Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey

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Abstract

Background

Globally per-capita, South Africa reports a disproportionately high number of multi- and extensively drug resistant tuberculosis cases (M/XDR-TB). We sought to estimate the prevalence of resistance provincially and nationally to TB drugs in newly diagnosed and retreated TB patients and compared these to the 2001-02 estimates.

Methods

A cross-sectional survey was done between June 15, 2012–June 14, 2014, using population proportionate randomised cluster sampling in the nine provinces in South Africa. 343 clusters were included, ranging between 31 and 48 per province. A patient was eligible for inclusion in the survey if he or she presented as a presumptive case during the intake period at a drug resistance survey enrolling facility. Consenting participants (≥18 years old) completed a questionnaire and had a sputum sample tested for resistance to first-line and second-line drugs. Analysis was by logistic regression with robust SEs, inverse probability weighted against routine data, and estimates were derived using a random effects model.

Findings

101 422 participants were tested in 2012–14. Nationally, the prevalence of MDR tuberculosis was 2·1% (95% CI 1·5–2·7) among new tuberculosis cases and 4·6% (3·2–6·0) among retreatment cases. The provincial point prevalence of MDR tuberculosis ranged between 1·6% (95% CI 0·9–2·9) and 5·1% (3·7–7·0). Overall, the prevalence of rifampicin-resistant tuberculosis (4·6%, 95% CI 3·5–5·7) was higher than the prevalence of MDR tuberculosis (2·8%, 2·0–3·6; p=0·01). Comparing the current survey with the previous (2001–02) survey, the overall MDR tuberculosis prevalence was 2·8% versus 2·9% and prevalance of rifampicin-resistant tuberculosis was 3·4% versus 1·8%, respectively. The prevalence of isoniazid mono-resistant tuberculosis was above 5% in all provinces. The prevalence of ethionamide and pyrazinamide resistance among MDR tuberculosis cases was 44·7% (95% CI 25·9–63·6) and 59·1% (49·0–69·1), respectively. The prevalence of XDR tuberculosis was 4·9% (95% CI 1·0–8·8). Nationally, the estimated numbers of cases of rifampicin-resistant tuberculosis, MDR tuberculosis, and isoniazid mono-resistant tuberculosis for 2014 were 13 551, 8249, and 17 970, respectively.

Conclusion

The overall prevalence of MDR tuberculosis in South Africa in 2012–14 was similar to that in 2001–02; however, prevalence of rifampicin-resistant tuberculosis almost doubled among new cases. Furthermore, the high prevalence of isoniazid mono-resistant tuberculosis, not routinely screened for, and resistance to second-line drugs has implications for empirical management.

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Introduction

Multi-drug resistant tuberculosis (MDR-TB) was declared a public health crisis by World Health Organization (WHO) in 2013 and recognized as a global health security risk by the World Health Assembly in 2014. South Africa (SA) remains one of the highest burdened countries in all three WHO-defined tuberculosis (TB) categories, including TB, MDR-TB and TB and human immunodeficiency virus (TB-HIV) co-infection cases. Rifampicin resistant (RR)-TB, often seen as a proxy for MDR-TB and treated as such has become increasingly relevant and constitutes MDR-TB and rifampicin-mono resistant (RMR)-TB cases. The difference fundamentally being the presence or absence of resistance to the second core TB drug – isoniazid. In 2014, SA reported the second highest absolute number of notified rifampicin-resistant (RR) cases, globally (18 734) [1], following India where 25 749 cases were recorded in a population 20 times greater than South Africa.

The previous tuberculosis drug resistance survey done in South Africa during 2001–02 reported the prevalence of MDR tuberculosis as 1·6% (95% CI 1·1–2·1) in new tuberculosis cases and 6·6% (4·9–8·2) in retreatment cases[2]. A t that time, the prevalence of tuberculosis and HIV was rising, late presentation was common, and tuberculosis-related mortality was high, whereas laboratory testing for drug-resistant tuberculosis was limited. In 2005, at the Tugela Ferry Hospital, KwaZulu-Natal province, an outbreak of extensively resistant (XDR) tuberculosis with high mortality was identified and was followed by the emergence of totally drug-resistant tuberculosis strains identified during 2008–09 in the Eastern Cape province.[3]

Treatment success based on notification data has remained low at approximately 50% for MDR tuberculosis and 20% for XDR tuberculosis cases.[4] However, the situation has potential for improvement with the introduction of bedaquiline, a new antimycobacterial agent, with improved outcomes compared with a background regimen[5]. Furthermore, implementation of new diagnostics for early detection of drug-resistant tuberculosis, in particular the Xpert MTB/RIF assay as the primary test, and an upscaling of the antiretroviral treatment programme were important advances in South Africa since the previous drug resistance survey in 2001–02. This cross-sectional survey was initiated in mid-2012 in South Africa to evaluate the prevalence of resistance to first-line and second-line agents in new and retreatment tuberculosis cases nationally and provincially, and provide estimates as to the burden of drug-resistant tuberculosis.

