survey has emerged as a concern. It was below 0.5% overall in the previous survey but has increased to 1.7% in the current survey. Provincial variation was observed in RMR-TB cases with several provinces showing similar prevalence rates of MDR and RMR-TB cases while Limpopo province showed higher RMR-TB prevalence than MDR-TB. The reason for the emergence of RMR-TB in the context of standardised combination therapy is unclear and should be further investigated.

The prevalence of any isoniazid resistance (9.3%; 95% CI: 7.9%-10.7%) was higher than that of any rifampicin resistance (4.6%; 95% CI: 3.5%-5.7%). A notable increase in isoniazid mono-resistance (IMR) was observed between the current survey (4.9%; 95% CI: 4.1%-5.8%) and the previous survey (2.7%; 95% CI: 2.2%-3.2%). This raises concerns for the future emergence of MDR-TB as these cases would in effect be on rifampicin mono-therapy in the continuation phase of standardised TB therapy and the current diagnostic algorithm which does not test for resistance to isoniazid. Strengthening the continuation phase regimen needs consideration and the potential role of isoniazid preventative therapy (IPT) as a driver of this increase in the South African context needs to be investigated. Furthermore, the effectiveness of IPT could be reduced as the prevalence of any isoniazid resistance is almost 10% which makes it essential to conduct a risk benefit assessment.

Second-line drug resistance prevalence among MDR-TB cases was for the first time evaluated in this survey and findings are concerning. The prevalence of resistance to ethionamide and pyrazinamide, both used empirically in the treatment of MDR-TB was found to be high at 44.7%% (95% CI: 25.9%-63.6%) and 59.1% (95% CI: 49.0%-69.1%) respectively. This compromises the effectiveness of the standard MDR regimen and could lead to further selection of resistance to other drugs. Additionally, resistance levels to the key drug classes, fluoroquinolones and injectable agents, were both 13% (95% CI: 5%-21%), highlighting the relatively high frequency of pre-extensively drug-resistant tuberculosis (XDR) cases among those with MDR confirmation and the need to identify these cases early. Taking into consideration the high pre-existent levels of second-line drug resistance and the loss of one or both key drugs among pre-XDR and XDR cases, achieving improved outcomes is likely to require the use of a new regimen incorporating newly introduced drugs.

The findings from the South African TB DRS 2012-14 provide important information which could potentially guide future planning and address the current poor outcomes among drug-resistant TB cases. The following **recommendations** are made based on the findings of the survey:

- Urgent implementation of interventions in Mpumalanga
  - Identify potential risk factors for targeted interventions
  - Improve cross-border co-operation with Swaziland and Mozambique, utilising existing agreements achieved through the SADC declaration
  - Conduct further research to fully define drivers of resistance in the province

- Develop interventions to curb IMR and its secondary effects
  - Strengthen current first-line regimen for continuation phase by adding ethambutol with or without pyrazinamide(RHE or RHZE), or institute appropriate measures for early identification of IMR
  - Assess the contribution and effectiveness of IPT in the light of increasing cases of resistance
- Monitor transmission of RMR, research underlying reasons for RMR and institute appropriate interventions
  - Regularly review transmission data from surveillance system
  - Review current rifampicin dosing and conduct rifampicin bioavailability studies in the four and two-drug combination with and without antiretroviral therapies (ARTs) in areas with high RMR occurrence
  - Undertake close monitoring of the quality of drugs used in the standard regimen
- Conduct randomised control trials (RCTs) and review existing standard of care data to assess effectiveness of existing first and second-line regimens
- Monitor use of Xpert MTB/Rif assay for early detection of rifampicin resistance and improve early detection of second-line drug resistance
- Optimise existing MDR regimen and consider shortening MDR regimen with triage algorithm for appropriate patient selection
- Design an appropriate regimen for pre-XDR/XDR patients using a combination of new drugs
- Maintain and enhance the routine surveillance system for monitoring existing and new drug resistance and reduce the proportion of diagnosed cases not started on treatment.

Introduction

Global Tuberculosis Epidemiology

According to the World Health Organization (WHO) in 2015, tuberculosis (TB) mortality has declined by 47% since 1990 and the number of new TB cases has been falling worldwide at an average rate of 1.5% per year since 2000[1]. Furthermore, the millennium development goal of halting and reversing TB incidence by 2015 has been achieved globally, in all six WHO regions and in 16 of the 22 TB high-burden countries (HBCs). Despite these positive developments, TB is now the leading cause of mortality worldwide, causing 1.4 million deaths in 2014. Amongst these TB-related deaths, 400 000 were also HIV positive. While the burden of TB is declining, the absolute numbers of drug-resistant TB cases continue to rise.

Multidrug-resistant (MDR) TB is defined by resistance to both rifampicin and isoniazid, the two core drugs used in the treatment of TB. MDR-TB requires an extended duration of treatment with less-effective drugs. Globally, 3.3% of new and 20% of previously treated TB cases have MDR-TB with an estimated caseload of 480 000 (range: 360 000–600 000) new cases of MDR-TB worldwide, annually, with only 111 000 (23%) cases initiated on appropriate treatment[2]. There were approximately 190 000 (range: 120 000–260 000) deaths from MDR-TB in 2014. The dismal situation has led the WHO to declare MDR-TB a global crisis[3].

Extensively drug-resistant (XDR) TB is MDR-TB that has developed further resistance to both key groups of second-line therapy, including the fluoroquinolones (e.g. moxifloxacin) and injectable agents (e.g. kanamycin), resulting in fewer therapeutic options, and an increased probability of a fatal outcome. An estimated 9.7% of people with MDR-TB have XDR-TB and XDR-TB has been reported in 105 countries by 2015. In 2014, 4 044 XDR-TB patients were enrolled on treatment in 49 countries. Most of XDR-TB cases in 2014 were notified from India (1 262), Ukraine (657), South Africa (562), Belarus (431), and Kazakhstan (318). Among XDR-TB patients in the 2012 cohort globally for whom outcomes were reported, treatment success was half that of MDR-TB at 26% (682/2 685) while mortality was twice as high at 30% (809/2 685).

In response, several new global initiatives have been introduced and aimed at improving the diagnosis and treatment of drug-resistant TB. In 2008, WHO endorsed line probe assays for the rapid detection of MDR-TB[4] and this was followed in 2011 by a strong recommendation for the use of Xpert MTB/RIF (Cepheid, USA; GXP)[5] as a primary diagnostic test; both were rapidly adopted by South Africa. These improvements in diagnostics were followed by the approval of Bedaquiline, a diarylquinoline derivative and delaminid, a dihydro-nitroimidazooxazole derivative, by the United States Food and Drug Administration (FDA)[6] and European Medicines Agency (EMA)[7]. These two drugs, representing two new anti-TB drug classes, became available for TB treatment in 2012 and 2013 respectively, more than 40 years after rifampicin was approved for TB treatment in 1974. Treatment with these two new drugs has resulted in improved patient outcomes among drug-resistant patients[8], but they are only available to patients in a limited number of countries and for select patient groups.

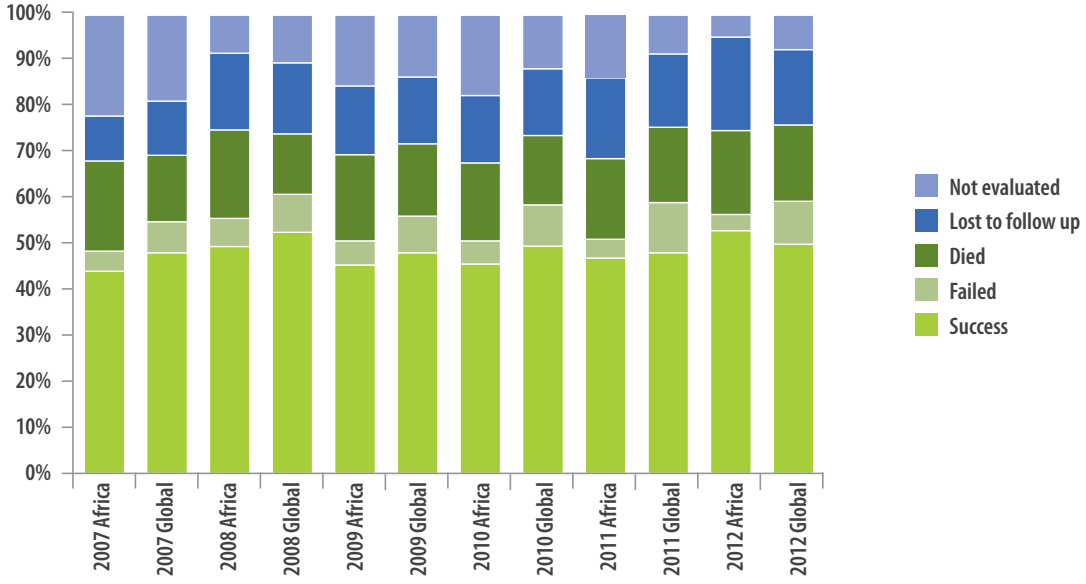


Figure 1: MDR-TB 2007 to 2012 cohort treatment outcomes in the African region and globally (adapted from WHO Global Report 2015)

Epidemiology of Tuberculosis in South Africa

South Africa remains one of the 22 highest TB-burdened countries globally and, has the second highest TB incidence rates in the world[1]. The high prevalence of HIV infection has been an important driver of TB in South Africa with TB incidence rates increasing from the early 1990s, coinciding with the increasing HIV prevalence. In response, South Africa has undertaken an aggressive programme aimed at controlling HIV and now has over 3 million people of its estimated 6.5 million HIV-infected on antiretroviral therapy (ART) [9]. This has led to improvements in the overall life expectancy in South Africa from 57.1 years in 2009 to 62.2 years in 2013[10]. This trend is possibly related to the decline in TB associated deaths, TB causes the most deaths in South Africa according to a report by Statistics South Africa, and remains a major public health threat[11].

TB notification data in South Africa has shown a decline from approximately 400,000 cases in 2009[12] to 300,000 in 2014[1]. This pattern was corroborated by a recent report showing a decrease in microbiologically confirmed pulmonary TB (mPTB) nationally and in South Africa's provinces from an annual incidence (per 100 000 population) of 848 (845–850) in 2008, to 774 (771–776) by 2012 (representing a 9% decrease from 2008 to 2012)[13]. Furthermore an inverse relationship was observed between incidence of mPTB and ART coverage among HIV-infected individuals nationally and in the provinces.

Annual incidence (per 100 000 population) of mPTB differed between the nine provinces, exceeding 1 000 in Northern Cape (2004–12), Western Cape (2004–09), Eastern Cape (2008–12), and KwaZulu-Natal (2007–12). Between 2004 and 2012, KwaZulu-Natal had the highest annual number of new cases, with absolute numbers in 2011 representing 31% of mPTB cases in South Africa that year.

Although this data represents only mPTB cases, it is a robust indicator of TB trends, which are declining in all provinces. An important observation was the heterogeneity in incidence rates between provinces suggesting that, apart from the impact of ART on TB control, other factors may be contributing.

Multidrug-Resistant Tuberculosis in South Africa

The first national survey of TB drug resistance in South Africa was undertaken between 2001 and 2002[14]. The study reported an MDR-TB rate in South Africa of 1.6% (1.1%-2.1%) in new cases and 6.6% (4.9%-8.2%) in retreatment cases. There was considerable variation by province, with the prevalence of resistance to rifampicin being lowest in the Western Cape at 0.9% and highest in Mpumalanga at 3.1% among new cases, and 3.9% and 16.0% respectively among retreatment cases.

Although the MDR prevalence appears to be low among primary TB cases, this needs to be interpreted in the context of a high incidence of TB in South Africa. In the *WHO Global TB Report 2015*, South Africa had the second highest absolute number of notified rifampicin-resistant (RR)/MDR cases globally (18 734)[1], with India ranked number one (25 748) but has a population 20 times that of South Africa.

The occurrence of laboratory-confirmed XDR-TB – a more resistant form of MDR – has long been recognised in South Africa, and was managed as difficult-to-treat MDR-TB cases. It was however, the outbreak of XDR-TB with a very high mortality rate in 2005 at the Church of Scotland Hospital in Tugela Ferry, KwaZulu-Natal in which 52 patients were confirmed with XDR TB, with about half being putative primary cases, that brought XDR into focus worldwide[15]. This outbreak highlighted the potential for nosocomial spread of MDR/XDR-TB and the possibility of a specific clone spreading rapidly among patients with HIV/AIDS, with fatal outcomes. The lack of universal testing for drug-resistant TB may lead to undiagnosed drug-resistant cases circulating in the community, with potential for further spread. Following the outbreak in KwaZulu-Natal, WHO has expressed concern over the emergence of virulent drug-resistant strains of *Mycobacterium tuberculosis* (MTB) and called for measures to be strengthened and implemented to prevent the global spread of these deadly MTB strains[16].

The outbreak in KwaZulu-Natal was followed six years later by a report of the emergence of "totally drug-resistant" TB in Eastern Cape, South Africa based on strains collected during the period of 2008-09[17]. The authors also identified an atypical Beijing strain type that was clustered in more than 80% of cases by strain typing, suggesting transmission. Although the term "totally drug-resistant" (TDR) TB is a misnomer as new drugs have been developed that offer new therapeutic options, the degree of resistance linked to clonal spread has been of great concern. This is especially true for a country like South Africa in which 18.9% of persons 15 years of age and above are infected with HIV[9].

It should be noted that these outbreaks occurred in geographically confined areas. However, with increasing population mobility, the emergence of drug-resistant strains is expected to disseminate more widely. Evidence from KwaZulu-Natal suggests that the overall XDR-TB incidence has indeed increased in this region between 2007 (3.1/100 000 population) and 2011 (3.5/100 000 population)[18]. Furthermore, the outcome of such highly resistant TB is known to be poor, especially among HIV-positive patients, as was most clearly shown in the Tugela Ferry outbreak with a case fatality of almost 100%.

Routine notification data has shown that the treatment success rate is approximately 50% in MDR-TB cases and 20% in XDR-TB patients[19]. Furthermore, many of these unsuccessfully treated patients die. The situation has however improved with the introduction of bedaquiline for which early programme data suggests improved outcomes[20].

Contrasted to the declining trend of new TB cases in South Africa, the number of drug-resistant cases diagnosed and recorded on treatment continues to rise[19]. The number of line probe assays (LPAs) performed was more than 100 000 at its peak in 2011[21] and was superseded by the Xpert MTB/RIF method with over 2 million tests performed in 2014, resulting in 218 231 TB cases having a primary test performed for rifampicin-resistant TB at baseline[1]. Thus the increase in case numbers is likely as a result of improved diagnostic options and intensified case finding leading to previously undiagnosed cases now being detected and treated rather than a true increase in burden.

Rationale for the Current Drug Resistance Survey

Much has changed in South Africa since the previous DRS with two recorded and published outbreaks of highly drug-resistant TB, an extensive scaling up of diagnostics, several intensive case-finding campaigns and the introduction of new drugs. The biggest change however has been the shift in policy with South Africa now home to the largest ART programme globally[22]. In addition the current WHO recommendation is to conduct a TB drug resistance survey every five years[23].

The need to assess the current status of drug-resistant TB in South Africa was thus seen as an important priority in the face of a multiplicity of events that have occurred since the DRS of 2001–02. As heterogeneity of drug-resistant TB has been observed in the previous survey and was also evident in specific provinces and outbreaks, it was necessary to ensure the survey would be powered to provide estimates for individual provinces. Additionally, there was a need to determine not only the MDR-TB prevalence rates but also those of XDR-TB, which has emerged as a particular concern in South Africa.

Objectives

Primary Objective

- To quantify and delineate the extent of drug resistance in new and retreatment TB patients nationally and provincially in South Africa.

Secondary Objective

- To compare the change in drug resistance prevalence nationally and provincially to that estimated in the previous survey, conducted during 2001-2002.

Methods

Study design

The survey was a population-based cross-sectional study conducted following the WHO Guidelines for surveillance of drug resistance in tuberculosis[23]. A population proportionate cluster sampling design was used to determine the sample size and select study sites, aimed at providing MDR estimates for each province, as well as nationally. The clusters were randomly selected and were either individual healthcare facilities or a combination of facilities.

Patients were eligible for inclusion in the survey if they were older than 18 and presented as a presumptive TB case, according to WHO/International Union against Tuberculosis and Lung Disease (IUATLD) definitions.

Definitions

New case

A "new case" is defined as a patient with a newly registered episode of TB who, in response to direct questioning, reports never having been treated for TB or reports having taken anti-TB drugs for less than one month; or where adequate documentation is available, for whom there is no evidence of having taken anti-TB drugs for one month or more.

Previously treated case

A "previously treated case" is defined as a patient with a newly registered episode of TB who, in response to direct questioning, reports having received one month or more of anti-TB drugs in the past; or where adequate documentation is available, there is evidence of having received one month or more of anti-TB drugs in the past.

Primary drug resistance

Patients who fulfilled the definition of being a "new case" of TB above, having no significant prior TB treatment exposure and who, based on laboratory testing, were found to have a drug-resistant MTB strain are considered to have primary drug-resistant TB.

Acquired drug resistance

Patients who fulfilled the definition of being a "previously treated case" of TB above, having received prior TB treatment for more than one month and who, based on laboratory testing, are found to have a drug- resistant MTB strain were considered to have acquired drug resistance.

MDR

Multidrug-resistant tuberculosis (MDR-TB) is defined as TB that is resistant to both isoniazid (INH) and rifampicin (RIF), two of the first-line drugs used in treating pulmonary tuberculosis.

Pre-XDR

Pre-XDR TB is defined as TB that is resistant to both isoniazid and rifampicin (RIF) and either a fluoroquinolone or second-line injectable agent but not both.

XDR

Extensively drug-resistant tuberculosis (XDR-TB) is defined as MDR-TB with additional resistance to any fluoroquinolone (FQ) and to at least one of three injectable second-line anti-tuberculosis drugs used in treatment (capreomycin [CPM], kanamycin [KM] or amikacin [AMK])

Study overview

All consecutive presumptive TB cases, who provided informed consent at the selected facilities during the survey period, had a case report form (CRF) completed through direct patient interview by a healthcare worker at the health facility and in addition had a survey-specific sputum sample collected, were included. The CRF with the corresponding sample was sent to the Centre for Tuberculosis at the National Institute for Communicable Diseases in Johannesburg, where smear microscopy, liquid mycobacterial culture and HIV testing on sputum was performed. This was followed by drug susceptibility testing against a panel of first-line and second-line anti-TB drugs on *Mycobacterium tuberculosis*-confirmed isolates (Figure 2). Data from the CRF and the laboratory testing process were collated and analysed.