Methods

Study design and participants

The survey was a population-based cross-sectional study, following WHO guidelines[6] as applied to the 2001–02 survey. A population proportionate, cluster-sampling design was used to determine sample size and select study sites to provide MDR tuberculosis prevalence estimates for each province and nationally. Clusters were randomly selected using a population-proportionate cluster-sampling approach based on a list of new sputum smear-positive cases per health facilities, per province, in the year when

the survey was designed, and were individual health-care facilities or a combination of facilities. A patient was eligible for inclusion in the survey if he or she presented as a presumptive case during the intake period at a drug resistance survey enrolling facility. A presumptive case was defined as a patient who had a persistent cough for more than 2 weeks or at least two of the following symptoms: fever, drenching night sweats, loss of appetite, unexplained weight loss (>1·5 kg/month), a general feeling of illness (malaise) and tiredness, and shortness of breath with chest pain. Only adults aged 18 years 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.

The survey received ethical approval from the University of Witwatersrand Research Ethics Committee on Nov 26, 2010 (ethics clearance number M081022). Clearance was also obtained from Centers for Disease Control and Prevention, Atlanta, GA, USA. The survey was initiated after approval from the respective provinces and the South Africa National Tuberculosis Control Programme was received.

Procedures

A survey-specific sputum sample, together with a questionnaire completed through direct patient interview by a health-care worker were collected from all patients with presumptive tuberculosis who provided informed consent at selected facilities during the June 15, 2012–June 14, 2014, survey period. Auramine smear microscopy, mycobacterial culture (MGIT 960; Becton Dickinson, Sparks, MD, USA), and HIV testing (Oraquick Advance Rapid HIV—1 & 2 Antibody Test; Orasure Technologies, Bethlehem, PA, USA) on sputum were done, followed by drug susceptibility testing against first-line and second-line antituberculosis drugs on *Mycobacterium tuberculosis* culture-confirmed isolates.[7] Data from case report forms and laboratory testing were collated and analysed.

A new case was defined as a patient with a newly registered episode of tuberculosis who, in response to direct questioning, reports never having been treated for tuberculosis or reports having taken antituberculosis drugs for less than 1 month; or where adequate documentation is available, for whom there is no evidence of having taken antituberculosis drugs for 1 month or more. A previously treated case was defined as a patient having a newly registered episode of tuberculosis who, in response to direct questioning, reports having received 1 month or more of antituberculosis drugs in the past; or where adequate documentation is available, there is evidence of having received 1 month or more of anti-tuberculosis drugs in the past. Drug resistance prevalence was determined among culture-confirmed tuberculosis cases in the survey.

Statistical analysis

Descriptive and statistical analyses accounting for the complex multistage sampling design and clustering of patients within primary sampling units, were done and compared with the 2001–02 survey. Multiple imputation was done for missing age, sex, and previous treatment history data, as well as final status for contaminated cultures and failed drug susceptibility testing. Logistic regression with robust SEs adjusting for clustering effects introduced by survey design and potential biases arising during implementation was used to determine provincial estimates of drug resistance prevalence among new

and previously treated cases, and by HIV status. These estimates were pooled to generate national estimates using a random effects model. Additionally, national estimates for prevalence of second-line drug resistance including XDR tuberculosis were calculated among subgroups of RMR tuberculosis and MDR tuberculosis cases. To determine the absolute burden expected to be diagnosed for 2014 in South Africa, the 95% CIs of the prevalence estimate for rifampicin-resistant tuberculosis, isoniazid monoresistant (IMR) tuberculosis and MDR tuberculosis was applied to the reported number of microbiologically confirmed tuberculosis cases reported for the same year.[8] Additional details are provided in the appendix (pp 2, 3) and estimates presented are the adjusted rates.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The South African Tuberculosis Drug Resistance Survey 2012–14 tested 101 422 people from 464 randomly selected facilities (figure 1) for tuberculosis by culture from all nine provinces in South Africa (table 1). Of those screened and not tested, 12 043 (6%) were younger than 18 years, 13 141 (7%) did not give consent, 10 012 (5%) did not fulfil criteria for presumptive tuberculosis cases, 864 (<1%) were currently on treatment, 1434 (1%) were already included at another survey site, 61 351 (31%) supplied insufficient specimen volume, and 91 (<1%) had incomplete forms (appendix p 4). Of 10 044 culture-confirmed tuberculosis cases detected, 5423 (55%) of 9793 were smear positive. Nationally, 2210 (22%) of the culture-positive cases reported having ever been previously treated for tuberculosis. The age and sex distributions are shown in the appendix (p 5). The prevalence of HIV coinfection among culture-confirmed tuberculosis cases was 63% nationally, ranging from 47% in Western Cape to 77% in Mpumalanga.