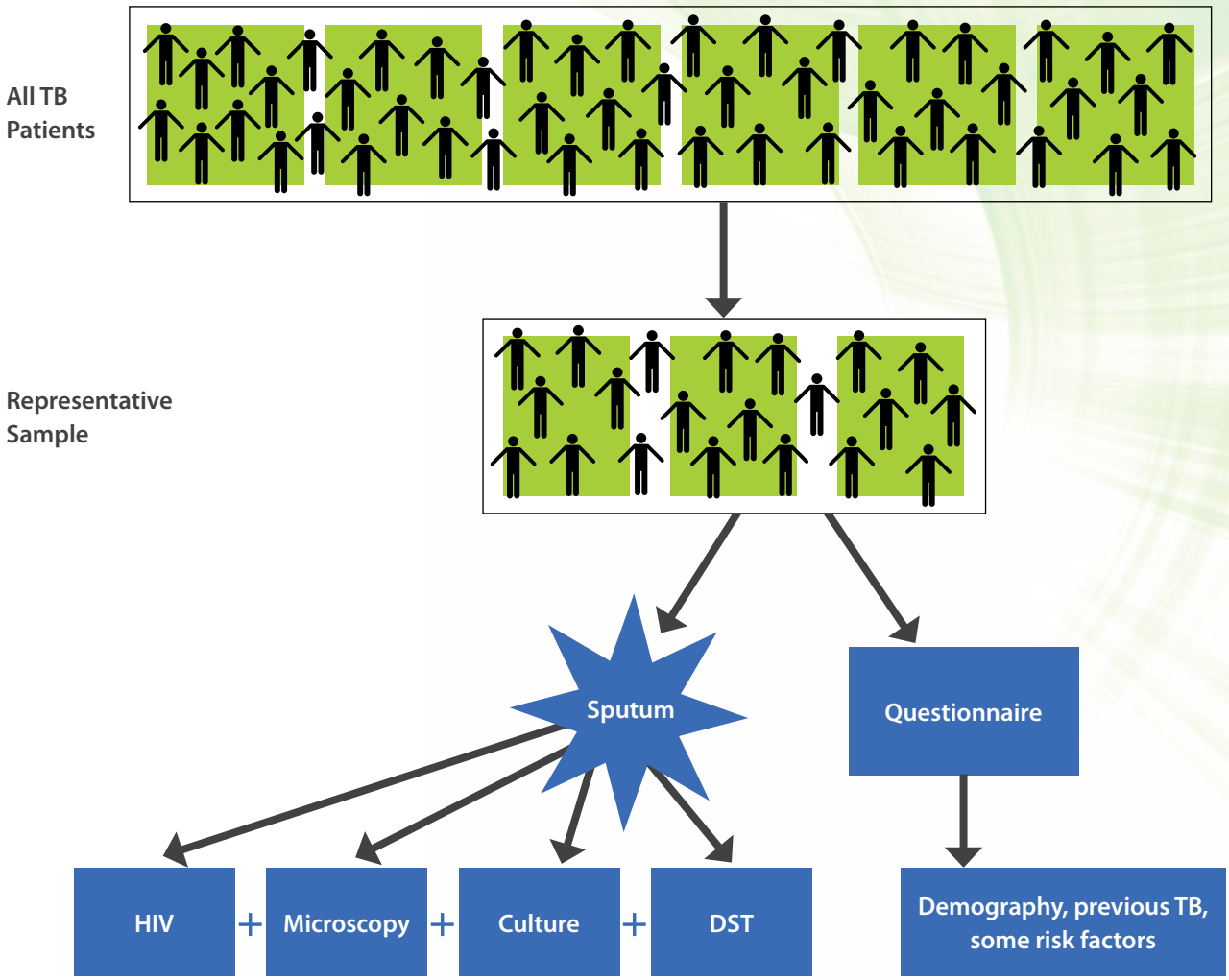


Figure 2: Flow diagram of sampling and enrolment process

Inclusion and exclusion criteria

A patient was eligible for inclusion in the survey if he/she presented as a presumptive case (new or previously treated patient), according to the WHO/IUATLD definitions during the intake period at a Drug Resistance Survey (DRS) enrolling facility. Only adults 18 years of age or older who could produce sufficient volumes of good quality sputum were included. Patients were excluded if they declined to give informed consent to participate in the survey.

Sample size and sampling

The sampling frame for this survey comprised all presumptive TB patients tested within a cluster who were subsequently shown to have culture-confirmed TB. As smear-positive disease is less frequent among HIV-infected TB patients, sputum smear-negative, culture-positive cases, as opposed to only smear-positive cases used in other WHO/IUATLD surveys, were included to reduce the likelihood of systematic exclusion of HIV co-infected patients who often present with paucibacillary disease, thus considerably increasing the sample size. The number of new patients required per province was determined using StatCalc in Epi-Info version[6], and was based on the following criteria:

- An estimate of culture-positive cases based on the total number of pulmonary cases detected in 2007 in each province for which a sputum sample was examined
- The expected prevalence of resistance to rifampicin of 0.9%, being the lowest level of primary multidrug-resistant TB detected in any province in the previous survey
- An absolute precision of 1-2%
- A confidence interval of 95% to estimate prevalence.

To adjust for the cluster design effect, the calculated sample size was multiplied by two. The design effect of two is the recommended default when information on variations in clusters is not available. As the proportion of suspects expected to test culture-positive varied by province (from 5-25% based on 2007 data), the required number of new patients was multiplied accordingly to obtain the number of suspects for screening. In order to accommodate the occurrence of a substantial number of previously treated cases - as experienced in a previous survey - the sample size was increased by the expected number of retreated cases in each province.

The calculated sample size was finally increased by 20% to account for expected loss of cases during the survey. These would include patients whose cultures were contaminated or failed to yield any MTB growth, as well as inconclusive drug susceptibility results. The sampled clusters per province with total positive cultures required are summarised in Table 1.

Table 1: Patient sampling schedule for survey, 2012-14

Province	Positive (Sm+) Cases Identified 2007	Positive Cultures Required for Survey	Doubling for Cluster Effect	Addition for Retreat-ment	Add 20% for Losses	Total Number Pos Cultures Required	Total (approx) Number TB Suspects Required	Numbers of Clusters Proposed	Number Pos Culture Patients per Cluster	No Suspects to Screen per Cluster	No Suspects to Screen	No Cases for DST
EC	27 146	463	926	1 323	1 654	1 654	16 536	32	**52**	516	16 512	1 664
FS	10 872	452	904	1 291	1 614	1 614	20 179	40	**40**	504	20 160	1 600
GP	29 881	463	926	1 323	1 654	1 654	16 536	35	**47**	472	16 520	1 645
KZN	48 748	468	936	1 337	1 671	1 671	16 714	32	**52**	522	16 704	1 664
LP	11 331	446	892	1 274	1 593	1 593	31 857	50	**32**	637	31 850	1 600
MP	11 566	444	888	1 269	1 586	1 586	15 857	38	**42**	417	15 846	1 596
NC	5 378	418	836	1 194	1 493	1 493	18 661	50	**30**	373	18 650	1 500
NW	14 673	456	912	1 303	1 629	1 629	16 286	40	**41**	407	16 280	1 640
WC	26 818	462	924	1 320	1 650	1 650	16 500	32	**52**	515	16 480	1 664
TOTAL	**186 413**	**4 072**	**8 144**	**11 634**	**14 544**	**14 544**	**169 126**				**169 002**	**14 573**

The DRS was planned for 349 randomly selected clusters (made up of 442 healthcare facilities) proportional to the patient load of sputum smear-positive TB cases reported to the National TB Control Programme in 2008. A total of 169 002 patients were targeted for recruitment during the intended year-long study period across the nine provinces in South Africa.

Sampling strategies

In order to ensure that specimens are representative for all TB patients in South Africa, the following methodological adjustments were made:

- To avoid the risk of missing the largest diagnostic facilities during randomisation, population-proportionate cluster sampling was used. Based on a list of new sputum smear-positive cases per diagnostic facilities, per province in the year 2007 when the survey was designed, diagnostic facilities included in the sampling frame for the study were weighted according to the expected patient caseload
- Clusters were chosen to ensure that the period of recruitment to achieve the required sample size of study subjects would be approximately 52 weeks in each province. The number of clusters was raised in some provinces, to increase the likelihood of reaching the required sample size of patients with positive cultures per cluster. Where the caseload per facility was still lower than the expected number of patients with positive cultures required per cluster, facilities in close proximity and within the same sub-district, were grouped together. This approach would allow direct estimation of the prevalence of resistance from the proportion calculated in the sample for each province
- The total number of new sputum smear-positive cases per province per year, divided by the number of clusters, was used as the sampling interval. A random number between one and the sampling interval was drawn to identify the facility in which the first cluster falls in each province. The sampling interval was sequentially added to this random number to locate the remaining clusters on the list. The number of study subjects per cluster was determined by dividing the required total sample size by the respective number of clusters in the province. If more than one cluster occurred in any diagnostic facility, the number of clusters needed was multiplied by the size of the cluster to calculate the total number of patients needed from that facility. In all selected diagnostic facilities, consecutive presumptive TB patients giving sputum samples were included in the survey until the number required for one or more clusters was reached. The clusters selected for this survey by province are presented in Appendix C and depicted in Figure 3.

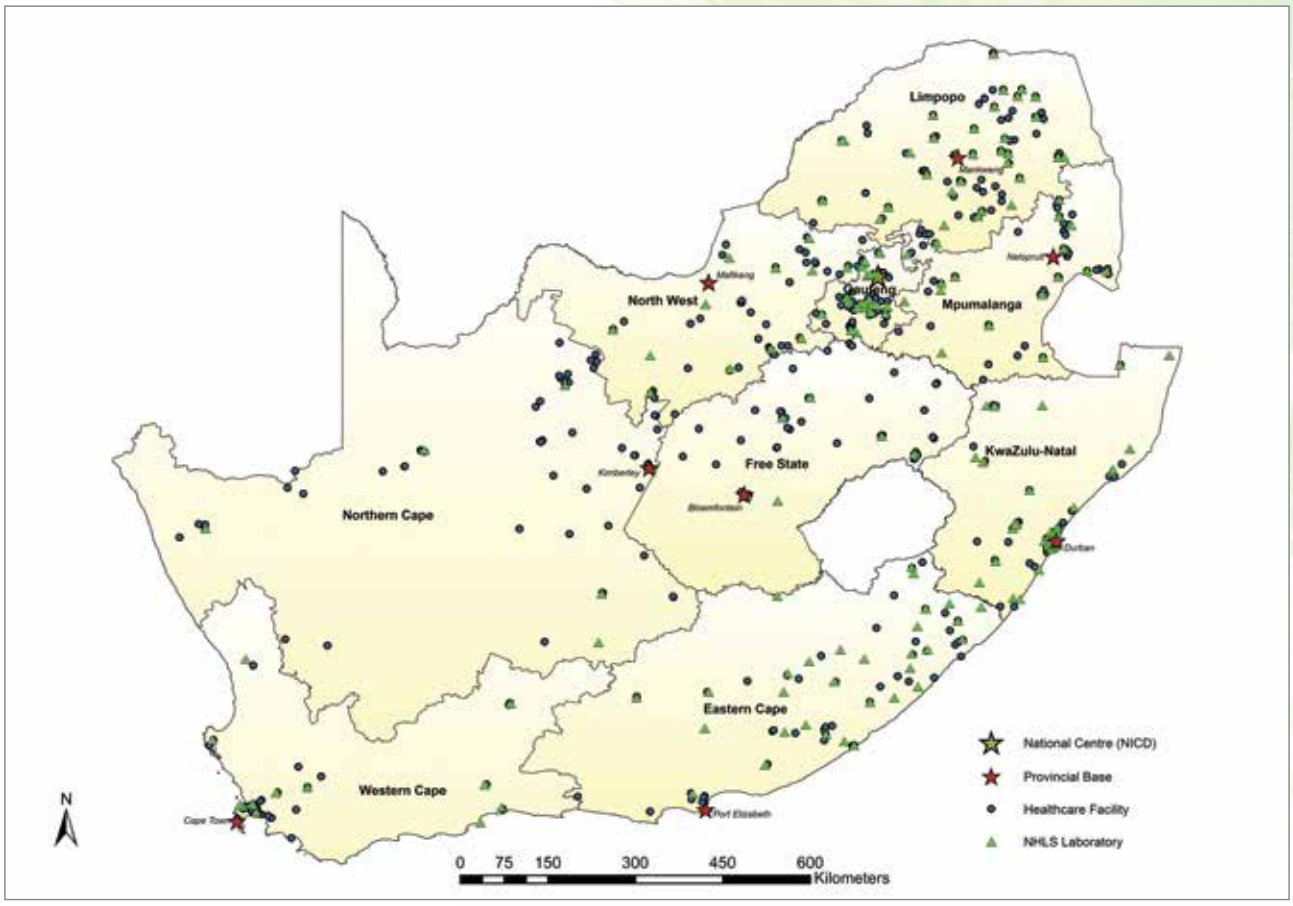


Figure 3: Map of randomly selected facilities included in the SA TB DRS 2012-14

Survey preparation and patient enrolments

Once ethical and provincial approvals were received, initial preparatory work was initiated, including sourcing survey-specific staff which included field project co-ordinators, laboratory and data capturing staff. Logistics and workflows were developed and initial piloting performed to identify and rectify any obvious challenges.

The roll-out of the survey was undertaken in a step-wise approach, initiating with one province per month. Province initiations were avoided during holiday periods. Thus the initiation of all 9 provinces took place over 12 months and the close out period followed a similar pattern. Thus the survey enrolment occurred between mid-2012 and mid-2014. A schematic presentation of the roll-out schedule is shown in Figure 4.

July-12	Aug-12	Sep-12	Oct-12	Nov-12	Feb-13	Feb-13	Mar-13	Apr-13
Gauteng	Gauteng	Gauteng	Gauteng	Gauteng	Gauteng	Gauteng	Gauteng	Gauteng
	North West	North West	North West	North West	North West	North West	North West	North West
		Northern Cape	Northern Cape	Northern Cape	Northern Cape	Northern Cape	Northern Cape	Northern Cape
			Limpopo	Limpopo	Limpopo	Limpopo	Limpopo	Limpopo
				Eastern Cape	Eastern Cape	Eastern Cape	Eastern Cape	Eastern Cape
					Mpumalanga	Mpumalanga	Mpumalanga	Mpumalanga
						Western Cape	Western Cape	Western Cape
							KwaZulu-Natal	KwaZul-Natal
								Free State

Figure 4: Roll-out plan for the 2012-14 SA TB DRS

A standardised case report form (CRF, Appendix A) was used at all survey facilities collecting demographic, clinical, enrolment criteria and risk factor information. This was accompanied by an informed consent form (Appendix B) which included a section related to HIV testing and reporting. In order to ensure that the information on the CRF was collected in a standardised manner, central training sessions were held in each province prior to initiation. During the training sessions, colleagues from participating facilities were reminded of the basic concepts of TB with specific attention paid to administering the questionnaire and collecting the extra sputum sample. The training comprised a combination of didactic presentations and role play. Training was also conducted on procedures for obtaining informed consent and clarification of issues related to the patient's voluntary participation. As not all staff were available for central training, this was followed up with on-site training at every participating facility where further role play was also conducted to ensure that the CRF was understood and completed correctly.

The training sessions included sputum collection and packaging procedures, as well as the handling and shipment of specimens. The former is a routine practice and training was aimed at reinforcing basic principles, including infection control measures. Specific training related to packaging was provided to deal with variation introduced by the requirement of an additional survey sample. At the DRS facilities, two 'spot' sputum samples were collected from consenting participants. The first sample was always used as the routine specimen, following the existing diagnostic algorithm at the time, and was intended for clinical management. During the course of the survey, the routine diagnostic algorithm was changed from smear and culture-based to the use of the Xpert MTB/RIF assay as the primary diagnostic tool. The second sample was the survey-specific sample. Both samples were individually packaged and placed into one 'maxi' shipment bag. A common sample label was used to link both samples and was pasted on the respective sputum containers and the accompanying DRS questionnaire. The DRS bag was identifiable with clear labeling and a unique red colour scheme, while members of staff from local laboratories who received these specimens were also trained on managing the two samples, keeping the first one for local diagnostic procedures and transferring the survey specific specimens to the Centre for Tuberculosis in Sandringham, Johannesburg.

Monitoring and evaluation

In each province, a provincial field co-ordinator was appointed to conduct close monitoring of the survey activities, on a day-to-day basis. This included following up on enrolment targets, retraining staff where necessary, liaison between the facilities and local laboratories, as well as providing feedback to the provincial programme on progress with survey implementation. Due to the large scale of the survey and the utilisation of routine health care workers for enrolment at the sites, challenges arose during enrolment that required regular retraining and support visits to survey sites due competing interests with routine work, movement of staff as well as insufficient human resource capacity at the health facilities over the survey period.

A monitoring plan that outlined indicators ensuring the survey was going to plan was developed prior to implementation. The plan described survey reports that would be published, their frequency, content and intended audience. A framework of indicators for monitoring all survey processes was included in the plan. The indicators were divided into four broad categories: sample size, laboratory testing, timeliness and data quality. Indicators were reported to the survey project manager and provincial co-ordinators for corrective action. This enabled the survey team to respond to challenges with the survey as they arose.

Lower than expected enrolment rates were problematic across the provinces and an additional period of two months was allocated to all provinces to ensure balance across sites, resulting in a total enrolment period of 14 months for each province. Several meetings with the respective provinces were also held to address low enrolments. The drive to increase enrolment was fairly successful but had unforeseen consequences: although the number of enrolments increased, a large number of samples were sub-optimal for processing upon receipt at NICD.

Registration of samples in the laboratory

On arrival at the peripheral laboratory of the National Health Laboratory Service (NHLS) registration of all samples was performed on the laboratory information system in use at the respective receiving laboratories. Registration entailed the creation of a unique laboratory number for each survey sample, as well as a survey specific location code. Once registered, all samples were subsequently transported through the existing (NHLS) logistics services, following standard procedures to the Centre for Tuberculosis (CTB), NICD in Johannesburg.

The non-testable samples were either of very low volume, making them unsuitable for processing due to the complexity of the test processing procedures, or had leaked from the container during transit. This necessitated re-fresher training for health facility staff on sputum collection and packaging procedures. In addition, the leaking of specimens was investigated and it was found that leakages were most likely due to the type of closing mechanism which differed from that of the routine sample container. This was corrected midway through the survey and other measures were also introduced to optimise the routing system for the samples aimed at minimising transit times.

Laboratory procedures

Once the samples arrived at the National TB Reference Laboratory (NTRL), receipt details were entered onto the respective Laboratory Information System (LIS) on which the sample was originally registered and the questionnaire separated and routed for data entry. The laboratory workflow is shown in Figure 5.

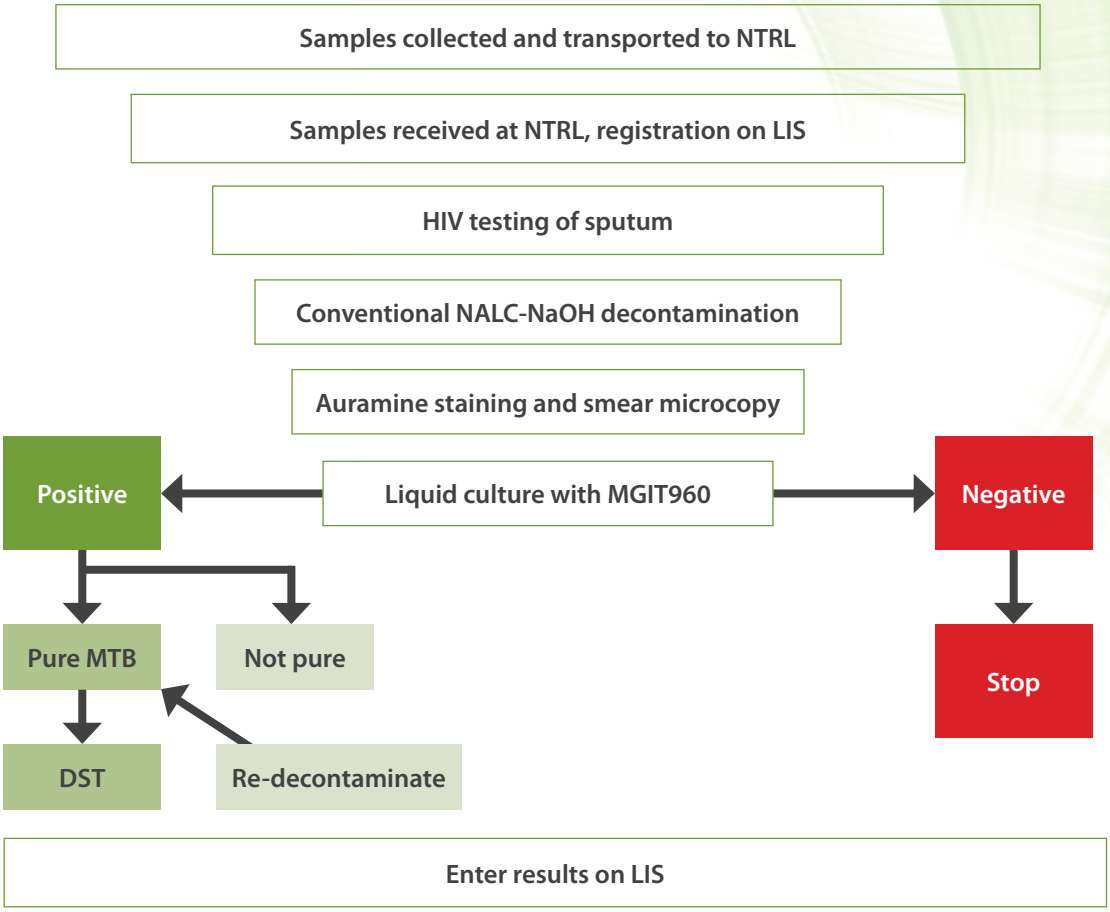


Figure 5: Workflow of laboratory testing

HIV testing was performed directly on the sputum sample using the Oraquick® Advance Rapid HIV – 1 & 2 Antibody Test (Orasure Technologies Inc) (Oraquick)[24] prior to decontamination if sufficient volume was available. Decontamination of specimens was performed using a conventional N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method[25]. The sediments were then stained with Auramine-O for fluorescent microscopy[26] and inoculated into liquid medium for mycobacterial growth detection (BD BACTEC™ mycobacterial growth indicator tube (MGIT)™ 960 System)[27]. Positive cultures were examined for acid-fast bacilli and purity, using the Ziehl-Neelsen (ZN) staining and blood agar inoculation. *Mycobacterium tuberculosis* complex (MTB Complex) was confirmed by testing for the presence of the MPT64 antigen, using the BD MGIT TBc identification test or the Genotype Mycobacterium CM assay (Hain Lifesciences, Germany) following manufacturer instructions.

Drug susceptibility testing (DST) using the MGIT system was performed on pure cultures and was based on WHO proposed critical concentrations for isoniazid (low and high concentrations), rifampicin, ethambutol, pyrazinamide, streptomycin, amikacin, kanamycin, capreomycin, ofloxacin, moxifloxacin, ethionamide and para-aminosalycilic acid (Table 2). Cultures that contained both mycobacteria and contaminating organisms were re-decontaminated using solid media (BD BBL™ Lowenstein-Jensen medium slants with Glycerol and PACT). Cultures that failed re-decontamination and showed the presence of acid fast bacilli, or where DSTs failed, were tested genotypically using the Genotype MTBDRplus version 2 assay (Hain Lifescience, Germany) to confirm the presence of MTB Complex and detect resistance to isoniazid and rifampicin.

Table 2: WHO-approved critical concentrations used for drug susceptibility testing

Drug Classification	Drug Name		Critical Concentrations (µg/ml) for Liquid Culture (MGIT)
First-line Agents:			
Group 1	Rifampicin		1.0
	Isoniazid		0.1
	Pyrazinamide		100
	Ethambutol		5
	Streptomycin		1
Second-line Agents (Group 2):			
Group A Fluoroquinolones	(Ofloxacin)		2.0
	Moxifloxacin		0.5
Group B Second-line injectable agents	Amikacin		1
	Capreomycin		2.5
	Kanamycin		2.5
	(Streptomycin)		1
Group C Other core second-line agents	Ethionamide		5.0
Group D Add-on agents *(not core MDR-TB regimen components)*	D1	Pyrazinamide	100
		Ethambutol	5.0
		High-dose isoniazid	0.4
	D2	-	
	D3	p-aminosalicylic acid	2.0

All laboratory work was carried out centrally under biosafety level three conditions at the NTRL within the Centre for Tuberculosis at the National Institute for Communicable Diseases. The laboratory is the national reference laboratory for the country and is accredited by the South African National Accreditation System (SANAS) following the ISO 15189:2012 standard and participates in the College of American Pathologists external quality assurance programme. It is also certified proficient for performing first and second-line DST by WHO and recently received full Supranational Reference Laboratory status by WHO.

Data management

Data for the survey were captured into three different data systems which included the case report form (CRF) on an SQL (structured query language) platform and the two laboratory information systems (Disalab & TrakCare) in use within NHLS. The data for the latter two systems were stored at the central data repository at the Corporate Data Warehouse (CDW) of the NHLS. An overview of the data flow is shown in Figure 6.

Completed DRS case report forms received from the facilities, including the printed unique laboratory number, were manually double-captured in provincial batches (blue pathway) with two individuals capturing the same form independently and their results compared and discordances resolved by a third independent person. The data manually captured were: laboratory number, specimen number, date of birth, age at survey, location of survey, gender, previous TB history and HIV status. For the remainder of the form there were multiple choice responses captured by image-scanning computer-based data extraction and manual review of exceptions.

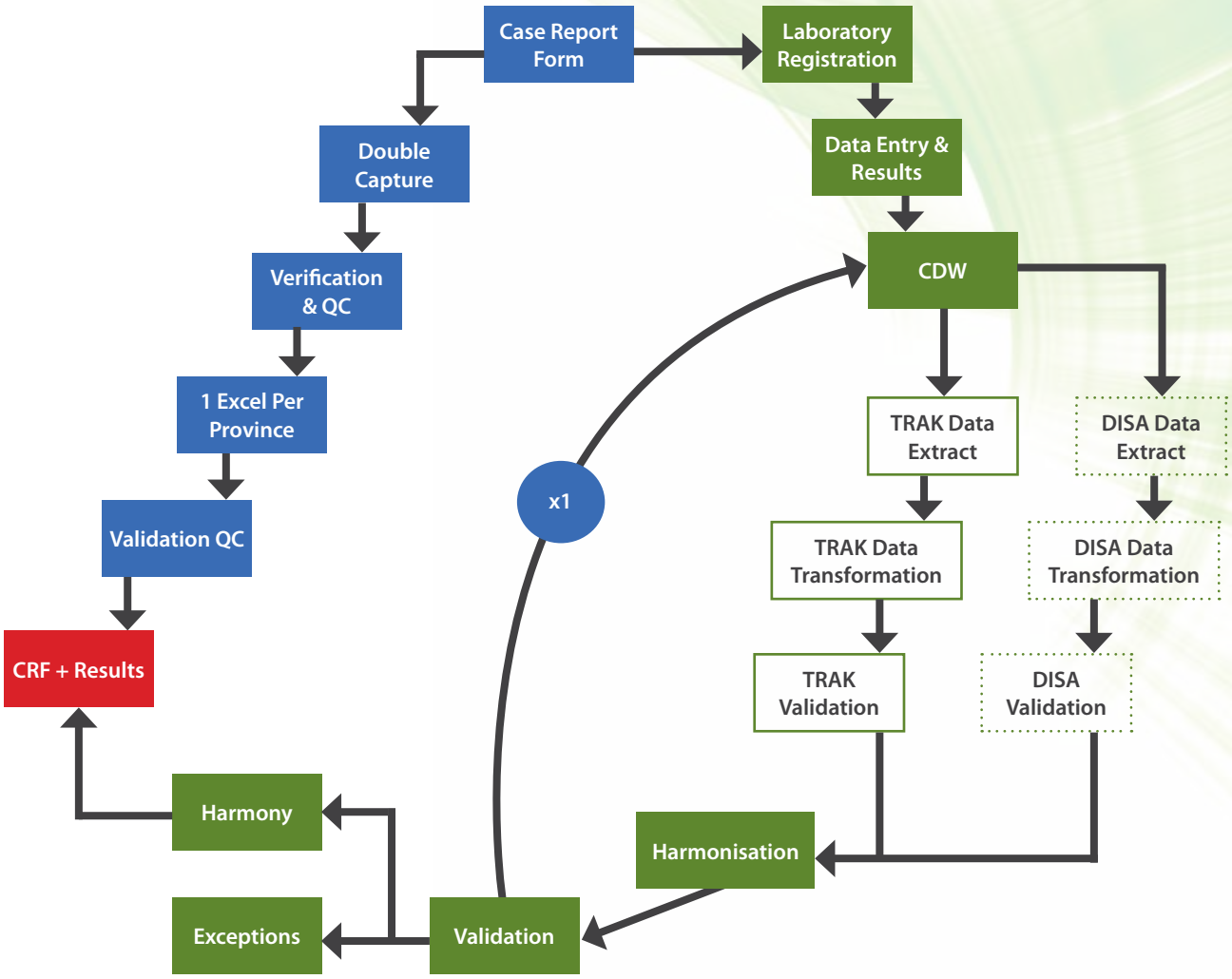


Figure 6: Overview of data management flow for the SA TB DRS 2012-14

Additional quality checks were also performed on a selection of forms by facility and the average error rate was 0.24 per 100 fields verified, ranging between 0.01 (Free State) to 0.51 (Gauteng). Further data cleaning was performed to identify and resolve duplicates and other errors prior to extraction of the laboratory data.

The variables used to extract the laboratory data were the laboratory number and specimen number. A unique set of laboratory numbers was retrieved from the CRF data and sent to the data warehouse to extract all test results and reject status associated with these laboratory numbers. Data extracted comprised the final reviewed results that were authorised either by a pathologist or other appropriately qualified senior laboratory staff member.

The finalised provincial CRF and laboratory sets were then harmonised and prepared for final analysis. This included data consistency and validity checks. The cases that were not tested in the survey had their final TB status determined using data from the routine sample tested which accompanied the survey sample.

Any resistance to rifampicin and isoniazid was defined as resistance to rifampicin and isoniazid, respectively, regardless of other drug susceptibility test results.

Mono-resistance to rifampicin and isoniazid was determined as rifampicin resistance and isoniazid susceptibility irrespective of other first-line resistance for the former and isoniazid resistance and rifampicin susceptibility irrespective of other first-line resistance in the latter. These were also further sub-classified in two ways to align with the previous report.

Strict mono-resistance was defined as resistance to rifampicin or isoniazid, with susceptibility to all other first-line drugs. Other mono-resistance was defined as resistance to rifampicin but not isoniazid and additional first-line drug resistance (streptomycin and/or ethambutol); similarly other isoniazid mono-resistance was defined as resistance to isoniazid but not rifampicin and other first-line drug resistance (streptomycin and/or ethambutol).

MDR status was assigned to confirmed TB cases with resistance to both rifampicin and isoniazid regardless of drug susceptibility test method used.

Second-line resistance was defined as resistance to any one of the second-line injectable therapeutic agents, while fluoroquinolone resistance was defined as resistance to ofloxacin. XDR-TB status was assigned to all TB confirmed cases that were MDR and that additionally had both second line and fluoroquinolone resistance.

Data on HIV status was obtained through self-reporting at interview and from the Oraquick testing on sputum samples. Self-reported HIV status is influenced by reporting bias especially for those reporting a HIV-negative status [28]. The Oraquick test on the other hand has reduced sensitivity (93.5%; 95% CI: 87.1%-97.3%) and negative predictive value (73.9%; 95% CI: 53.4%-88.7%) when tested on sputum more than 24 hours after collection which was most often the case in this survey[29]. Additionally, HIV-infected individuals who are receiving HAART may produce false negative results[30]. Thus to determine a final HIV status the Oraquick result was used and for those cases where the test was negative and the self-report was positive, these cases were regarded as HIV positive.

Data analysis

Both descriptive and statistical analysis accounting for the complex multistage sampling strategy and clustering of patients within primary sampling units were performed. Simple descriptive statistics compared demographic and laboratory parameters between provinces including age, sex, smear, culture and HIV positivity rates. For those with missing age or sex these were extracted from the laboratory registration data for the matched routine sample if this data was available. Culture positivity rates were calculated as the proportion of culture positives with confirmed TB among the presumptive TB cases enrolled and tested by culture. The smear positivity rates were calculated among TB culture- positive cases.

Statistical analysis aimed at determining population level first-line drug resistance estimates, at a provincial level, and both first and second-line population level resistance estimates at a national level among TB cases. Additionally, national second-line estimates were calculated among the sub-group of MDR cases. The provincial estimates were determined after adjusting for the clustering effect introduced by the survey design and any potential biases that may have arisen during implementation. The provincial estimates were pooled to generate national estimates.

The data for the population level analysis was initially analysed to assess the bias potentially introduced through challenges with sampling and with missing data. The sampling risk was that not all attendees at the facilities were enrolled and among participants not all had a culture performed as some of the cultures and drug susceptibility testing were unsuccessful. Age-sex structures were assessed at each cascade of potential loss using routine laboratory surveillance data to assess representativeness. This included an assessment of those participants that were enrolled but whose sputa could not be tested, those tested but with a contaminated culture and those with failed drug susceptibility testing (DST).

Additionally, patterns of missing data in key variables were tabulated by cluster and province. These variables included: cluster, province, age, sex, previous treatment history and an assessment made on the randomness of the missing values. After performing these tasks, a consultation was held with technical support from the WHO and the US Centers for Disease Control and Prevention (CDC) and several different approaches discussed and evaluated before coming to a final determination of the most robust approach to be used to correct for any biases identified.

Multiple imputation was selected as an appropriate method and used to impute missing age, gender, previous treatment history, final culture status of those with contaminated cultures and DST results for failed susceptibility testing. Rifampicin and isoniazid were imputed individually to determine the final MDR status the same was done for ofloxacin and the class of second-line injectable agents to determine the XDR status.

Inverse probability weighting was applied post-imputation, using the variables age, gender and cluster, in order to address potential bias in enrolments. The numerator for these weights was composed of all culture-positive MTB cases detected in the DRS and all cases that were enrolled in the DRS but had untestable DRS samples yet were smear, culture or Xpert-positive for MTB through routine testing. The denominator consisted of all culture-positive MTB cases detected in the DRS.

The estimates were then tabulated for resistance among new and retreatment cases, as well as overall and compared to the individual level crude analysis and cluster level analysis for each province. These were then also compared during analysis using logistic regression with robust standard errors (RSE) prior to imputation, RSE with multiple imputation and RSE with multiple imputation and inverse probability weighting. The results showed consistency in the estimates with no significant difference in the methods applied. The final results presented are based on the model using both multiple imputation and inverse probability weighting as these factor in the potential bias mentioned previously.

In order to determine the national estimate for first and second-line resistance among TB cases the individual province estimates were pooled, and weighting was applied using the notification data for TB cases in each province in the year 2012, stratified by new and previously treated cases irrespective of smear result. Additionally for the national estimate of second line and XDR resistance estimates among MDR cases, the imputed provincial data for the second lines were pooled and weighted against the number of notified MDR cases on treatment by province in 2012.

Ethical considerations

Patients were invited to participate in the survey and those who volunteered were required to provide informed consent prior to enrolment. This was performed by healthcare workers at the DRS enrolling sites who had received training on completing the task.

As HIV is strongly associated with risk for TB, the need for HIV testing and reporting was considered important. Concerns however, arose that this may lead to a bias in enrolment if HIV testing was compulsory for participation. After discussions and approval from the ethics committees, it was decided to perform testing on all sputum samples for HIV with a voluntary option in the informed consent procedure on whether patients wish to receive the result or not.

In addition, and to ensure that patients were provided the best care, all patients enrolled in the survey were encouraged to have a routine HIV test performed and any participant that consented to receive the HIV test result, was referred for voluntary counseling on the advantages and implications of undergoing standard testing for HIV. The survey-specific HIV test was deemed suitable for surveillance purposes but not routine clinical management.

The survey received ethical approval from the University of Witwatersrand Research Ethics Committee on the 26/11/2010 (Ethics clearance No. M081022). Clearance was also received from the Centers for Disease Control and Prevention, Atlanta, USA. The survey was initiated after consultation and approval from the respective provinces and the National TB Control Programme.

Results

Demographic and laboratory characteristics

A total of 200 358 patients aged ≥18 years were screened and enrolled into the survey across 343 participating clusters. Of these, Limpopo province had the largest number of patients screened (31 503) and was also the province with the largest number of clusters (48). This was followed by Free State (26 288) and Northern Cape (23 107) provinces. The majority of participants nationally were female (55.2%) and the median age was 39 years (IQR: 30-51). The age and gender distribution is presented in Table 3. The proportion of females varied between 47.1% enrolled in the Western Cape to 59.8% in Limpopo province. The median age ranged from 35 years to 43 years, being lowest in KwaZulu-Natal and highest in Limpopo province respectively.

Table 3: Demographic characteristics of patients screened into the survey

Province	# DRS Clusters	Gender					Median Age in Years (IQR)
		Male	Female	Missing	Total	% Female	
Eastern Cape	32	8 522	10 585	242	19 349	**55.4%**	**39 (29-53)**
Free State	39	11 286	14 579	423	26 288	**56.4%**	**42 (31-53)**
Gauteng	38	8 895	10 877	329	20 101	**55.0%**	**38 (30-47)**
KwaZulu-Natal	31	8 699	11 459	218	20 376	**56.8%**	**35 (28-46)**
Limpopo	48	12 371	18 398	734	31 503	**59.8%**	**43 (32-56)**
Mpumalanga	38	9 185	12 434	120	21 739	**57.5%**	**39 (30-51)**
North West	35	8 716	10 535	338	19 589	**54.7%**	**40 (30-51)**
Northern Cape	47	11 217	11 644	246	23 107	**50.9%**	**41 (31-51)**
Western Cape	35	9 540	8 483	283	18 306	**47.1%**	**38 (29-48)**
South Africa	**343**	**88 431**	**108 994**	**2 933**	**200 358**	**55.2%**	**39 (30-51)**

Among 200 358 participants screened, a total of 101 422 (50.6%) were tested by mycobacterial culture (Table 4). *Mycobacterium tuberculosis* Complex was identified in 10 044 participants giving an overall culture positivity rate of 9.9% for MTB. The provinces with the highest number of MTB-positive cultures were Western (1 487) and Northern Cape (1 372) with all but two provinces having more than 1 000 MTB confirmed cases. The MTB culture positivity rate was lowest in the Free State at 6.4% while most provinces had a culture positivity rate close to the national average of 9.9%. History of prior TB treatment exposure varied across the nine provinces ranging between 14% and 35%. The provinces with the highest retreatment proportion among TB confirmed cases were in the Western (35%), Northern (28%) and Eastern Cape (27%) provinces. Nationally the proportion of prior treatment exposure was 22%. Further details are provided in Table 4.