The national MDR tuberculosis prevalence estimate was 2·1% (95% CI 1·5–2·7) in new tuberculosis cases, and higher among retreatment cases (4·6%, 3·2–6·0), with an overall estimate of 2·8% (2·0–3·6; table 2). Provincial MDR tuberculosis prevalence in six of nine provinces was below 2·0% among new cases (table 3). Mpumalanga province had the highest overall prevalence of MDR tuberculosis (5·1%, 95% CI 3·7–7·0), including both new (4·2%, 2·8–5·6) and retreatment cases (7·6%, 3·2–12·0), whereas Limpopo province had the lowest at 1·6% (0·9–2·9) overall, 1·4% (0·4–2·4) new, and 2·5% (0–5·1) retreatment cases.

Compared with the MDR tuberculosis point prevalence estimate nationally, rifampicin-resistant tuberculosis prevalence was significantly higher overall at 4·6% (95% CI 3·5–5·7, p=0·01), and in new cases at 3·4% (2·5–4·3, p=0·03), whereas in retreatment cases it was 7·1% (4·8–9·5, p=0·07; table 2). The rifampicin-resistant tuberculosis prevalence ranged between 3·0% (95% CI 2·1–4·2) and 4·9% (3·2–7·5) for eight of the provinces, whereas Mpumalanga province again had the highest prevalence at 8·4% (6·5–11·0). The higher prevalence in Mpumalanga province was observed in both new and retreatment