Table 4: Participant enrolment cascade and previous treatment exposure

Province	# Screened	# Tested by Culture	# Culture Positive	# Culture Positive MTB	MTB Positivity among Tested (%)	Previous treatment history among TB cases (%)
Eastern Cape	19 349	8 548	1 123	1 033	**12.1%**	**27%**
Free State	26 288	14 079	1 155	907	**6.4%**	**21%**
Gauteng	20 101	11 188	1 423	1 123	**10.0%**	**18%**
KwaZulu-Natal	20 376	9 082	899	784	**8.6%**	**22%**
Limpopo	31 503	14 016	1 442	1 121	**8.0%**	**14%**
Mpumalanga	21 739	11 800	1 418	1 193	**10.1%**	**17%**
North West	19 589	10 344	1 370	1 024	**9.9%**	**20%**
Northern Cape	23 107	13 376	1 688	1 372	**10.3%**	**28%**
Western Cape	18 306	8 989	1 537	1 487	**16.5%**	**35%**
South Africa	**200 358**	**101 422**	**12 055**	**10 044**	**9.9%**	**22%**

The distribution of cases screened and confirmed to have MTB compared with the respective numbers expected by province is presented in Table 5. The number of presumptive TB cases required to be screened into the survey was above target for eight of the nine provinces and is in part due to the additional two months given to all provinces because of the low enrolments initially and to accommodate for the large number of patients screened that did not have a testable sample. Despite the increased number screened, nationally only 86.3% of the required number of MTB culture-positive cases were detected, with only two of the nine provinces exceeding the target. One of these was the Northern Cape (105.3%), which was the only province excluded from the previous survey because of poor enrolment.

Table 5: Numbers of screened and culture-positive MTB cases detected in the survey against those required by province

Province	# Screened	# Required to be Screened	% Screened of Target	# Culture positive MTB	# Required Culture positive MTB	% MTB of Target
Eastern Cape	19 349	16 512	117%	1 033	1 323	**78.1%**
Free State	26 288	20 160	130%	907	1291	**70.3%**
Gauteng	20 101	16 520	122%	1 123	1 323	**84.9%**
KwaZulu-Natal	20 376	16 704	122%	784	1337	**58.6%**
Limpopo	31 503	31 850	99%	1 121	1 274	**88.0%**
Mpumalanga	21 739	15 846	137%	1 193	1 269	**94.0%**
North West	19 589	18 650	105%	1 024	1 194	**85.8%**
Northern Cape	23 107	16 280	142%	1 372	1 303	**105.3%**
Western Cape	18 306	16 480	111%	1 487	1 320	**112.7%**
South Africa	**200 358**	**169 002**	**119%**	**10 044**	**11 634**	**86.3%**

Although the screening process in the North West province had to be repeated in the previous survey due to poor enrolments, in the current survey this province performed well and achieved an 85.8% response rate, despite a major mining strike in Marikana that disrupted services in certain parts of the province during the survey period. Additionally, one mining health facility which was included in the original sampling for the North West province, experienced difficulties due to lack of agreement between mine management and labourers, as well as other problems related to participation in the survey and after several attempts to include this site were unsuccessful, it was excluded from the survey. Another mine health facility included in the sampling for the Free State province completed enrolments for less than six months during the survey period, after problems requiring prolonged engagement had been resolved.

Among those with culture-confirmed TB included in the survey, the age grouping by province stratified by gender is shown in Figure 7 and Figure 8. The percentage of cases above 65 years of age was low across all provinces (1.1%-5.5%) in both genders while the percentage of individuals aged 44 years and below accounted for more than 70% of males in six of the nine provinces and all nine provinces among females. The proportion of patients in the 18-24 year group among females was also notably higher in all provinces compared to males. The proportion of female participants aged 18-24 years was above 10% in seven of the nine provinces while among males in the same age group this was true for only three of nine provinces.

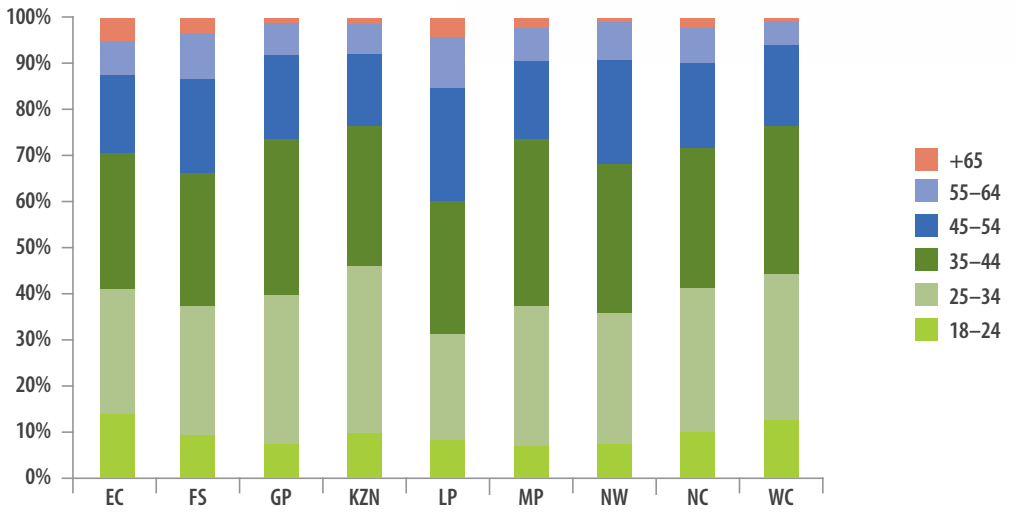


Figure 7: Age distribution among males by province among confirmed TB cases in the survey

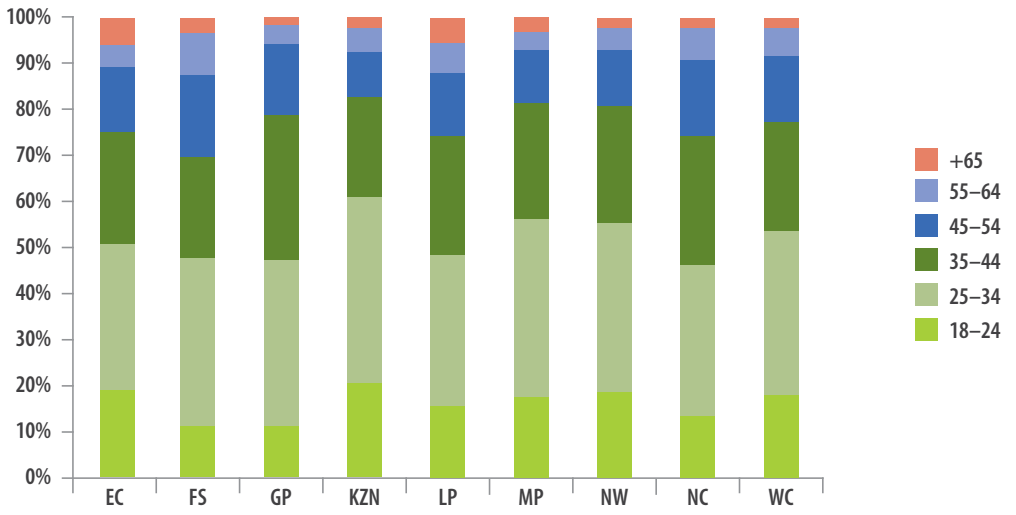


Figure 8: Age distribution of females by province among confirmed TB cases in the survey

A total of 12 056 cultures were positive for mycobacteria and of these 2 012 were identified as non-tuberculosis mycobacteria (NTM) (Table 6). The percentage of NTM among culture-positive cases nationally was 17% with the highest percentage in the North West province (25%) which is also the province associated with large mining activities. The lowest percentages were in the Western Cape (3%) and Eastern Cape (8%) provinces. Inland provinces generally had higher proportions of NTMs compared with the coastal provinces.

Table 6: Non-tuberculosis mycobacteria among culture-positive cases in the survey

Province	Culture Positive	MTB	NTM	% NTM
Eastern Cape	1 123	1 033	90	**8%**
Free State	1 155	907	248	**21%**
Gauteng	1 423	1 123	300	**21%**
KwaZulu-Natal	899	784	115	**13%**
Limpopo	1 443	1 121	322	**22%**
Mpumalanga	1 418	1 193	225	**16%**
North West	1 370	1 024	346	**25%**
Northern Cape	1 688	1 372	316	**19%**
Western Cape	1 537	1 487	50	**3%**
South Africa	**12 056**	**10 044**	**2 012**	**17%**

Among culture-positive TB cases, the smear positivity rate was 55% nationally, indicating that just less than half the number of cases were detectable only on culture. The lowest culture positivity rate was in the Free State province at 45% while the highest observed was in the North West province at 68%. Of the nine provinces, four had smear positivity rates below 50% among TB cases (Table 7).

Table 7: Smear positivity among culture-positive TB cases by province

Province	Smear Negative	Smear Positive	Smear Invalid	Total TB Culture Positive	% Smear Positivity
Eastern Cape	439	569	25	1 033	**56%**
Free State	472	390	45	907	**45%**
Gauteng	441	676	6	1 123	**61%**
KwaZulu-Natal	405	350	29	784	**46%**
Limpopo	467	639	15	1 121	**58%**
Mpumalanga	576	543	74	1 193	**49%**
North West	329	688	7	1 024	**68%**
Northern Cape	504	868	0	1 372	**63%**
Western Cape	737	700	50	1 487	**49%**
South Africa	**4 370**	**5 423**	**251**	**10 044**	**55%**

The HIV positivity rate among culture-confirmed cases nationally was 63.2% and varied by province (Table 8). Gauteng and Mpumalanga were the provinces with the highest HIV positivity rates at 74.6% and 76.8% respectively. The lowest rates were in the Western Cape and Northern Cape at 47.4% and 51.7% respectively.

Table 8: HIV co-infection among culture-confirmed TB cases by province

Province	Negative	Positive	Missing	Total	HIV Positivity
Eastern Cape	434	543	56	1 033	**55.6%**
Free State	244	578	85	907	**70.3%**
Gauteng	280	823	20	1 123	**74.6%**
KwaZulu-Natal	226	507	51	784	**69.2%**
Limpopo	394	688	39	1 121	**63.6%**
Mpumalanga	260	859	74	1 193	**76.8%**
North West	325	691	8	1 024	**68.0%**
Northern Cape	637	682	53	1 372	**51.7%**
Western Cape	684	617	186	1 487	**47.4%**
South Africa	**3 484**	**5 988**	**572**	**10 044**	**63.2%**

National first-line drug resistance estimates

The national estimates for first-line resistance are presented in Table 9. As expected, levels of MDR resistance and resistance to the individual drugs rifampicin and isoniazid were higher in previously treated cases compared with new cases in the current survey. The MDR resistance level was 2.1% (95% CI: 1.5%-2.7%) in new cases and 4.6% (95% CI: 3.2%-6.0%) in retreatment cases with an overall, MDR estimate of 2.8%; (95%CI: 2.0%-3.6%). Overall, the prevalence of rifampicin resistance (4.6%; 95% CI: 3.5%-5.7%) was higher than that of MDR (2.8%; 95%CI: 2.0%-3.6%). Of note was the level of rifampicin mono-resistance at 1.7% (95% CI: 1.1-2.2%), regardless of treatment history. The prevalence of any isoniazid resistance (9.3%; CI 95%: 7.9%-10.7%) was higher than that of any rifampicin resistance (4.6%; 95% CI: 3.5%-5.7%).

Among rifampicin and isoniazid mono-resistant cases, including those with resistance to other first-line drugs, no significant differences were observed in the prevalence of resistance between new and previously treated cases. The isoniazid mono-resistance levels were similar in new cases at 5.5% (95% CI: 4.6%-6.5%) while in previously treated cases it was 6.5% (95% CI: 5.1%-7.9%). The levels of resistance to the other first line agents in order of highest to lowest overall were: streptomycin (4.5%), pyrazanimide (3.7%) and ethambutol (2.5%). These were lower than the point estimate for rifampicin resistance. In each of these cases, the prevalence rates were higher among retreatment cases than in new cases.

Table 9: National first-line drug resistance estimates among TB cases, 2012-14 survey

2012-14	New (%, 95%CI)	Previously Treated (%, 95%CI)	Overall (%, 95%CI)
MDR	2.1 (1.5-2.7)	4.6 (3.2-6.0)	2.8 (2.0-3.6)
Any rifampicin	3.4 (2.5-4.3)*	7.1 (4.8-9.5)	4.6 (3.5-5.7)
Rifampicin mono†	1.4 (0.9-1.8)	2.5 (1.2-3.7)	1.7 (1.1-2.2)
Rifampicin mono (strict)[1]	0.9 (0.5-1.3)*	1.8 (0.7-2.9)	1.1 (0.6-1.7)*
Rifampicin mono (other) [2]	0.4 (0.1-0.7)*	0.7 (0.2-1.2)	0.5 (0.2-0.8)*
Any isoniazid††	7.6 (6.4-8.7)	11.1 (9.1-13.1)	9.3 (7.9-10.7)
Isoniazid mono	5.5 (4.6-6.5)	6.5 (5.1-7.9)	6.1 (5.1-7.1)
Isoniazid mono (strict) [1]	4.5 (3.6-5.3)*	5.5 (4.3-6.8)*	4.9 (4.1-5.8)*
Isoniazid mono (other) [2]	1.1 (0.3-1.8)	1.0 (0.4-1.6)	1.1 (0.4-1.7)
Ethambutol	2.0 (1.2-2.8)*	3.5 (2.2-4.8)	2.5 (1.7-3.3)*
Streptomycin	3.9 (2.8-5.1)	5.1 (3.8-6.5)*	4.5 (3.5-5.5)*
Pyrazinamide	2.9 (2.2-3.6)	5.2 (3.8-6.7)	3.7 (2.9-4.5)

† rifampicin-resistant & isoniazid susceptible

†† rifampicin susceptible & isoniazid resistant

[1] strict (without resistance to another first-line drug: streptomycin/ethambutol)

[2] other (with resistance to another first-line drug: streptomycin/ethambutol)

*non-overlapping 95% confidence intervals between 2012-14 and 2001-02

Table 10: National first-line drug resistance estimates among TB cases, 2001-2002 survey [14]

2001-2	New (%, 95%CI)	Previously Treated (%, 95%CI)	Overall (%, 95%CI)
MDR	1.6 (1.1-2.1)	6.6 (4.9-8.2)	2.9 (2.4-3.5)
Any rifampicin	1.8 (1.3-2.3)	7.5 (5.7-9.2)	3.4 (2.8-3.9)
Rifampicin mono (strict) [1]	0.2 (0.1-0.4)	0.8 (0.4-1.2)	0.4 (0.2-0.5)
Rifampicin mono (other)[2]	0	0.1 (0-0.4)	0.02 (0.0-0.1)
Any isoniazid	5.7 (4.9-6.5)	11.8 (9.3-14.4)	7.4 (6.5-8.3)
Isoniazid mono (strict) [1]	2.6 (2.0-3.2)	2.9 (1.9-4.0)	2.7 (2.2-3.2)
Isoniazid mono (other) [2]	1.5 (1.2-1.9)	2.3 (1.5-3.2)	1.7 (1.4-2.1)
Ethambutol	0.8 (0.4-1.1)	2.4 (1.5-3.3)	1.2 (0.8-1.6)
Streptomycin	4.3 (3.5-5.0)	8.1 (6.6-9.6)	5.3 (4.7-5.9)

[1] strict (without resistance to another first-line drug: streptomycin/ethambutol)

[2] other (with resistance to another first-line drug: streptomycin/ethambutol)

Provincial first-line drug resistance estimates

The MDR resistance levels by province are shown in Table 11. Mpumalanga province showed the highest overall MDR estimate at 5.1% (3.7%-7.0%); notably higher than four other provinces: Eastern Cape 2.1% (1.3-3.6%); Limpopo 1.6% (0.9%-2.9%), North West 2.6% (1.8%-3.9%) and Northern Cape 1.7% (1.0-2.8%). The point estimate among new cases was less than 2% in six of nine provinces and varied considerably among retreatment cases, ranging from 2.5% to 7.6%.

The level of any rifampicin resistance across all cases (Table 12) was consistently higher than the MDR levels for each province; and was almost double or more among previously treated cases in all provinces with Mpumalanga showing the highest level of rifampicin resistance. Rifampicin mono-resistance (RMR) overall had a point estimate ≥1% in all provinces (Table 13), being the lowest in the Eastern Cape (1.1%) and Western Cape (1.2%) and the highest in Mpumalanga (3%). Comparing the ratio of MDR to RMR estimates (Table 14), Limpopo had the lowest ratio at 0.7 implying that there were less MDR cases than RMR cases while for the Free State there was a 1:1 ratio of MDR cases to rifampicin mono-resistant cases.

Isoniazid resistance levels are shown in Table 15 and isoniazid mono-resistance levels in Table 16. The overall point estimates of isoniazid resistance for four of nine provinces was at or above 10%. The point estimates for IMR was greater than 5% in all provinces with the highest observed in Northern Cape (8.1%) followed by Free State (7.3%) and Western Cape (7.3%). There were no significant differences in resistance levels among new and previously treated cases and levels of resistance were close between the two populations.

Resistance levels to the other first-line drugs including ethambutol, streptomycin and pyrazinamide are shown in Tables 17-19, respectively. Resistance levels were variable with no differences in levels of resistance between new and retreatment cases for some drugs and some provinces. The province with the highest relative ethambutol resistance overall was the Free State (4%), with Gauteng having the highest prevalence of streptomycin resistance (5.3%). The pyrazinamide resistance level ranged between 2.2% and 5.3% and was the lowest in the Limpopo province (2.2%) and Western Cape (2.5%). Complete tables of first-line resistance profiles by individual provinces are provided in Appendix D. Additionally, RMR and IMR tables stratified by the presence or absence of resistance to the other first-line drugs streptomycin and ethambutol are provided in Appendix C.

Table 11: Provincial multi-drug resistance prevalence among TB cases

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	1.7	0.8	2.6	2.7	0.5	5	2.1	1.3	3.6
Free State	1.8	0.8	2.8	3.9	0.8	7	2.3	1.5	3.6
Gauteng	2.7	1.3	4.1	6.4	2.6	10.3	3.4	2.3	5.2
KwaZulu-Natal	1.8	0.6	3	6.4	2.3	10.4	2.9	1.8	4.5
Limpopo	1.4	0.4	2.4	2.5	0	5.1	1.6	0.9	2.9
Mpumalanga	4.2	2.8	5.6	7.6	3.2	12	5.1	3.7	7
North West	1.9	0.8	3.1	4.3	1.4	7.1	2.6	1.8	3.9
Northern Cape	1.3	0.4	2.1	2.6	0.8	4.3	1.7	1	2.8
Western Cape	2	0.7	3.2	4.5	2.1	7	3	2.1	4.2

Table 12: Provincial any rifampicin resistance prevalence among TB cases

Province	New Cases			Previously Treated Cases			Overall		
	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	2.7	1.5	3.9	4	1.5	6.5	3.3	2.2	4.9
Free State	3.5	2	5.1	7.3	2.5	12.1	4.6	3.2	6.6
Gauteng	3.6	2.1	5.2	9.3	4.8	13.8	4.8	3.4	6.8
KwaZulu-Natal	3.5	1.6	5.5	8.8	3	14.6	4.9	3.2	7.5
Limpopo	3.4	2	4.7	6.2	2.6	9.7	3.9	2.8	5.5
Mpumalanga	6	4.4	7.7	15.5	9.2	21.7	8.4	6.5	11
North West	3.1	1.5	4.6	9.7	5.9	13.4	4.9	3.6	6.8
Northern Cape	2	1.1	3	5	2.5	7.5	3	2.1	4.2
Western Cape	2.9	1.5	4.3	6.1	3.6	8.6	4.2	3.2	5.5

Table 13: Provincial rifampicin mono-resistance (isoniazid susceptible) prevalence among TB cases

Province	New Cases			Previously Treated Cases			Overall		
	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	1	0.3	1.7	1.2	0	2.4	1.1	0.6	2
Free State	1.8	0.4	3.1	3.4	0.5	6.3	2.2	1.2	3.9
Gauteng	1	0.3	1.6	2.8	0.3	5.3	1.3	0.8	2.2
KwaZulu-Natal	1.7	0.2	3.2	2.4	0	4.9	1.9	1	3.8
Limpopo	2	1.1	2.9	3.5	0.5	6.5	2.2	1.5	3.4
Mpumalanga	1.8	0.9	2.7	7.8	3.5	12.1	3	2	4.5
North West	1.1	0.2	2	5.3	2.8	7.9	2.2	1.4	3.5
Northern Cape	0.8	0.1	1.4	2.4	0.5	4.3	1.3	0.7	2.3
Western Cape	0.9	0.3	1.6	1.5	0.5	2.5	1.2	0.8	1.8

Table 14: Ratio of MDR: Rifampicin mono-resistance estimates among TB cases by province

Province	MDR: Rif Mono ratio		
	New Cases	Previously Treated Cases	Overall
Eastern Cape	1.7	2.3	1.9
Free State	1.0	1.1	1.0
Gauteng	2.7	2.3	2.6
KwaZulu-Natal	1.1	2.7	1.5
Limpopo	0.7	0.7	0.7
Mpumalanga	2.3	1.0	1.7
North West	1.7	0.8	1.2
Northern Cape	1.6	1.1	1.3
Western Cape	2.2	3.0	2.5

Table 15: Provincial isoniazid resistance prevalence among TB cases

Province	New Cases			Previously Treated Cases			Overall		
	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	7.1	4.9	9.3	10	6.1	13.9	8.9	6.6	12
Free State	8.8	6.4	11.1	10.1	5.2	14.9	10	7.8	12.9
Gauteng	7.5	5.4	9.5	12.8	7.3	18.3	9.2	7.2	11.7
KwaZulu-Natal	6.6	3.5	9.7	12.5	6.4	18.5	8.5	5.9	12.4
Limpopo	6.6	4.7	8.4	7.1	3.2	11	7.1	5.5	9.1
Mpumalanga	10.6	8	13.1	14.6	7.6	21.6	12.7	9.8	16.5
North West	7.7	6	9.5	9.4	5.6	13.2	8.9	7.2	11
Northern Cape	8.5	6.4	10.7	10.7	7.2	14.1	10.1	8.2	12.5
Western Cape	8.9	6.5	11.3	11.2	7	15.3	10.8	8.5	13.7

Table 16: Provincial isoniazid mono-resistance (rifampicin susceptible) prevalence among TB cases

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	5.4	3.3	7.5	7.2	4.1	10.3	6.4	4.6	9
Free State	7	4.9	9.1	6.1	2.2	10	7.3	5.6	9.6
Gauteng	4.8	3.3	6.3	6.3	2.7	10	5.3	4.1	6.9
KwaZulu-Natal	4.8	2.1	7.4	6	2.3	9.8	5.3	3.3	8.5
Limpopo	5.1	3.8	6.5	4.5	1.3	7.6	5.3	4.1	6.9
Mpumalanga	6.3	4	8.7	6.9	2.6	11.2	6.9	4.8	9.9
North West	5.8	4.3	7.2	5.1	2.1	8.1	6	4.6	7.7
Northern Cape	7.3	5.4	9.2	8.1	4.8	11.4	8.1	6.4	10.3
Western Cape	6.9	5.1	8.7	6.6	3.7	9.5	7.3	5.5	9.7

Table 17: Provincial ethambutol resistance prevalence among TB cases

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	2	0.2	3.7	2.2	0.1	4.2	2.1	1.2	3.9
Free State	3	1.5	4.5	6.2	1.4	11	4	2.7	5.9
Gauteng	1.9	0.6	3.2	4.8	1	8.5	2.4	1.4	4.1
KwaZulu-Natal	1.9	0.3	3.4	6	1.3	10.8	2.9	1.6	5
Limpopo	1.8	0.3	3.2	1.2	0	3.5	1.7	0.9	3.5
Mpumalanga	4.2	2.8	5.7	2.8	0	5.7	4.2	3	5.8
North West	1	0.2	1.9	1.8	0	4.3	1.3	0.6	2.8
Northern Cape	1.7	0.6	2.8	2.6	0.8	4.5	2.1	1.3	3.3
Western Cape	1.8	0.9	2.7	2.7	1	4.4	2.1	1.4	3.4

Table 18: Provincial streptomycin resistance prevalence among TB cases

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	2.6	1.3	4	6.1	3.4	8.8	4.1	2.8	5.9
Free State	3.3	1.4	5.2	4.1	0	8.3	3.7	2.3	5.9
Gauteng	5.3	3.7	6.9	5.3	1.4	9.1	5.6	4.2	7.5
KwaZulu-Natal	4.5	1.8	7.2	5.7	1.4	9.9	5	3	8.2
Limpopo	2.9	1.7	4	2.2	0	5.3	2.9	2	4.2
Mpumalanga	5.4	3.3	7.5	5.8	2.5	9.1	5.8	4.2	8.1
North West	2.1	1	3.2	4.9	1.5	8.3	2.9	1.9	4.5
Northern Cape	4.3	2.7	6	3.8	1.4	6.1	4.4	3.1	6.1
Western Cape	3.6	2.1	5.1	4	1.8	6.2	3.9	2.8	5.4

Table 19: Provincial pyrazinamide resistance prevalence among TB cases

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	3	1.3	4.8	4.2	1.4	7	3.6	2.4	5.5
Free State	2.8	1.4	4.1	2.9	0	6.1	2.9	1.9	4.6
Gauteng	4.5	2.6	6.5	7.4	2.3	12.5	5.3	3.6	7.8
KwaZulu-Natal	2.3	1	3.7	8.1	3.1	13	3.7	2.6	5.4
Limpopo	1.8	1	2.6	4	1	7.1	2.2	1.5	3.2
Mpumalanga	3.7	2	5.4	4.4	0.9	7.9	4	2.6	6.1
North West	3.6	1.8	5.3	5	1.6	8.4	4.1	2.7	6.1
Northern Cape	2.5	1.4	3.5	5.4	2.9	8	3.5	2.5	4.8
Western Cape	2	0.8	3.3	3.3	1.5	5.1	2.5	1.7	3.7

National second-line drug resistance estimates among TB cases

The prevalence of ofloxacin resistance was 1.4% (0.9%-1.8%) overall and was similar in new and previously treated cases (Table 20). Second-line injectable resistance was higher overall (3.9%; 95%CI: 2.8%-4.9%) than ofloxacin resistance and across all categories, but similar to the levels of streptomycin resistance (4.5%; 95% CI: 3.5%-5.5%); another injectable drug but classed as a first-line agent. The XDR resistance level overall among TB cases was 0.5% (0.2%-0.7%) nationally and was no different between new and previously treated cases.

Table 20: National Second Line Drug Resistance among TB Cases

Drug	New (%, 95%CI)	Previously treated (%, 95%CI)	Overall (%, 95%CI)
Ethionamide	3.8 (2.9-4.7)	4.6 (3.1-6.1)	4.3 (3.5-5.1)
P-aminosalicylic acid	2.2 (1.3-3.1)	2.4 (1.5-3.4)	2.4 (1.6-3.2)
Second-line injectable	3.4 (2.3-4.5)	4.6 (3.3-5.9)	3.9 (2.8-4.9)
Ofloxacin	1.2 (0.7-1.7)	1.5 (0.7-2.2)	1.4 (0.9-1.8)
XDR-TB	0.4 (0.2-0.7)	0.6 (0.2-1.0)	0.5 (0.2-0.7)

National second-line drug resistance estimates among MDR-TB cases

Second-line resistance among MDR cases is shown in Table 21. Ofloxacin resistance among MDR cases was 13.0% (5.0-21.0%) and was similar for second-line injectable resistance among MDR cases, 13.0% (5.0%-20.9%). The point estimate among MDR cases was the lowest for p-aminosalicylic acid (PAS) (5.3%), while the levels of resistance for ethionamide and pyrazinamide among MDR cases were higher than those of the second-line injectables and ofloxacin. The lowest bound of resistance was 25.9% for ethionamide and 49.0% for pyrazinamide among MDR cases. These high levels of resistance among MDR cases were also observed for streptomycin and ethambutol, which are first-line drugs but considered for use in MDR cases, with lower bounds of the resistance estimates at 52.8% and 30.2 % respectively.

Table 21: National second-line drug resistance among MDR cases

Drug	Overall (%, 95%CI)
Pyrazinamide	59.1 (49.0-69.1)
Ethambutol	44.1 (30.2-58.0)
Streptomycin	63.0 (52.8-73.2)
Ethionamide	44.7 (25.9-63.6)
P-aminosalicylic acid	5.3 (2.2-8.3)
Second-line injectable	13.0 (5.0-20.9)
Ofloxacin	13.0 (5.0-21.0)
XDR-TB	4.9 (1.0-8.8)

Cross-resistance among selected drugs

Isoniazid was tested at two concentrations and cross-resistance among isoniazid resistant strains at the higher concentration was 84% (Table 22). For the second-line injectable agents showing kanamycin resistance almost half of the strains showed susceptibility to capreomycin. Fluoroquinolones tested were ofloxacin and the moxifloxacin, a newer generation drug of the same class and showed that 71% of ofloxacin strains were also resistant to moxifloxacin tested at the epidemiological cut-off of 0.5ug/ml.

Table 22: Cross-resistance among selected drugs (crude data)

Drug	r	N	%
Isoniazid 0.1ug/ml	232	232	100%
Isoniazid 0.4ug/ml	196	232	84%
Kanamycin	27	27	100%
Amikacin	23	27	85%
Capreomycin	16	27	59%
Ofloxacin	21	21	100%
Moxifloxacin 0.5ug/ml	15	21	71%

Discussion

The South African TB Drug Resistance Survey, as part of an extended global endeavour of the WHO and the International Union against Tuberculosis and Lung Disease (IUATLD), was the largest of its kind conducted to date anywhere in the world, with over 100 000 persons suspected of suffering from TB tested. All nine provinces were represented in the survey and for the first time laboratory-based information on drug resistance survey data has become available for the Northern Cape province, which was excluded from the last survey. An important consideration of the current survey was to ensure that smear-negative TB cases which occur commonly in South Africa were included in the survey. Almost half of all TB cases in the survey were smear-negative and this finding correlates well with estimates of smear positivity in high HIV-prevalence settings. The TB-HIV co-infection rate was 63.2% nationally and is similar to the 61% reported in the WHO Global Report 2015, confirming the important role of HIV infection in the TB epidemic.

In the previous study the culture positivity rate in survey participants was almost 23.9% while in the current survey it was 9.9%. A concern in the previous survey was the high culture positivity rate that may have been associated with late presentation of presumed TB cases whereas in the current survey positivity rate was close to the 10% recommended by the WHO. This is a positive finding suggesting that the efforts aimed at community awareness have yielded results and more persons are presenting earlier than before for TB investigations.

The number of presumptive cases screened into the survey was higher than targeted and this was a result of the two month extension provided to all provinces to overcome losses encountered during the survey and to ensure that sufficient numbers of culture-positive MTB cases were detected to achieve the required power for the survey. The power calculation was based on the lowest point estimate of MDR-TB cases amongst provinces included in the previous survey of 0.9%; however, in the current survey the lowest provincial point estimate was 1.4%, resulting in robust estimates being made even for the seven provinces that did not achieve the expected number of culture-positive MTB cases. Both first-line and second-line drug resistance estimates were determined for this survey. Determination of resistance to the second-line drugs has become increasingly relevant as new drugs are being introduced and new regimens formulated.

MDR-TB prevalence

The national prevalence of MDR-TB has remained relatively unchanged over a period of more than ten years between the two surveys: The overall MDR-TB rate was 2.9% (95% CI: 2.4%-3.5%) in 2001-2 (Table 10) and was determined at 2.8% (95% CI: 2.0%-3.6%) in the current survey (Table 9). Among new cases the MDR-TB prevalence is 2.1% (95% CI: 1.5%-2.7%) comparable with the global WHO estimate[1] of 3.3% (95% CI: 2.2%-4.4%). The prevalence of MDR-TB in previously treated cases, at 4.6% (95% CI: 3.2%-6.0%) was however lower than the global average of 20% (95% CI: 14%-27%). This may be due to the high mortality rate in the local setting among this sub-group or possibly the impact of the introduction of diagnostics for the early detection of drug-resistant TB (e.g. LPA and GXP) that may have resulted in cases within this higher risk group being effectively treated and cured.

Provincial MDR-TB prevalence rates have varied with the highest observed in Mpumalanga province with an overall rate of 5.1% (95% CI: 3.7%-7.0%) which was higher than the national rate (2.8%; 95% CI: 2.0%-3.6%). In the previous survey, Mpumalanga also showed the highest MDR-TB rate. There were no significant differences observed in MDR-TB rates between the other provinces in the current survey and point estimates ranged from 1.8% (Limpopo and Northern Cape) to 3.2% (Gauteng). Compared with the provincial MDR-TB prevalence in the previous survey, there were no major differences among new cases; however among retreatment cases greater variability was noted in the estimates for the previous survey (Table 23).

Concerns about the high MDR-TB rates in Mpumalanga were noted in the previous survey and again feature prominently in the present survey. Several potential contributory factors are at play, one of which is cross-border migration: Swaziland, an immediate neighbour has the highest MDR-TB rate in the region with estimates from 2009[31] of 7.7% (95% CI: 4.9%-10.5%) among new cases which, although higher, was not significantly different from those of Mpumalanga (4.2%; 95% CI: 2.8%-5.6%). Sub-analysis (data not presented) has shown higher MDR-TB rates in facilities selected in the DRS that were closer to the Swaziland border than those elsewhere in Mpumalanga. Thus regional efforts and co-ordination are required and are essential in dealing with this emerging problem.

Gold mining in the province has also expanded in the recent past, adding to the risk of TB transmission and will need to be closely monitored. Mpumalanga is also one of the provinces in South Africa where HIV prevalence has been above the national average and the Gert Sibande district in particular has one of the highest rates nationally[32]. Compounded to the existent problems are the high levels of poverty and general deficiencies in health system activities. A comprehensive multi-sectorial intervention aimed at dealing with the issues is required.

Rifampicin and isoniazid resistance

For any rifampicin and any isoniazid resistance, regardless of other drug susceptibility findings, increases in point estimates were observed between the previous and current surveys but these were not significant. Significant increases were, however, observed specifically in new cases when the previous and current surveys are compared with any rifampicin resistance almost doubling from 1.8% (95% CI: 1.3%-2.3%) to 3.4% (95% CI: 2.5%-4.3%; see Table 24). When assessed provincially, the same pattern holds with increases in the point estimate seen across all provinces among new rather than previously treated cases, although significance was not shown at this level. Increases among new cases are indicative of primary resistance driven by transmission and this is concerning as South Africa has one of the highest rates of people living with HIV in the world and thus also has high numbers of persons at an increased risk for TB infection.

The introduction of Xpert MTB/RIF as a primary diagnostic tool targeting all presumptive TB cases in South Africa[33], has the added advantage of not only detecting TB but also rifampicin resistance. Widespread adoption could lead to early diagnosis of primary drug-resistant cases which would be missed if only retreatment cases were tested. Ensuring that these technologies are effectively used and acted upon is thus an important target for improving TB control efforts. Although the methodology used for susceptibility testing was the WHO-approved MGIT system, recent data has emerged indicating that MGIT testing may record false susceptible findings in strains harbouring specific *rpoB* mutations[34]; thus the estimates for rifampicin resistance may be underestimated in the survey but would be detected routinely with the currently used molecular diagnostic methods.

Rifampicin mono-resistance

The prevalence of RMR-TB has shown significant increases from the previous survey, notably among new cases and raises similar issues regarding transmission. Although the point estimate overall is above 1% nationally, this phenomenon was noted in the previous survey and has continued to increase. A study from KZN[35] has also noted a higher rate of RMR-TB to what is generally encountered and younger patients (25-29y) were at increased risk. This correlates with the notable increase in new cases we have observed as patients 25-29 years of age are less likely to have had a second episode by this stage of life.

It is unusual for resistance to a single drug to emerge when treatment for TB comprises standardised combination therapy. A study from France[36] showed that patients who were either HIV- infected or abused alcohol were at an increased risk for RMR. Adequate dosage levels are critical and concerns have been raised about the current dosing used for rifampicin being too low[37]. This is further compounded in patients who use alcohol or who are on ART during TB therapy, as these could also affect the bioavailability of the drug. A survey from another African setting in Burundi has also recorded the RMR phenomenon[38] and noted that irregular drug intake could also lead to inadequate therapeutic levels. A study including patients from South Africa has shown the use of a higher dose for rifampicin improved culture conversion without a significant increase in adverse events[39]. Furthermore, a smaller study from Japan[40] has highlighted the clonality of strains with RMR in their treatment environment. Such a situation is plausible in our setting since the biggest increase in RMR-TB, has been noted in new cases nationally, suggesting transmission. Although such strains may have originated through the selection of rifampicin resistance during treatment in higher risk patients, transmission will further increase its occurrence.

The increase in RMR-TB is a concern; however, it does leave an effective oral first-line drug isoniazid, available for treatment. Furthermore, the predictive value of rifampicin as a marker of MDR-TB is diminished with wide variability amongst provinces. For the Western Cape the ratio of MDR to RMR cases was 2.5, suggesting that for every RMR case there were 2-3 times as many MDR cases, while for several provinces the ratio was equal or close to one, suggesting equal distribution of the two rifampicin resistance profiles. However, Limpopo province was unusual in having a ratio below one, indicating a higher number of rifampicin mono-resistant than MDR cases. The loss of rifampicin, a potent sterilising drug, is significant, requiring prolonged courses of treatment for cure; however the inclusion of isoniazid is warranted in all rifampicin-resistant cases pending the result of drug susceptibility testing for isoniazid.

Isoniazid mono-resistance

Significant increases have also been noted for isoniazid mono-resistance (IMR) without any other first-line drug resistance, shifting from 2.7% (95% CI: 2.2%-3.2%) in the previous survey to 4.9% (95% CI: 4.1%-5.8%) in the current survey, irrespective of previous treatment history. There was no significant difference by treatment history in the current and previous surveys, suggesting that prior TB combination therapy is not likely to be a contributory factor. The point estimate of IMR, irrespective of resistance to other first-line drugs, was above 5% in all provinces. Comparing the IMR prevalence between the two surveys by province (Table 25), increases in the point estimate can be observed across all provinces, indicating that the increase observed nationally is not being driven by any particular province.

The occurrence of IMR has been observed across many settings in the world and has been recorded by Menzies and colleagues in a meta-analysis to account for almost half of all TB drug resistance[41]. A study from Iran[42] has observed significant increases of IMR prevalence over time, from 4.4% in 2003 to 9.4% in 2011 while a study from the USA[43] has shown that the prevalence IMR has remained

unchanged despite declines observed for RMR and MDR in their setting. Among countries neighbouring South Africa whose data were reported in peer-reviewed publications, IMR levels in new cases, irrespective of additional resistance to streptomycin and ethambutol have been 5.1% in Swaziland[44], 4.2% in Mozambique[45] and 5.1% in Botswana[46].