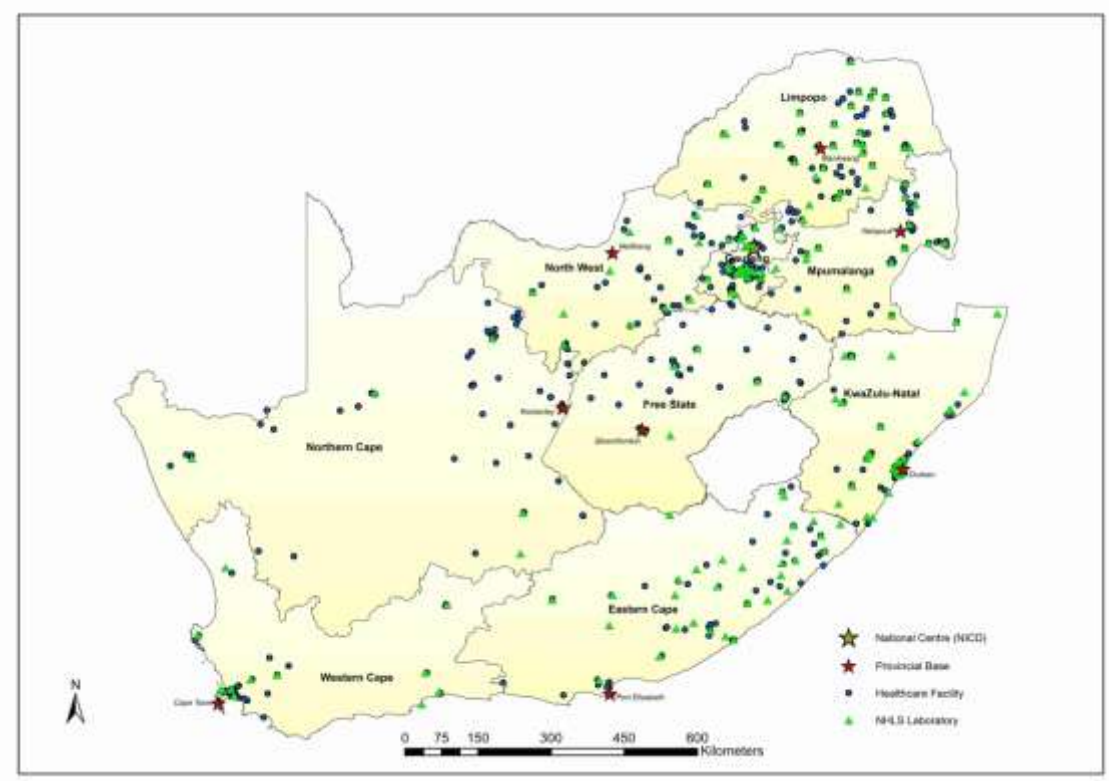

Figure 1: Map of randomly selected facilities included in the SA TB DRS 2012-14

Table 1: Participant enrollment cascade including previous TB treatment and HIV-TB co-infection among culture confirmed TB cases, South Africa – 2012-14

Province	No. Clusters	No. Screened	No· Tested by Culture	Proportion Tested of Screened (%)	No· Culture Positive	Proportion Culture Positive of Tested (%)	No· Culture Positive MTB*	Proportion MTB* of culture positive	Previous History of TB Treatment (%)$	HIV-TB co-infection (%)$
Eastern Cape	32	19349	8548	44%	1123	13%	1033	92%	27%	55·6%
Free State	39	26288	14079	54%	1155	8%	907	79%	21%	70·3%
Gauteng	38	20101	11188	56%	1423	13%	1123	79%	18%	74·6%
KwaZulu-Natal	31	20376	9082	45%	899	10%	784	87%	22%	69·2%
Limpopo	48	31503	14016	44%	1442	10%	1121	78%	14%	63·6%
Mpumalanga	38	21739	11800	54%	1418	12%	1193	84%	17%	76·8%
North West	35	19589	10344	53%	1370	13%	1024	75%	20%	68·0%
Northern Cape	47	23107	13376	58%	1688	13%	1372	81%	28%	51·7%
Western Cape	35	18306	8989	49%	1537	17%	1487	97%	35%	47·4%
South Africa	**343**	**200358**	**101422**	**51%**	**12055**	**12%**	**10044**	**83%**	**22%**	**63·2%**

*MTB: *Mycobacterium tuberculosis* complex

$among culture positive MTB cases

Table 2: National first-line drug resistance estimates among TB cases, 2012-14 and 2001-2 surveys[2], in South Africa

Resistance Type	New Case (%, 95% CI)		Previously Treated (%, 95% CI)		Overall (%, 95% CI)	
	2001-2	2012-4	2001-2	2012-4	2001-2	2012-4
Multi-drug resistant	1·6 (1·1-2·1)	2·1 (1·5-2·7)	6·6 (4·9-8·2)	4·6 (3·2-6·0)	2·9 (2·4-3·5)	2·8 (2·0-3·6)
Rifampicin	1·8 (1·3-2·3)	3·4 (2·5-4·3) *	7·5 (5·7-9·2)	7·1 (4·8-9·5)	3·4 (2·8-3·9)	4·6 (3·5-5·7)
Rifampicin mono (R_RH_S)		1·4 (0·9-1·8)		2·5 (1·2-3·7)		1·7 (1·1-2·2)
Rifampicin mono ($R_RH_SZ_SE_S$)	0·2 (0·1-0·4)	0·9 (0·5-1·3) *	0·8 (0·4-1·2)	1·8 (0·7-2·9)	0·4 (0·2-0·5)	1·1 (0·6-1·7) *
Rifampicin mono ($R_RH_SZ_RE_S$ / $R_RH_SZ_SE_R$)	0	0·4 (0·1-0·7) *	0·1 (0-0·4)	0·7 (0·2-1·2)	0·02 (0·0-0·1)	0·5 (0·2-0·8) *
Isoniazid	5·7 (4·9-6·5)	7·6 (6·4-8·7)	11·8 (9·3-14·4)	11·1 (9·1-13·1)	7·4 (6·5-8·3	9·3 (7·9-10·7)
Isoniazid mono (R_SH_R)		5·5 (4·6-6·5)		6·5 (5·1-7·9)		6·1 (5·1-7·1)
Isoniazid mono ($R_SH_RZ_SE_S$)	2·6 (2·0-3·2)	4·5 (3·6-5·3) *	2·9 (1·9-4·0)	5·5 (4·3-6·8) *	2·7 (2·2-3·2)	4·9 (4·1-5·8) *
Isoniazid mono ($R_SH_RZ_RE_S$ / $R_SH_RZ_SE_R$)	1·5 (1·2-1·9)	1·1 (0·3-1·8)	2·3 (1·5-3·2)	1·0 (0·4-1·6)	1·7 (1·4-2·1)	1·1 (0·4-1·7)
Ethambutol	0·8 (0·4-1·1)	2·0 (1·2-2·8) *	2·4 (1·5-3·3)	3·5 (2·2-4·8)	1·2 (0·8-1·6)	2·5 (1·7-3·3) *
Streptomycin	4·3 (3·5-5·0)	3·9 (2·8-5·1)	8·1(6·6-9·6)	5·1 (3·8-6·5)*	5·3 (4·7-5·9)	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)
Ofloxacin		1·2 (0·7-1·7)		1·5 (0·7-2·2)		1·4 (0·9-1·8)