Risk factors for IMR include prior TB therapy and IPT reported by Catamanchi *et al.*[47], while in another study, younger age groups were identified as being at increased risk[48]. A study by Hazbon and colleagues[49] has analysed the genetic basis of isoniazid resistance, comparing IMR with MDR and noted a significant difference with much higher frequency of *inhA* mutations in IMR; a mutation conferring low-level isoniazid resistance. Thus prior exposure to isoniazid treatment, especially with inconsistent use, could be a contributory factor to the emergence of IMR.

The outcomes from a study in the USA, comparing treatment of IMR patients with that of matched fully susceptible cases were not different, although for the former group of patients, approximately half had received more than six months of therapy. A meta-analysis has highlighted the considerable lack of evidence on therapeutic regimens for IMR cases while failure rates ranged between 18% and 44%. Improved outcomes were associated with longer duration of rifampicin therapy, daily therapy and treatment with a greater number of effective drugs. Furthermore, the authors noted that none of the studies included HIV co-infected individuals. Another study[50] has shown higher cure rates when a fluoroquinolone was included in the regimen used for treatment of IMR cases.

An association between IPT and IMR or other drug resistance has not been shown based on a WHO-initiated review of published data[51]. However, a model-based study on community-administered IPT[52], has suggested that this is likely to occur at a population level and could be missed when analysing studies involving small numbers of patients. A presentation at a local HIV conference has summarised data from studies in South Africa asking the question "Is IPT a priority for SA?" and highlights the lack of evidence for IPT benefit and emphasised the increased risk for resistance based on local data[53]. Although the increased use of isoniazid mono-therapy in IPT is specifically indicated for person/people living with HIV (PLHIV) without active TB. Identification of the latter however, is not always conclusive, especially in HIV patients for whom screening based on absence of symptoms suggestive of TB is applied. Further studies to investigate the relationship between IPT and IMR at a population level are warranted.

The occurrence of IMR could also potentially have implications for the effectiveness of isoniazid preventive therapy as latent organisms are likely to share the same resistance profile as active TB, being its progenitor. A study using whole genome sequencing[54] has demonstrated the ability of organisms of latent TB infection (LTBI) to 'develop' resistance mutations at the same rate as the organisms of active TB and that LTBI is also affected by environmental pressure. Although this is a new concept, and requires further investigation, it may change our understanding and management of LTBI in the future.

The increase in IMR observed in the current survey compounds the risk of MDR-TB over time, as undetected cases would be effectively receiving rifampicin mono-therapy in the continuation phase of standard therapy, leading to the development of further drug resistance. A study from China has demonstrated the effect of IMR in driving increase in MDR over time in rural communities[55]. Although MDR-TB rates have remained relatively stable, this could change if IMR is not effectively managed. The use of Xpert MTB/RIF as the first-line diagnostic test has been invaluable in detecting rifampicin-resistant (RR) and MDR but does not test for isoniazid resistance and raises the need to consider additional testing for IMR or alternatively, strengthening the current empiric regimen. This would require further studies to advise on the most appropriate approach.

Other first-line resistance

One of the primary goals of multidrug regimens is to reduce the emergence of drug resistance and this requires treatment with a combination of drugs to which current strains of MTB have been shown to be susceptible at a population level. The current survey has shown an increase in individual first-line drug resistance, relative to the previous survey, for not only isoniazid and rifampicin, but also ethambutol with overall rates in the current survey of 9.3%, 4.9% and 2.5%, respectively. The present rate of pyrazinamide resistance which was not assessed in the previous survey is estimated at 3.7%. However, pyrazinamide phenotypic resistance testing is known to overestimate resistance[56] and the true rate is likely to be lower. Thus three of the four drugs used for intensive phase treatment have rates of resistance below 5% and use of the four-drug regimen is likely to continue to be effective. Rational treatment strategies could be enhanced by using Xpert MTB/RIF as a primary test and treating only rifampicin-susceptible cases with the current regimen, since a fair proportion of other first-line drug resistance is associated with rifampicin resistance.

Attempts at reducing the emergence of resistance are not limited to providing effective combination therapy but require high levels of adherence to treatment and the reduction of undetected or untreated cases. These factors remain a concern as pre-treatment loss to follow up rates above 20% have been reported [13,57] and could be amplified in a high-risk HIV endemic setting prevailing in South Africa.

Estimating the burden of RR/MDR-TB

During 2014, 332 783 microbiologically confirmed TB cases were reported through the laboratory system. Applying the point estimate and 95% confidence intervals for RR TB cases from this survey, the expected number of RR cases would be 15 308 (11 647-18 969). This correlates with the reported RR cases for that year, being 18 734; however, the RR/MDR numbers include both PTB and extra-pulmonary TB (EPTB) drug-resistant cases and thus would be lower if restricted to PTB cases. Additionally, the current estimates are based on the MGIT methodology which may underestimate rifampicin resistance compared to Xpert MTB/RIF and other molecular assays. For the same year, a total of 272 078 cases were reported as having started on TB treatment. Applying the point estimate and confidence intervals for RR TB cases from this survey, the expected number of RR cases on treatment would be 12 516 (9 523–15 508). In the same year, 11538 RR/MDR cases were reported to have started on treatment. It is reassuring that there is no gross under or overdetection of treatment cases, however the range between the lowest and highest estimates across the two sources is quite wide.

Further investigation is warranted to identify reasons for the difference and to ensure that all diagnosed cases are started on treatment. In the WHO Global TB Report 2015, the trends in the number of cases diagnosed and on treatment are shown for the 27 high-burden countries globally, and included South Africa. The pattern observed in South Africa is not much different to global trends or those in many high-burden countries. Rates of loss to follow up are expected to be higher in drug-resistant TB than in drug-susceptible TB cases, due to a variety of factors, including duration of treatment, use of injectable agents and poor outcomes. New drugs and shorter, patient-friendly treatment regimens associated with good patient outcomes are urgently needed. The increased availability of new diagnostics needs to be paralleled with acceleration in new drug discovery and clinical trials for the evaluation of new treatment regimens.

Fluoroquinolone and pyrazinamide resistance among TB cases

Evidence from early bactericidal studies evaluating different combinations of drugs has shown good outcomes when new agents were combined with fluoroquinolones and pyrazinamide[58]. The present survey records relatively low resistance rates for both these drugs, with fluoroquinolone resistance overall at 1.4% (95% CI: 0.9%-1.8%), using ofloxacin as an indicator of fluoroquinolone resistance. This finding is encouraging and supports the use of this class of drugs in new regimens for TB treatment. When assessing cross-resistance among ofloxacin-resistant strains, 71.2% were susceptible to moxifloxacin tested at 0.5ug/ml – the tentative epidemiological 'breakpoint' concentration for this TB treatment drug. However, this breakpoint concentration has been disputed by some, suggesting that it should be lowered to 0.25ug/ml, though resistance estimates at this concentration would still likely to be below that for ofloxacin. The breakpoint concentration currently suggested for clinical management is 2.0ug/ml and is similar to that of ofloxacin. Resistance estimates would be considerably lower if tested at this much higher concentration.

Pyrazinamide resistance showed an overall national rate of 3.7% (2.9%-4.5%), however based on sequencing of strains for Gauteng and KwaZulu-Natal conducted as part of a multi-country study, resistance rates were 3.1% and 3.9% respectively for the two provinces, compared with 5.3% and 3.7% respectively using phenotypic methods (publication under review). Sequencing of the *pncA* gene is emerging as the preferred standard test for pyrazinamide resistance[59]. The higher rates by phenotype in Gauteng relate to this province being the first to enrol in the current national DRS and pyrazinamide resistance reconfirmation was not done in the first few months of the survey, as data only emerged later on high false resistance being recorded on phenotypic testing. The KwaZulu-Natal MGIT-based estimates for pyrazinamide resistance are much closer to the sequencing estimates and retesting using conventional MGIT-based methodology was fully instituted by the time of this provinces' enrolment.

Second-line drug resistance levels among MDR cases

The outcomes among RR/MDR cases have been shown to be very poor and even worse for XDR-TB cases. On assessing the extent of resistance to currently used drugs among MDR-TB cases, it is clear that, although the frequency of resistance to second-line drugs and specifically fluoroquinolones is relatively low at 13% for the latter, high rates of resistance to the companion drugs prevail. The ethionamide resistance rate is 44.7% (25.9%-63.6%) and even using the lower limit of the 95% confidence interval, isolates from one in four empirically treated MDR-TB patients would be resistant to ethionamide. This estimate holds true as MDR-TB cases are by definition isoniazid-resistant and mutations in the *inhA* promoter region, accounting for approximately 8-43% of isoniazid resistant strains[60] would confer cross-resistance to ethionamide. Furthermore, ethionamide resistance can only partially be attributed to *inhA* mutations. Pyrazinamide, another drug used in drug resistance regimens is known to have potent sterilising activity but very high resistance rates of between 50% and 70% have been recorded for this drug. This is corroborated by a recent study which analysed the prevalence of PZA resistance among MDR strains from the previous survey and found 52.1% to be PZA resistant[61]. Similarly, studies both within South Africa and elsewhere have shown a high prevalence of pyrazinamide resistance among MDR TB cases[59,62-64]. With these high rates of resistance, further selection of resistance and consequently poor patient outcomes are likely to occur.

Ideally, a new treatment regimen would be required, but the challenge facing DR-TB programmes is the limited number of drugs available to deal with M/XDR-TB cases. A pragmatic approach would be the use of available drugs to which the causative strain has been shown to be susceptible and targeting a tailored therapeutic regimen at least in MDR-TB cases but this would require rapid susceptibility testing of the isolates to second-line drugs. One drug to which very low levels of resistance have been encountered has been p-aminosalicylic acid (PAS) which provides a good option, although tolerability concerns associated with this drug have limited its use.

Second-line injectable agents, kanamycin, amikacin and capreomycin, remain a cornerstone of the treatment for drug-resistant cases and the estimate of 13.0% (95% CI: 5.0%-20.9%) was based on resistance to any one of the three injectable agents; thus, despite considerable cross-resistance, resistance to individual drugs would be somewhat lower. When confronted with an MDR-TB patient with kanamycin resistance and thus by definition a pre-XDR TB case, cross-resistance would be lower for amikacin (85%) and lowest for capreomycin (59%). This is a useful management option but would only be relevant for such cases. However, treatment of these cases would be most suited to novel regimens as adding individual drugs to the regimens of these cases could lead to acquisition of resistance to the added drug, taking into account the high levels of resistance observed for the other drugs.

XDR-TB estimates

The XDR-TB estimate among MDR-TB cases in this survey of 4.9% (95% CI: 1.0%-8.8%) was lower than that reported globally at 9.7% (7.4%-12%) but the difference was not statistically significant. This suggests that the XDR-TB problem that has seen two outbreaks during the period between the two surveys has not become widespread across the country. A contributory factor could have been the high mortality associated with these cases. In addition, XDR-TB cases may have been confined to certain provinces or districts and this survey may not have been powered to assess the distribution of such cases at the required level of detail.

Limitations

The survey has produced important findings which will need to be discussed and will additionally require the development of a plan for implementation of recommendations. However, the survey had certain limitations and the findings should be seen in context. The recording of previous treatment history is prone to recall bias; however the retreatment rates reported here are comparable to what was observed in the previous survey. Additionally, the national estimate generated in the previous survey excluded the Northern Cape province; however, when the analysis for the current survey was repeated without this province there were no discernable differences. This is expected since this province accounts for only 2.2% of the South African population[65] and has a low burden of MDR-TB. Furthermore, comparing two time points does not necessarily allow a trend to be assessed and the time between the surveys has been just over ten years. Thus the situation could have been worse around the time when the Tugela Ferry outbreak occurred and could have recovered to the present levels, or the trajectory could still be increasing. Further analysis of routine surveillance data is underway to elucidate these questions.

There were also three specific issues that are relevant to the current survey and the analysis. First, large losses of screened candidates occurred due to no or low-specimen volumes, despite the interventions that were applied. The losses were however widespread with no discernable concentration in a specific geographic area and were therefore likely to be random. Low-volume specimens can be expected in an HIV endemic setting and this has been documented previously[2,66]. Additionally, TB DRS surveys are based on microbiologically confirmed TB cases and thus would inherently exclude cases that could not be tested.

Second, while the study design required consecutive recruitment of presumptive TB cases, it also required informed consent, which to some extent depended on the level of engagement of the local TB nurse. Therefore enrolment was not consistent and could potentially have introduced bias if recruiting nurses were to be more insistent to recruit patients that they thought were more likely to have TB. However this is unlikely to have occurred as the study population included presumptive TB cases, and predicting TB in patients would be difficult, even for experienced clinicians. The survey included smear-negative cases and thus ensured that HIV patients would be included. Furthermore, these concerns have been addressed by imputing and applying inverse probability weighting. The estimates were however similar to the unadjusted but we have reported on the adjusted data.

Third, DST, especially for second-line drugs, is often less reliable; however the survey was conducted following established procedures at an ISO-accredited reference laboratory which is part of the WHO supranational reference laboratory network and showed good performance in the external quality assurance (EQA) programme for both first-line and second-line drug testing. In addition and where available, sequencing performed on strains was used to cross-check resistance profiles and correlate findings with those published in the literature.

Table 23: Provincial MDR prevalence among TB cases in the 2012-14 and 2001-2 surveys

	DRS 2012/14						DRS 2001/02					
	New Cases			Previously Treated Cases			New Cases			Previously Treated Cases		
Province	%	95% CI		%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	1.7	0.8	2.6	2.7	0.5	5	1	0	2.3	7.4	4.7	11.1
Free State	1.8	0.8	2.8	3.9	0.8	7	1.7	0	3.4	1.7	0	5
Gauteng	2.7	1.3	4.1	6.4	2.6	10.3	1.4	0.1	2.6	5.4	2.5	10.1
KwaZulu-Natal	1.8	0.6	3	6.4	2.3	10.4	1.7	0.8	3.1	7.7	4.5	12.2
Limpopo	1.4	0.4	2.4	2.5	0	5.1	2.4	1.2	4.3	6.8	2.5	14.3
Mpumalanga	4.2	2.8	5.6	7.6	3.2	12	2.7	1.5	4	13.7	9	19.7
North West	1.9	0.8	3.1	4.3	1.4	7.1	2.3	1.2	3.7	6.9	3.7	11.5
Northern Cape	1.3	0.4	2.1	2.6	0.8	4.3	N/A	N/A		N/A	N/A	
Western Cape	2	0.7	3.2	4.5	2.1	7	0.9	0	2.4	3.9	1.8	7.3

Table 24: Provincial rifampicin resistance prevalence among TB cases in the 2012-14 and 2001-2 surveys

	DRS 2012/14						DRS 2001/02					
	New Cases			Previously Treated Cases			New Cases			Previously Treated Cases		
Province	%	95% CI		%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	2.7	1.5	3.9	4	1.5	6.5	1.2	1.2	3.7	7.8	4.1	13.8
Free State	3.5	2	5.1	7.3	2.5	12.1	2.4	0.9	5.6	2.9	0.6	9.7
Gauteng	3.6	2.1	5.2	9.3	4.8	13.8	1.7	0.6	4.1	6.1	2.3	14.2
KwaZulu-Natal	3.5	1.6	5.5	8.8	3	14.6	1.8	0.7	4.3	8.7	4.3	16.3
Limpopo	3.4	2	4.7	6.2	2.6	9.7	2.4	1	5.7	10.2	3.6	24
Mpumalanga	6	4.4	7.7	15.5	9.2	21.7	3.1	1.7	5.7	16	9.3	25.7
North West	3.1	1.5	4.6	9.7	5.9	13.4	2.7	1.3	5.3	9.6	4.7	17.8
Northern Cape	2	1.1	3	5	2.5	7.5	N/A	N/A		N/A	N/A	
Western Cape	2.9	1.5	4.3	6.1	3.6	8.6	0.9	0.2	3.7	3.9	1.4	9.9

Table 25: Provincial isoniazid mono-resistance without additional ethambutol/streptomycin resistance prevalence among TB cases in the 2012-14 and 2001-2 surveys

	DRS 2012/14						DRS 2001/02					
	New Cases			Previously Treated Cases			New Cases			Previously Treated Cases		
Province	%	95% CI		%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	4.7	2.7	6.8	5.2	2.4	8	3.8	1.9	7.1	3.2	1.1	8
Free State	5.7	3.6	7.7	5.4	1.7	9.1	3.3	1.5	6.8	4	1.2	11.2
Gauteng	3.8	2.6	5	5.6	2.2	9	1.9	0.7	4.4	0.6	0	6.6
KwaZulu-Natal	3.3	1.8	4.8	5.2	1.6	8.7	2.5	1.1	5.2	4.3	1.5	10.8
Limpopo	4.4	3.2	5.7	4	0.8	7.3	1.3	0.3	4.2	4.5	0.8	16.7
Mpumalanga	5.6	3.6	7.5	6.9	2.6	11.2	3.1	1.7	5.7	2.3	0.4	8.8
North West	5.2	3.7	6.6	4.8	1.8	7.7	2.2	1	4.7	0.5	0.5	5.8
Northern Cape	6.3	4.6	8.1	7.6	4.4	10.9	N/A	N/A		N/A	N/A	
Western Cape	5.8	4.1	7.4	6.3	3.5	9	2.6	1	6	2.2	0.5	7.5

Conclusion

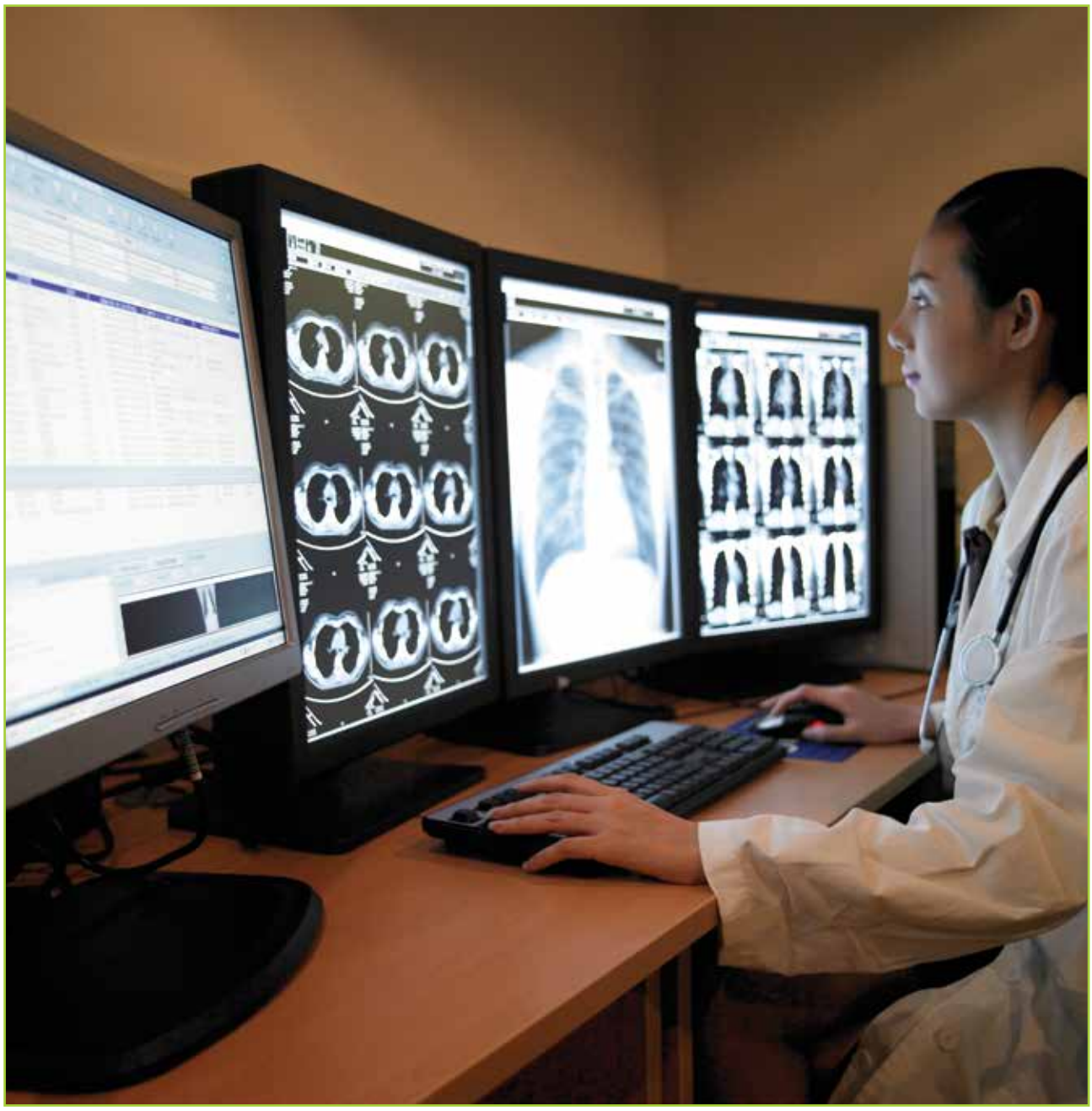
South Africa has experienced a stable MDR-TB epidemic spanning a ten-year period; however, resistance to individual drugs is on the increase. The increase of rifampicin mono-resistance is concerning and increases primarily among new cases are suggestive of transmission; however, underlying reasons for its occurrence may relate to sub-optimal dosing of rifampicin, the bioavailability of rifampicin being affected by drug interactions, as well as intermittent compliance with treatment. The increased occurrence of isoniazid mono-resistance is another concern and would be missed with the current diagnostic algorithm. Although its impact on patient outcomes is poorly defined, isoniazid mono-resistance could potentially impact MDR-TB levels in the future as undetected cases may effectively continue to receive rifampicin mono-therapy. The province of greatest concern is Mpumalanga with higher MDR-TB rates than the national average which was also observed in the previous survey.