R: Rifampicin, H: Isoniazid, Z: Pyrazinamide, E: Ethambutol

Subscripts $_R$: Resistant, $_S$: Susceptible

* Non-overlapping 95% confidence intervals between the two surveys

Table 3: Provincial RR, MDR and RMR prevalence among TB cases, South Africa – 2012-14

Province	New Cases						Previously Treated Cases						Overall					
	RR		MDR		RMR		RR		MDR		RMR		RR		MDR		RMR	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Eastern Cape	2·7	1·5-3·9	1·7	0·8-2·6	1	0·3-1·7	4	1·5-6·5	2·7	0·5-5	1·2	0-2·4	3·3	2·2-4·9	2·1	1·3-3·6	1·1	0·6-2
Free State	3·5	2-5·1	1·8	0·8-2·8	1·8	0·4-3·1	7·3	2·5-12·1	3·9	0·8-7	3·4	0·5-6·3	4·6	3·2-6·6	2·3	1·5-3·6	2·2	1·2-3·9
Gauteng	3·6	2·1-5·2	2·7	1·3-4·1	1	0·3-1·6	9·3	4·8-13·8	6·4	2·6-10·3	2·8	0·3-5·3	4·8	3·4-6·8	3·4	2·3-5·2	1·3	0·8-2·2
KwaZulu Natal	3·5	1·6-5·5	1·8	0·6-3	1·7	0·2-3·2	8·8	3-14·6	6·4	2·3-10·4	2·4	0-4·9	4·9	3·2-7·5	2·9	1·8-4·5	1·9	1-3·8
Limpopo	3·4	2-4·7	1·4	0·4-2·4	2	1·1-2·9	6·2	2·6-9·7	2·5	0-5·1	3·5	0·5-6·5	3·9	2·8-5·5	1·6	0·9-2·9	2·2	1·5-3·4
Mpumalanga	6	4·4-7·7	4·2	2·8-5·6	1·8	0·9-2·7	15·5	9·2-21·7	7·6	3·2-12	7·8	3·5-12·1	8·4	6·5-11	5·1	3·7-7	3	2-4·5
North West	3·1	1·5-4·6	1·9	0·8-3·1	1·1	0·2-2	9·7	5·9-13·4	4·3	1·4-7·1	5·3	2·8-7·9	4·9	3·6-6·8	2·6	1·8-3·9	2·2	1·4-3·5
Northern Cape	2	1·1-3	1·3	0·4-2·1	0·8	0·1-1·4	5	2·5-7·5	2·6	0·8-4·3	2·4	0·5-4·3	3	2·1-4·2	1·7	1-2·8	1·3	0·7-2·3
Western Cape	2·9	1·5-4·3	2	0·7-3·2	0·9	0·3-1·6	6·1	3·6-8·6	4·5	2·1-7	1·5	0·5-2·5	4·2	3·2-5·5	3	2·1-4·2	1·2	0·8-1·8

RR: Rifampicin resistance (R_R), MDR: Multi-drug resistance (R_RH_R), RMR: Rifampicin mono-resistance (R_RH_S)

cases. Regional variation was observed in RMR tuberculosis cases with point prevalence of MDR tuberculosis and RMR tuberculosis notably different in Gauteng province at 3·4% (95% CI 2·3–5·2) and 1·3% (0·8–2·2), and in Western Cape province it was 3·0% (2·1–4·2) and 1·2% (0·8–1·8), respectively (table 3). The MDR to RMR ratio of the point estimates was close to 1:1 for several provinces (appendix p 9). Prevalence among HIV-positive cases was higher than HIV-negative cases for both rifampicin-resistant tuberculosis (4·9%, 95% CI 3·8–6·1 *vs* 3·2%, 2·1–4·3) and MDR tuberculosis (3·1%, 2·2–4·0 *vs* 2·0%, 1·1–2·8; appendix p 8).