Rates of resistance to fluoroquinolones and pyrazinamide, both considered companion drugs for new regimens for TB treatment have shown to be low among TB cases, rendering these regimens suitable for implementation within South Africa. Contrasted with this are the high rates of resistance to ethionamide and pyrazinamide among MDR–TB cases, which may be contributory factors to the poor outcomes seen in these cases. XDR-TB rates nationally were below 5% among MDR-TB cases and lower than the global average, indicating that the problem is not widespread across the country.

Recommendations

- Urgent implementation of interventions in Mpumalanga
  - › Identify potential risk factors for targeted interventions
  - › Improve cross-border co-operation with Swaziland and Mozambique, utilising existing agreements achieved through the SADC declaration
  - › Conduct further research to fully define drivers of resistance in the province
- Develop interventions to curb IMR and its secondary effects
  - › Strengthen current first-line regimen for continuation phase by adding ethambutol with or without pyrazinamide(RHE or RHZE), or institute appropriate measures for early identification of IMR
  - › Assess the contribution and effectiveness of IPT in the light of increasing cases of resistance
- Monitor transmission of RMR, research underlying reasons for RMR and institute appropriate interventions
  - › Regularly review transmission data from surveillance system
  - › Review current rifampicin dosing and conduct rifampicin bioavailability studies in the four and two-drug combination with and without antiretroviral therapies (ARTs) in areas with high RMR occurrence
  - › Undertake close monitoring of the quality of drugs used in the standard regimen
- Conduct randomised control trials (RCTs) and review existing standard of care data to assess effectiveness of existing first and second-line regimens
- Monitor use of Xpert MTB/Rif assay for early detection of rifampicin resistance and improve early detection of second-line drug resistance
- Optimise existing MDR regimen and consider shortening MDR regimen with triage algorithm for appropriate patient selection
- Design an appropriate regimen for pre-XDR/XDR patients using a combination of new drugs
- Maintain and enhance the routine surveillance system for monitoring existing and new drug resistance and reduce the proportion of diagnosed cases not started on treatment.

Appendixes



Appendix A: Case Report Form

NORTHERN CAPE

SA National TB Drug Resistance Survey **Questionnaire**

Lab Request Form Bar-code

SECTION 1: Patient Demographics (please complete in writing or ✓ as appropriate)

Date D D / M M / Y Y Y Y

District NAMAQUA

Name of clinic/hospital POFFADER CLINIC

Patient's clinic/hospital no.

Surname

First Name

Other Name

Please indicate time of specimen collection (✓): 08:00 | 09:00 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 15:00 | 16:00 | 17:00

Gender: M F

Date of Birth (DOB) D D / M M / Y Y Y Y or Age if DOB is unavailable (in years)

Country of residence for last 6 months SA Other Specify

How would you describe yourself in terms of population group? Black | White | Indian | Coloured Other:

Has patient consented to participate in the DRS? Yes No **If no, return questionnaire to Centre for Tuberculosis**

SECTION 2: Screening Clinical Information

2.1 Exclusion Criteria

Patient is less than 18 years old Yes No

Patient is currently on TB treatment Yes No **If any of these questions are answered Yes, please exclude patient from the survey**

Patient has already been included for DRS at another clinic Yes No

2.2 Inclusion Criteria

Does patient present with the following symptom:

Persistent cough for more than 2 weeks Yes No

OR a combination of at least two of the symptoms listed below:

Fever for more than 2 weeks Yes No

Drenching night sweats Yes No

Loss of appetite Yes No

Unexplained weight loss (more than 1.5kg /month) Yes No

A general feeling of illness (malaise) and tiredness Yes No

Shortness of breath and chest pain Yes No

If patient has cough for more than 2 weeks OR a combination of any 2 of the other symptoms, then classify as a TB suspect.

Does patient qualify as a TB suspect? Yes No

If yes, continue with questionnaire. If no, consider other diagnosis.

2.3 Previous TB History

Have you ever been previously diagnosed with TB? Yes No

If yes, have you ever been previously treated for TB? Yes No

If Yes, for how many months?

If patient is unsure, please answer the following questions:

Have you ever been in hospital with a chest illness? Yes No

If yes, did you receive treatment for >1 month? Yes No

SECTION 3: Relevant occupational and social history

Has anyone in your household been previously diagnosed with TB? Yes No

Has anyone in your household been previously treated for TB? Yes No If Yes, how long ago (in months)?

Have you previously tested positive for HIV? Yes No If Yes, are you currently on ARVs? Yes No

Do you smoke currently or have you smoked in the last 5 years? Yes No If yes, how many cigarettes do you smoke per day? ≤5 | 6-20 | >20

Do you currently use drugs (e.g. dagga, tik, mandrax) or have you regularly used drugs in the last 5 years Yes No

Do you currently drink alcohol? Yes No In the last month how often did you have at least 5 units of alcohol in a single day (a unit=1 quart of beer / 2 glasses of wine or 2 shots of spirits)

What is the highest level of education that you have completed? No formal | Primary | Secondary | Tertiary

How many bedrooms do you have in your household?

How many people reside in your household?

Have you ever been imprisoned? Yes No If yes, for how long? <2 years | ≥2-5 years | >5 years | >10 years

Have you ever worked in mines/quarry/sandblasting? Yes No If yes, for how long? <2 years | ≥2-5 years | >5 years | >10 years

Have you worked in a clinic / hospital / medical lab? Yes No If yes, for how long? <2 years | ≥2-5 years | >5 years | >10 years

drs@nicd.ac.za **Centre for Tuberculosis, NICD, 1 Modderfontein Road, Sandringham, Johannesburg** 011 386 6476

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Appendix B: Informed Consent Form

SA National TB Drug Resistance Survey **Informed Consent Form**

This information must be communicated by the staff member to the client in a language the client is familiar with. After verbal agreement, the staff member must indicate if the client wants the results of the tests sent back to the clinic where it would be available for clinical care.

Good morning, I am (_____). I would like to ask you for a few minutes of your time to explain something to you and to ask for your assistance with some work we are doing.

Most people who are infected with tuberculosis (TB) can be treated and cured with the usual medicine which must be taken for six months. Some people are infected with a form of TB that these drugs cannot treat. They need to be treated with different drugs for a longer time. We call this form of TB "drug-resistant". The National Department of Health and the National Health Laboratory Services are doing a survey in South Africa to see how many people have drug-resistant TB and what drugs will be best to treat this. The results of this survey will be important for the Department of Health to ensure that people with TB are getting the best treatment as well as to improve service delivery.

If you agree to participate in this survey, we are asking you to give two samples of sputa instead of one. The first sample will be tested as usual and the result will be given to you by the clinic. The second sample will be sent to a central laboratory in Johannesburg and will be tested for TB and to see what drugs should be used to treat the TB. Further testing will also be done to understand why TB may affect you. You will also need to answer some questions that will help us make a proper diagnosis. It is possible that you may be asked to re-do this interview at a later date.

No harm will come to you through participating in this survey. If you are found to have TB, you will benefit directly, as your sputum will be tested against all available TB drugs to determine the best treatment for you.

It is important to know your HIV status, especially if you have TB, so that the doctors can give you the best treatment. If you wish to have a standard HIV test today, then the clinic sister will tell you where this can be done. An HIV test will also be done on the sputum we are collecting today. If you wish to know the results they will be sent back to the clinic for follow up. If you do not want to receive the sputum HIV test result, tell me and those results will not be sent back from the laboratory. The care you receive at the facility will be the same, even if you do not want to take part in the study, or refuse to answer any of the questions or have your HIV result.

The results and all information you provide will be kept confidential and will not be given to any relatives or employers.

I _____ confirm that I have explained the purpose of this study and the tests that will be performed to the patient.

Patient_____verbally agreed ☐ / declined ☐ to participate in the study *(please ✓ as appropriate)*.

If this patient consented to participate in the survey, does he/she want to be informed of the results of the sputum HIV test if positive: Yes ☐ No ☐ *(please ✓ as appropriate)*

Interviewer name: _____

Signature of interviewer: _____ Date: _____

drs@nicd.ac.za Centre for Tuberculosis, NICD, 1 Modderfontein Road, Sandringham, Johannesburg 011 386 6476

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Appendix C: Mono-resistance tables by province (strict and other)

Table C1: Provincial rifampicin mono-resistance – strict prevalence (without resistance to another first-line drug: streptomycin/ethambutol)

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	-	-	-	0.4	0	1.1	0.3	0	2.2
Free State	1.3	0.2	2.4	1.5	0	3.9	1.4	0.7	2.8
Gauteng	0.6	0.1	1.2	1.9	0	4.1	0.9	0.5	1.7
KwaZulu-Natal	1.2	0	2.3	1.6	0	3.9	1.3	0.5	3.1
Limpopo	1.5	0.7	2.4	3.3	0.4	6.1	1.8	1.1	3
Mpumalanga	1.3	0.3	2.4	6.8	2.5	11	2.4	1.4	4
North West	0.8	0.1	1.6	4.1	1.6	6.5	1.7	1	2.9
Northern Cape	0.5	0	1	2.2	0.5	4	1	0.5	2
Western Cape	0.7	0.1	1.2	1.4	0.3	2.4	0.9	0.6	1.5

Table C2: Provincial rifampicin mono-resistance – other prevalence (with resistance to another first-line drug: streptomycin/ethambutol)

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	0.5	0	1.3	0.9	0	2	0.7	0.2	1.8
Free State	0.5	0	1.3	1.6	0	4.1	0.7	0.1	3.3
Gauteng	0.3	0	0.7	0.8	0	2.2	0.4	0.1	1.2
KwaZulu-Natal	0.4	0	1.3	0.6	0	2	0.5	0.1	2.5
Limpopo	0.4	0	0.9	0.5	0	1.3	0.4	0.1	1.2
Mpumalanga	0.4	0	1	0.9	0	2.5	0.5	0.2	1.5
North West	0.2	0	0.6	1.2	0	2.8	0.5	0.1	1.5
Northern Cape	0.2	0	0.7	0.3	0	0.8	0.2	0	1.4
Western Cape	0.3	0	0.6	0.2	0	0.6	0.2	0	0.9

Table C3: Provincial isoniazid mono-resistance – strict prevalence (without resistance to another first-line drug: streptomycin/ethambutol)

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	4.7	2.7	6.8	5.2	2.4	8	5.2	3.5	7.6
Free State	5.7	3.6	7.7	5.4	1.7	9.1	5.9	4.3	8.2
Gauteng	3.8	2.6	5	5.6	2.2	9	4.3	3.2	5.6
KwaZulu-Natal	3.3	1.8	4.8	5.2	1.6	8.7	3.8	2.5	5.8
Limpopo	4.4	3.2	5.7	4	0.8	7.3	4.6	3.5	6
Mpumalanga	5.6	3.6	7.5	6.9	2.6	11.2	6.2	4.4	8.8
North West	5.2	3.7	6.6	4.8	1.8	7.7	5.3	4	7.1
Northern Cape	6.3	4.6	8.1	7.6	4.4	10.9	7.2	5.6	9.2
Western Cape	5.8	4.1	7.4	6.3	3.5	9	6.3	4.8	8.4

Table C4: Provincial isoniazid mono-resistance – other prevalence (with resistance to another first-line drug: streptomycin/ethambutol)

	New Cases			Previously Treated Cases			Overall		
Province	%	95% CI		%	95% CI		%	95% CI	
Eastern Cape	0.6	0	1.2	2	0.5	3.5	1.1	0.6	2.2
Free State	1.3	0.2	2.4	0.9	0	2.6	1.2	0.5	2.7
Gauteng	1	0.2	1.8	0.7	0	1.9	1	0.5	2
KwaZulu-Natal	1.5	0	3.8	0.8	0	2.1	1.4	0.3	5.4
Limpopo	0.7	0.2	1.2	0.6	0	1.6	0.6	0.3	1.4
Mpumalanga	0.8	0	1.6	0.7	0	1.7	0.6	0.2	1.9
North West	0.6	0	1.2	0.5	0	1.4	0.5	0.2	1.4
Northern Cape	0.9	0.2	1.7	0.5	0	1.1	0.8	0.4	1.6
Western Cape	1.1	0.3	1.9	0.4	0	1.2	0.9	0.5	1.6

Appendix D: Individual Province Results

Eastern Cape

Summary		
DRS Clusters	# DRS Clusters	32
Screened	Female	10 585
	Missing	242
	Total	19 349
	% Female	55.4%
	Median Age in Years (IQR)	39 (29-53)
	# Required to be Screened	16 512
	% Screened of Target	117%
Tested by Culture	# Tested by Culture	8 548
	# Culture Positive	1 123
	# Culture Positive MTB	1 033
	MTB Positivity among Tested (%)	12.1%
	# Culture Positive MTB	1 033
	# Required Culture Positive MTB	1 323
	% MTB of Target	78.1%
	# Culture Positive NTM	90
	% NTM among Culture Positive	8%
Characteristics of Culture Confirmed TB	Smear Positive	569
	Smear Invalid	25
	Total TB Culture Positive	1 033
	% Smear Positivity	56%
	HIV Positive	543
	HIV Invalid	56
	Total	1 033
	HIV Positivity among Culture Positive MTB	55.6%
	Previous Treatment History among TB Cases (%)	27%

	New Cases		Previously Treated Cases		Overall	
Resistance Pattern	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Multidrug resistance	1.7	0.8 - 2.6	2.7	0.5 - 5	2.1	1.3 - 3.6
Any rifampicin resistance	2.7	1.5 - 3.9	4	1.5 - 6.5	3.3	2.2 - 4.9
Rifampicin mono-resistance	1	0.3 - 1.7	1.2	0 - 2.4	1.1	0.6 - 2
Rifampicin mono-resistance (strict)	-	- - -	0.4	0 - 1.1	0.3	0 - 2.2
Rifampicin mono-resistance (other)	0.5	0 - 1.3	0.9	0 - 2	0.7	0.2 - 1.8
Any isoniazid resistance	7.1	4.9 - 9.3	10	6.1 - 13.9	8.9	6.6 - 12
Isoniazid mono-resistance	5.4	3.3 - 7.5	7.2	4.1 - 10.3	6.4	4.6 - 9
Isoniazid mono-resistance (strict)	4.7	2.7 - 6.8	5.2	2.4 - 8	5.2	3.5 - 7.6
Isoniazid mono-resistance (other)	0.6	0 - 1.2	2	0.5 - 3.5	1.1	0.6 - 2.2
Ethambutol resistance	2	0.2 - 3.7	2.2	0.1 - 4.2	2.1	1.2 - 3.9
Streptomycin resistance	2.6	1.3 - 4	6.1	3.4 - 8.8	4.1	2.8 - 5.9
Pyrazinamide resistance	3	1.3 - 4.8	4.2	1.4 - 7	3.6	2.4 - 5.5

Free State

Summary		
DRS Clusters	# DRS Clusters	39
Screened	Female	14 579
	Missing	423
	Total	26 288
	% Female	56.4%
	Median Age in Years (IQR)	42 (31-53)
	# Required to be Screened	20 160
	% Screened of Target	130%
Tested by Culture	# Tested by Culture	14 080
	# Culture Positive	1 155
	# Culture Positive MTB	907
	MTB Positivity among Tested (%)	6.4%
	# Culture Positive MTB	907
	# Required Culture Positive MTB	1 291
	% MTB of Target	70.3%
	# Culture Positive NTM	248
	% NTM among Culture Positive	21%
Characteristics of Culture Confirmed TB	Smear Positive	390
	Smear Invalid	45
	Total TB Culture Positive	907
	% Smear Positivity	45%
	HIV Positive	578
	HIV Invalid	85
	Total	907
	HIV Positivity among Culture Positive MTB	70.3%
	Previous Treatment History among TB Cases (%)	21%

	New Cases			Previously Treated Cases			Overall		
Resistance Pattern	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Multidrug resistance	1.8	0.8	2.8	3.9	0.8	7	2.3	1.5	3.6
Any rifampicin resistance	3.5	2	5.1	7.3	2.5	12.1	4.6	3.2	6.6
Rifampicin mono-resistance	1.8	0.4	3.1	3.4	0.5	6.3	2.2	1.2	3.9
Rifampicin mono-resistance (strict)	1.3	0.2	2.4	1.5	0	3.9	1.4	0.7	2.8
Rifampicin mono-resistance (other)	0.5	0	1.3	1.6	0	4.1	0.7	0.1	3.3
Any isoniazid resistance	8.8	6.4	11.1	10.1	5.2	14.9	10	7.8	12.9
Isoniazid mono-resistance	7	4.9	9.1	6.1	2.2	10	7.3	5.6	9.6
Isoniazid mono-resistance (strict)	5.7	3.6	7.7	5.4	1.7	9.1	5.9	4.3	8.2
Isoniazid mono-resistance (other)	1.3	0.2	2.4	0.9	0	2.6	1.2	0.5	2.7
Ethambutol resistance	3	1.5	4.5	6.2	1.4	11	4	2.7	5.9
Streptomycin resistance	3.3	1.4	5.2	4.1	0	8.3	3.7	2.3	5.9
Pyrazinamide resistance	2.8	1.4	4.1	2.9	0	6.1	2.9	1.9	4.6