The prevalence of any isoniazid resistance nationally (9·3%, 95% CI 7·9–10·7) was higher than that of any rifampicin resistance (4·6%, 3·5–5·7; table 2). The point prevalence of IMR tuberculosis ranged between 5·3% and 8·1% across the nine provinces with no notable difference by previous tuberculosis treatment history (appendix p 6). The prevalence of resistance among tuberculosis cases was relatively low for pyrazinamide (3·7%, 95% CI 2·9–4·5) and the fluoroquinolone ofloxacin (1·4%, 0·9–1·8; table 2).

Second-line drug resistance prevalence was determined among MDR tuberculosis and RMR tuberculosis cases. The prevalence of resistance to drugs used empirically in the treatment of MDR tuberculosis was 44·7% (95% CI 25·9–63·6) for ethionamide and 59·1% (49·0–69·1) for pyrazinamide, contrasting with the point estimate of 5·3% (2·2–8·3) for resistance to para-aminosalicylic acid (table 4). However, among RMR tuberculosis cases, the prevalence of resistance to ethionamide (11·2%, 95% CI 0–23·8) and pyrazinamide (13·9%, 2·0–25·9) were notably lower. Resistance prevalence to the key second-line drug classes, fluoroquinolones (ofloxacin) and injectable antituberculosis drugs, were both 13·0% (95% CI 5·0–21·0; table 4; appendix). Cross-resistance between selected drugs was also assessed (appendix p 7). The XDR tuberculosis prevalence among MDR tuberculosis confirmed cases was 4·9% (95% CI 1·0–8·8) nationally (table 4).

The midpoint estimate of the MDR-TB case burden for 2014 was 8249 and was similar to the number reported as microbiologically confirmed (8035; Table 5). For RR-TB, the estimated burden ranged between 10311 and 16792 while the case burden reported (18631) was higher than the estimate and this was also observed in 3 of the 9 provinces. The number of IMR-TB cases reported in 2014 was 851 and was much lower than the mid-point estimate (17 970; 95%CI: 15 024 – 20 916).

Discussion

The South African Tuberculosis Drug Resistance Survey 2012–14 provides an update of the population level first-line and second-line drug resistance estimates in a country with the highest per capita incidence of tuberculosis globally. The emergence of RMR tuberculosis among new cases and the high levels of second-line resistance are major causes of concern and have important implications for the introduction of new rapid technologies for diagnosis, as well as the use of short regimens and new therapeutic agents. Furthermore, although the number of cases of rifampicin-resistant tuberculosis and MDR tuberculosis diagnosed in the country was comparable to the burden reported through standard-of-care testing, the problem of IMR tuberculosis was largely undetected using current routine testing algorithms. To our knowledge, the present survey was the largest of its kind done globally, with over

Table 4: National second-line drug resistance among MDR and RMR cases, South Africa – 2012-14

Drug	Overall (%, 95% CI)	
	MDR	RMR
Pyrazinamide	59·1 (49·0-69·1)	13·9 (2·0-25·9)
Ethambutol	44·1 (30·2-58·0)	19·3 (0-45·9)
streptomycin	63·0 (52·8-73·2)	16·7 (3·4-30·1)
Ethionamide	44·7 (25·9-63·6)	11·2 (0-23·8)
P-aminosalicylic acid	5·3 (2·2-8·3)	16·2 (0-35·5)
Second-line injectable	13·0 (5·0-20·9)	17·8 (0-41·7)
Ofloxacin	13·0 (5·0-21·0)	10·4 (0-28·3)
XDR-TB	4·9 (1·0-8·8)	

MDR: Multi-drug resistance (R_RH_R), RMR: Rifampicin mono-resistance (R_RH_S)

Table 5: Estimated burden of RR-TB and MDR-TB compared with numbers reported for 2014 in South Africa

Province	*mPTB 2014 reported[8]	RR-TB				MDR-TB			
		lower limit	mid-point	upper limit	**reported	lower limit	mid-point	upper limit	**reported
Eastern Cape	60518	1331	1997	2965	3923	787	1271	2179	2071
Free State	15833	507	728	1045	1008	237	364	570	309
Gauteng	46467	1580	2230	3160	2530	1069	1580	2416	730
KwaZulu Natal	72743	2328	3564	5456	5075	1309	2110	3273	2354
Limpopo	15921	446	621	876	717	143	255	462	113
Mpumalanga	18439	1199	1549	2028	1680	682	940	1291	528
North West	17790	640	872	1210	1036	320	463	694	327
Northern Cape	9607	202	288	403	508	96	163	269	261
Western Cape	37272	1193	1565	2050	2154	783	1118	1565	1342
South Africa	**294590**	**10311**	**13551**	**16792**	**18631**	**5892**	**8249**	**10605**	**8035**

* mPTB: microbiologically confirmed pulmonary TB; source – www.nicd.ac.za **The WHO Global Report 2015 (reports 18734 cases, however it includes 103 cases with an unassigned province).

100 000 people tested. The consistently higher prevalence across provinces of rifampicin and multidrug resistance among HIV-infected individuals, confirms the importance of HIV infection in the tuberculosis epidemic.

The national prevalence of MDR tuberculosis in 2012–14 remained relatively unchanged (2·8%) compared with that reported in 2001–02 (2·9%). Among new cases, the prevalence of MDR tuberculosis was 2·1%, and similar to the global estimate[1] of 3·3%, whereas in previously treated cases it was much lower (4·6% compared with 20%, respectively). This finding might be related to a high mortality rate in the local setting, which was twice as high in 2002 compared with 2013[9] particularly in HIV-infected individuals not on antiretroviral therapy, and thus a second episode would not have occurred. Alternatively, introduction of new diagnostics (eg, line-probe assays in 2008 and Xpert MTB/RIF in 2011) that tested for drug resistance irrespective of treatment history, might have resulted in an effective cure and prevented a recurrence. The absolute number of cases reported for 2014 was in line with the estimate derived from the survey, which is encouraging. However, the survey estimates only included microbiologically confirmed cases; whereas 34·3% of cases notified in South Africa in 2014 were clinically diagnosed,[1] the absolute number is likely an underestimate on our part.

Contributory factors for the higher provincial prevalence of MDR tuberculosis observed in Mpumalanga (5·1%) than the national estimate (2·8%), might include cross-border migration from neighbouring countries such as Swaziland, which has reported the highest MDR tuberculosis prevalence in the region.[10] This finding illustrates the need for a regional approach in dealing with efforts aimed at combating drug-resistant tuberculosis. Additionally, this province also had the highest prevalence of tuberculosis–HIV coinfection (77%) in the current survey, and other socioeconomic factors might also have contributed.

The significant difference between the rifampicin-resistant tuberculosis and MDR tuberculosis estimates highlights the increasing relevance of RMR tuberculosis, which contributes to the growing drug-resistant tuberculosis crisis and counters the simplistic dogma that rifampicin-resistant tuberculosis and MDR tuberculosis are synonymous. Significant increases in rifampicin resistance among new cases compared with the previous survey (table 2) were observed, almost doubling from 1·8% to 3·4%. The same trend was evident at the provincial level, with increases in point estimates observed across all provinces among new cases. Increases in rifampicin resistance among new cases indicate primary resistance driven by transmission, which is of concern in the South African context with its high rates of HIV infection, now being coupled to an increased risk of acquiring rifampicin-resistant *M tuberculosis* complex infection. The introduction of Xpert MTB/RIF as a primary diagnostic tool targeting presumptive tuberculosis cases enables simultaneous detection of tuberculosis and rifampicin resistance. Widespread adoption is essential for early diagnosis of primary drug-resistant cases, which would be missed if only retreatment cases were tested. Additionally, the higher prevalence of rifampicin-resistant tuberculosis and MDR tuberculosis among HIV-infected cases, highlights the importance of using this technology universally in high burdened HIV-infected settings.

The WHO-approved mycobacteria growth indicator tube methodology was used for drug susceptibility testing in the present survey, but recent data indicate that this methodology might record false

susceptible findings in strains harbouring specific *rpoB* mutations and could account for more than 10% of cases.[11] Thus, our rifampicin-resistant tuberculosis estimates might be an underestimate in the survey, but would have been detected with the currently used molecular diagnostic methods. This possibly explains the higher case burden reported in 2014 compared with the burden estimate derived from the survey findings (table 5).

The prevalence of RMR tuberculosis in South Africa has also increased substantially since the 2001–02 survey, notably among new cases, and is the primary reason for the doubling in rifampicin-resistant tuberculosis. Clonal transmission has been shown to be an important driver of RMR tuberculosis and other drug resistance.[12, 13, 14] Younger patients (aged 25–29 years), who are less likely to have had previous tuberculosis treatment exposure have also been shown to be at increased risk of RMR tuberculosis.[15] Emergence of single drug resistance is unusual when standard combination therapy is used. Adequate dosage concentrations are crucial and concerns raised about the current rifampicin dosage being too low have important global implications.[16] This problem is compounded when patients are on concurrent antiretroviral therapy, abuse alcohol, or take treatment irregularly, all of which have been associated with rifampicin mono-resistance related to deficient drug bioavailability.[17, 18] Although RMR strains might have originated through selection of rifampicin resistance during treatment, transmission will increase if left unchecked.