Gauteng

Summary		
DRS Clusters	# DRS Clusters	38
Screened	Female	10 877
	Missing	329
	Total	20 101
	% Female	55%
	Median Age in Years (IQR)	38 (30-47)
	# Required to be Screened	16 520
	% Screened of Target	122%
Tested by Culture	# Tested by Culture	11 188
	# Culture Positive	1 423
	# Culture Positive MTB	1 123
	MTB Positivity among Tested (%)	10%
	# Culture Positive MTB	1 123
	# Required Culture Positive MTB	1 323
	% MTB of Target	84.9%
	# Culture Positive NTM	300
	% NTM among culture positive	21%
Characteristics of Culture Confirmed TB	Smear Positive	676
	Smear Invalid	6
	Total TB Culture Positive	1 123
	% Smear Positivity	61%
	HIV Positive	823
	HIV Invalid	20
	Total	1 123
	HIV Positivity among Culture Positive MTB	74.6%
	Previous Treatment History among TB Cases (%)	18%

	New Cases			Previously Treated Cases			Overall		
Resistance Pattern	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Multidrug resistance	2.7	1.3	4.1	6.4	2.6	10.3	3.4	2.3	5.2
Any rifampicin resistance	3.6	2.1	5.2	9.3	4.8	13.8	4.8	3.4	6.8
Rifampicin mono-resistance	1	0.3	1.6	2.8	0.3	5.3	1.3	0.8	2.2
Rifampicin mono-resistance (strict)	0.6	0.1	1.2	1.9	0	4.1	0.9	0.5	1.7
Rifampicin mon- resistance (other)	0.3	0	0.7	0.8	0	2.2	0.4	0.1	1.2
Any isoniazid resistance	7.5	5.4	9.5	12.8	7.3	18.3	9.2	7.2	11.7
Isoniazid mono-resistance	4.8	3.3	6.3	6.3	2.7	10	5.3	4.1	6.9
Isoniazid mono-resistance (strict)	3.8	2.6	5	5.6	2.2	9	4.3	3.2	5.6
Isoniazid mono-resistance (other)	1	0.2	1.8	0.7	0	1.9	1	0.5	2
Ethambutol resistance	1.9	0.6	3.2	4.8	1	8.5	2.4	1.4	4.1
Streptomycin resistance	5.3	3.7	6.9	5.3	1.4	9.1	5.6	4.2	7.5
Pyrazinamide resistance	4.5	2.6	6.5	7.4	2.3	12.5	5.3	3.6	7.8

KwaZulu-Natal

Summary		
DRS Clusters	# DRS Clusters	31
Screened	Female	11 459
	Missing	218
	Total	20 376
	% Female	56.8%
	Median Age in Years (IQR)	35 (28-46)
	# Required to be Screened	16 704
	% Screened of Target	122%
Tested by Culture	# Tested by Culture	9 082
	# Culture Positive	899
	# Culture Positive MTB	784
	MTB Positivity among Tested (%)	8.6%
	# Culture Positive MTB	784
	# Required Culture positive MTB	1 337
	% MTB of Target	58.6%
	# Culture Positive NTM	115
	% NTM among Culture Positive	13%
Characteristics of Culture Confirmed TB	Smear Positive	350
	Smear Invalid	29
	Total TB Culture Positive	784
	% Smear Positivity	46%
	HIV Positive	507
	HIV Invalid	51
	Total	784
	HIV Positivity among Culture Positive MTB	69.2%
	Previous Treatment History among TB Cases (%)	22%

	New Cases		Previously Treated Cases		Overall	
Resistance Pattern	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Multidrug resistance	1.8	0.6 - 3	6.4	2.3 - 10.4	2.9	1.8 - 4.5
Any rifampicin resistance	3.5	1.6 - 5.5	8.8	3 - 14.6	4.9	3.2 - 7.5
Rifampicin mono-resistance	1.7	0.2 - 3.2	2.4	0 - 4.9	1.9	1 - 3.8
Rifampicin mono-resistance (strict)	1.2	0 - 2.3	1.6	0 - 3.9	1.3	0.5 - 3.1
Rifampicin mono-resistance (other)	0.4	0 - 1.3	0.6	0 - 2	0.5	0.1 - 2.5
Any isoniazid resistance	6.6	3.5 - 9.7	12.5	6.4 - 18.5	8.5	5.9 - 12.4
Isoniazid mono-resistance	4.8	2.1 - 7.4	6	2.3 - 9.8	5.3	3.3 - 8.5
Isoniazid mono-resistance (strict)	3.3	1.8 - 4.8	5.2	1.6 - 8.7	3.8	2.5 - 5.8
Isoniazid mono-resistance (other)	1.5	0 - 3.8	0.8	0 - 2.1	1.4	0.3 - 5.4
Ethambutol resistance	1.9	0.3 - 3.4	6	1.3 - 10.8	2.9	1.6 - 5
Streptomycin resistance	4.5	1.8 - 7.2	5.7	1.4 - 9.9	5	3 - 8.2
Pyrazinamide resistance	2.3	1 - 3.7	8.1	3.1 - 13	3.7	2.6 - 5.4

Limpopo

Summary		
DRS Clusters	# DRS Clusters	48
Screened	Female	18 398
	Missing	734
	Total	31 503
	% Female	59.8%
	Median Age in Years (IQR)	43 (32-56)
	# Required to be Screened	31 850
	% Screened of Target	99%
Tested by Culture	# Tested by Culture	14 016
	# Culture Positive	1 442
	# Culture Positive MTB	1 120
	MTB Positivity among Tested (%)	8%
	# Culture Positive MTB	1 120
	# Required Culture Positive MTB	1 274
	% MTB of Target	87.9%
	# Culture Positive NTM	322
	% NTM among Culture Positive	22%
Characteristics of Culture Confirmed TB	Smear Positive	638
	Smear Invalid	15
	Total TB Culture Positive	1 120
	% Smear Positivity	58%
	HIV Positive	687
	HIV Invalid	39
	Total	1 120
	HIV Positivity among Culture Positive MTB	63.6%
	Previous Treatment History among TB Cases (%)	14%

	New Cases		Previously Treated Cases		Overall	
Resistance Pattern	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Multidrug resistance	1.4	0.4 - 2.4	2.5	0 - 5.1	1.6	0.9 - 2.9
Any rifampicin resistance	3.4	2 - 4.7	6.2	2.6 - 9.7	3.9	2.8 - 5.5
Rifampicin mono-resistance	2	1.1 - 2.9	3.5	0.5 - 6.5	2.2	1.5 - 3.4
Rifampicin mono-resistance (strict)	1.5	0.7 - 2.4	3.3	0.4 - 6.1	1.8	1.1 - 3
Rifampicin mono-resistance (other)	0.4	0 - 0.9	0.5	0 - 1.3	0.4	0.1 - 1.2
Any isoniazid resistance	6.6	4.7 - 8.4	7.1	3.2 - 11	7.1	5.5 - 9.1
Isoniazid mono-resistance	5.1	3.8 - 6.5	4.5	1.3 - 7.6	5.3	4.1 - 6.9
Isoniazid mono-resistance (strict)	4.4	3.2 - 5.7	4	0.8 - 7.3	4.6	3.5 - 6
Isoniazid mono-resistance (other)	0.7	0.2 - 1.2	0.6	0 - 1.6	0.6	0.3 - 1.4
Ethambutol resistance	1.8	0.3 - 3.2	1.2	0 - 3.5	1.7	0.9 - 3.5
Streptomycin resistance	2.9	1.7 - 4	2.2	0 - 5.3	2.9	2 - 4.2
Pyrazinamide resistance	1.8	1 - 2.6	4	1 - 7.1	2.2	1.5 - 3.2

Mpumalanga

Summary		
DRS Clusters	# DRS Clusters	38
Screened	Female	12 434
	Missing	120
	Total	21 739
	% Female	57.5%
	Median Age in Years (IQR)	39 (30-51)
	# Required to be Screened	15 846
	% Screened of Target	137%
Tested by Culture	# Tested by Culture	11 800
	# Culture Positive	1 418
	# Culture Positive MTB	1 193
	MTB Positivity among Tested (%)	10.1%
	# Culture Positive MTB	1 193
	# Required Culture Positive MTB	1 269
	% MTB of Target	94%
	# Culture Positive NTM	225
	% NTM among Culture Positive	16%
Characteristics of Culture Confirmed TB	Smear Positive	543
	Smear Invalid	74
	Total TB Culture Positive	1 193
	% Smear Positivity	49%
	HIV Positive	859
	HIV Invalid	74
	Total	1 193
	HIV Positivity among Culture Positive MTB	76.8%
	Previous Treatment History among TB Cases (%)	17%

	New Cases		Previously Treated Cases		Overall	
Resistance Pattern	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Multidrug resistance	4.2	2.8 - 5.6	7.6	3.2 - 12	5.1	3.7 - 7
Any rifampicin resistance	6	4.4 - 7.7	15.5	9.2 - 21.7	8.4	6.5 - 11
Rifampicin mono-resistance	1.8	0.9 - 2.7	7.8	3.5 - 12.1	3	2 - 4.5
Rifampicin mono-resistance (strict)	1.3	0.3 - 2.4	6.8	2.5 - 11	2.4	1.4 - 4
Rifampicin mono-resistance (other)	0.4	0 - 1	0.9	0 - 2.5	0.5	0.2 - 1.5
Any isoniazid resistance	10.6	8 - 13.1	14.6	7.6 - 21.6	12.7	9.8 - 16.5
Isoniazid mon-resistance	6.3	4 - 8.7	6.9	2.6 - 11.2	6.9	4.8 - 9.9
Isoniazid mono-resistance (strict)	5.6	3.6 - 7.5	6.9	2.6 - 11.2	6.2	4.4 - 8.8
Isoniazid mono-resistance (other)	0.8	0 - 1.6	0.7	0 - 1.7	0.6	0.2 - 1.9
Ethambutol resistance	4.2	2.8 - 5.7	2.8	0 - 5.7	4.2	3 - 5.8
Streptomycin resistance	5.4	3.3 - 7.5	5.8	2.5 - 9.1	5.8	4.2 - 8.1
Pyrazinamide resistance	3.7	2 - 5.4	4.4	0.9 - 7.9	4	2.6 - 6.1

North West

Summary		
DRS Clusters	# DRS Clusters	35
Screened	Female	10 535
	Missing	338
	Total	19 589
	% Female	54.7%
	Median Age in Years (IQR)	40 (30-51)
	# Required to be Screened	18 650
	% Screened of Target	105%
Tested by Culture	# Tested by Culture	10 344
	# Culture Positive	1 370
	# Culture Positive MTB	1 024
	MTB Positivity among Tested (%)	9.9%
	# Culture Positive MTB	1 024
	# Required Culture Positive MTB	1 194
	% MTB of Target	85.8%
	# Culture Positive NTM	346
	% NTM among Culture Positive	25%
Characteristics of Culture Confirmed TB	Smear Positive	688
	Smear Invalid	7
	Total TB Culture Positive	1 024
	% Smear Positivity	68%
	HIV Positive	691
	HIV Invalid	8
	Total	1 024
	HIV Positivity among Culture Positive MTB	68%
	Previous Treatment History among TB Cases (%)	20%

	New Cases		Previously Treated Cases		Overall	
Resistance Pattern	**%**	**95% CI**	**%**	**95% CI**	**%**	**95% CI**
Multi-drug resistance	1.9	0.8 - 3.1	4.3	1.4 - 7.1	2.6	1.7 - 3.9
Any rifampicin resistance	3.1	1.5 - 4.6	9.7	5.9 - 13.4	4.9	3.6 - 6.8
Rifampicin mono-resistance	1.1	0.2 - 2	5.3	2.8 - 7.9	2.2	1.4 - 3.5
Rifampicin mono-resistance (strict)	0.8	0.1 - 1.6	4.1	1.6 - 6.5	1.7	1 - 2.9
Rifampicin mono-resistance (other)	0.2	0 - 0.6	1.2	0 - 2.8	0.5	0.1 - 1.5
Any isoniazid resistance	7.7	6 - 9.5	9.4	5.6 - 13.2	8.9	7.2 - 11
Isoniazid mono-resistance	5.8	4.3 - 7.2	5.1	2.1 - 8.1	6	4.6 - 7.7
Isoniazid mono-resistance (strict)	5.2	3.7 - 6.6	4.8	1.8 - 7.7	5.3	4 - 7.1
Isoniazid mono-resistance (other)	0.6	0 - 1.2	0.5	0 - 1.4	0.5	0.2 - 1.4
Ethambutol resistance	1	0.2 - 1.9	1.8	0 - 4.3	1.3	0.6 - 2.8
Streptomycin resistance	2.1	1 - 3.2	4.9	1.5 - 8.3	2.9	1.9 - 4.5
Pyrazinamide resistance	3.6	1.8 - 5.3	5	1.6 - 8.4	4.1	2.7 - 6.1

Northern Cape

Summary		
DRS Clusters	# DRS Clusters	47
Screened	Female	11 644
	Missing	246
	Total	23 107
	% Female	50.9%
	Median Age in Years (IQR)	41 (31-51)
	# Required to be Screened	16 280
	% Screened of Target	142%
Tested by Culture	# Tested by Culture	13 376
	# Culture Positive	1 688
	# Culture Positive MTB	1 372
	MTB Positivity among Tested (%)	10.3%
	# Culture Positive MTB	1 372
	# Required Culture Positive MTB	1 303
	% MTB of Target	105.3%
	# Culture Positive NTM	316
	% NTM among Culture Positive	19%
Characteristics of Culture confirmed TB	Smear Positive	868
	Smear Invalid	0
	Total TB Culture Positive	1 372
	% Smear Positivity	63%
	HIV Positive	682
	HIV Invalid	53
	Total	1 372
	HIV Positivity among Culture Positive MTB	51.7%
	Previous Treatment History among TB Cases (%)	28%

	New Cases			Previously Treated Cases			Overall		
Resistance Pattern	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Multi-drug resistance	1.3	0.4	2.1	2.6	0.8	4.3	1.7	1	2.8
Any Rifampicin resistance	2	1.1	3	5	2.5	7.5	3	2.1	4.2
Rifampicin mono-resistance	0.8	0.1	1.4	2.4	0.5	4.3	1.3	0.7	2.3
Rifampicin mono-resistance (strict)	0.5	0	1	2.2	0.5	4	1	0.5	2
Rifampicin mono-resistance (other)	0.3	0	0.7	0.3	0	0.8	0.2	0	1.4
Any isoniazid resistance	8.5	6.4	10.7	10.7	7.2	14.1	10.1	8.2	12.5
Isoniazid mono-resistance	7.3	5.4	9.2	8.1	4.8	11.4	8.1	6.4	10.3
Isoniazid mono-resistance (strict)	6.3	4.6	8.1	7.6	4.4	10.9	7.2	5.6	9.2
Isoniazid mono-resistance (other)	0.9	0.2	1.7	0.5	0	1.1	0.8	0.4	1.6
Ethambutol resistance	1.7	0.6	2.8	2.6	0.8	4.5	2.1	1.3	3.3
Streptomycin resistance	4.3	2.7	6	3.8	1.4	6.1	4.4	3.1	6.1
Pyrazinamide resistance	2.5	1.4	3.5	5.4	2.9	8	3.5	2.5	4.8

Western Cape

Summary		
DRS Clusters	# DRS Clusters	35
Screened	Female	8 483
	Missing	283
	Total	18 306
	% Female	47.1%
	Median Age in Years (IQR)	38 (29-48)
	# Required to be Screened	16 480
	% Screened of Target	111%
Tested by Culture	# Tested by Culture	8 989
	# Culture Positive	1 537
	# Culture Positive MTB	1 487
	MTB Positivity among Tested (%)	16.5%
	# Culture Positive MTB	1 487
	# Required Culture Positive MTB	1 320
	% MTB of Target	112.7%
	# Culture Positive NTM	50
	% NTM among Culture Positive	3%
Characteristics of Culture Confirmed TB	Smear Positive	700
	Smear Invalid	50
	Total TB Culture Positive	1 487
	% Smear Positivity	49%
	HIV Positive	617
	HIV Invalid	186
	Total	1 487
	HIV Positivity among Culture Positive MTB	47.4%
	Previous Rreatment History among TB Cases (%)	35%

	New Cases			Previously Treated Cases			Overall		
Resistance Pattern	**%**	**95% CI**		**%**	**95% CI**		**%**	**95% CI**	
Multidrug resistance	2	0.7	3.2	4.5	2.1	7	3	2.1	4.2
Any rifampicin resistance	2.9	1.5	4.3	6.1	3.6	8.6	4.2	3.2	5.5
Rifampicin mono-resistance	0.9	0.3	1.6	1.5	0.5	2.5	1.2	0.8	1.8
Rifampicin mono-resistance (strict)	0.7	0.1	1.2	1.4	0.3	2.4	0.9	0.6	1.5
Rifampicin mono-resistance (other)	0.3	0	0.6	0.2	0	0.6	0.2	0	0.9
Any isoniazid resistance	8.9	6.5	11.3	11.2	7	15.3	10.8	8.5	13.7
Isoniazid mono-resistance	6.9	5.1	8.7	6.6	3.7	9.5	7.3	5.5	9.7
Isoniazid mono-resistance (strict)	5.8	4.1	7.4	6.3	3.5	9	6.3	4.8	8.4
Isoniazid mono-resistance (other)	1.1	0.3	1.9	0.4	0	1.2	0.9	0.5	1.6
Ethambutol resistance	1.8	0.9	2.7	2.7	1	4.4	2.1	1.4	3.4
Streptomycin resistance	3.6	2.1	5.1	4	1.8	6.2	3.9	2.8	5.4
Pyrazinamide resistance	2	0.8	3.3	3.3	1.5	5.1	2.5	1.7	3.7

Steering Committee

Lindiwe Mvusi, Nazir Ahmed Ismail, Chikwe Ihekweazu, Sanni Babatunde, Thabo Molebatsi, Martie van der Walt, Limenako Matsoso, Adeboye Adelekan, Varough Deyde, Shabir Madhi.

Technical Work Group Members

Ananta Nanoo, Andries Dreyer, Shaheed V Omar, Relebohile Ncha, Maanda Mudau, Beki Magazi, Patricia Ntsele.

National and Provincial TB Programme Managers

David Mametja (NDoH), Norbert Ndjeka (NDoH), Sicelo Dlamini (NDoH), Ms Miyakazi Nokwe (EC), Ms Matshidiso Morigihlane and Ms Sonja van der Merwe (FS), Dr Dimakatso Moloi (GP), Ms Jacqui Ngozo (KZN), Dr Harold Hlope (LP), Ms Dudu Mbambo and Dr Andrew Shija (MP), Ms Simangele Ngcombela (NW), Ms Phyllis Baitsiwe (NC), Mr Jimmy Ledwaba and Ms Alvera Swart (WC).

DRS Provincial Co-ordinators

Lifukazi Ngcwangu (EC), Aziza Rangunwala (FS), Danny Lathane (GP), Jovan Dutt (KZN), Dorah Lekalakala (LP), Mbhekiseni Khumalo (MP), Portia Madumo (NC), Moira Mothibi (NW), Phumeza Vazi (WC)

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Centre for Tuberculosis

1 Modderfontein Road
Sandringham, Johannesburg

Tel: 011 386 6000
www.nicd.ac.za

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES

Division of the National Health Laboratory Service