The WHO estimate in 2014 for MDR tuberculosis cases in South Africa was 6200, whereas 18 734 rifampicin-resistant tuberculosis and MDR tuberculosis cases were reported from the country.[1] This WHO estimate for MDR tuberculosis alone was lower than the survey estimate (n=8249) and importantly did not take into account RMR tuberculosis. The latter is essentially managed as MDR tuberculosis, and as observed in the current survey, accounts for a large (39% of all rifampicin-resistant strains) and expanding burden. This major shortcoming in WHO reporting has been addressed in the 2016 report and more accurately reflects the true burden of drug-resistant tuberculosis in South Africa, which is now estimated at 20 000 cases.

Furthermore, the number of rifampicin-resistant and MDR tuberculosis cases reported by South Africa accounts for 18 734 (73%) of 25 531 cases in Africa, while among notified tuberculosis cases it was 318 193 (24%) of 1 342 400 cases. The disproportionately higher number of rifampicin-resistant and MDR tuberculosis cases from South Africa appears to be an outlier. However, the survey does confirm the high case burden of MDR tuberculosis notified from South Africa, which is likely undetected in less resourced African countries and could undermine WHO's END-TB strategy if improvement in access to laboratory testing is not addressed.

Significant increases in overall IMR have also been noted, increasing from 2·7% in the 2001–02 survey to 4·9% in the current survey. There was no significant difference in IMR prevalence between new and previously treated cases, suggesting that previous tuberculosis combination therapy is unlikely to contribute to IMR. The IMR point estimate, irrespective of resistance to other first-line drugs, was 5% or more in all provinces (appendix p 6). IMR tuberculosis prevails across many settings in the world and in a meta-analysis by Menzies and colleagues[19] it accounted for almost half of all tuberculosis drug resistance.

Globally, South Africa has one of the largest isoniazid preventative therapy programmes and both previous isoniazid preventative therapy and previous tuberculosis therapy, and younger age groups were identified as risk factors.[20] An association between isoniazid preventative therapy and IMR or other drug resistance has not been shown in a WHO-initiated review of published data.[21] However, a model-based study on community-administered isoniazid preventative therapy[22] has suggested that this is likely to occur at a population level and could be missed when analysing studies involving small numbers of patients. The use of rifapentine in combination with isoniazid as preventative therapy does offer a promising approach to prevent the emerging risk of IMR tuberculosis and results of clinical trials are awaited.

The estimated case burden of IMR tuberculosis in 2014 was almost 20-fold higher than the reported number of diagnosed cases through the public sector laboratories in the country. Xpert MTB/RIF, which has ensured every newly diagnosed case of tuberculosis in South Africa can be concurrently tested for rifampicin resistance, does not test for isoniazid resistance. A review on IMR tuberculosis has noted poorer clinical outcomes in such cases[23] and thus consideration needs to be given for all tuberculosis cases diagnosed being tested for isoniazid resistance or alternatively strengthening of the continuation regimen with a third agent. Furthermore, inadequate treatment of these cases would in effect result in rifampicin monotherapy during the continuation phase and over time lead to an increase in MDR tuberculosis, as has been previously shown.[24]

The present survey is the first to provide population level estimates of second-line resistance in South Africa. Although the frequency of resistance to fluoroquinolones was relatively low at 13% among MDR tuberculosis cases, high rates of resistance to companion drugs prevailed. The ethionamide resistance rate was 44·7%. MDR tuberculosis cases are by definition isoniazid-resistant and mutations in the *inhA* promoter region, accounting for approximately 8–43% of isoniazid-resistant strains,[25] would confer cross-resistance to ethionamide. Information on *inhA* mutations is available through the use of line-probe assays and could be used to guide therapeutic decision making. Pyrazinamide, another drug used in drug resistance regimens, has potent sterilising activity but among MDR cases more than half showed resistance to this drug (59·1%, 95% CI 49·0–69·1). Our finding is corroborated by other studies showing similarly high prevalence of pyrazinamide resistance.[24, 26, 27] With these high rates of resistance, further selection of resistance and consequently poor patient outcomes are likely to persist, unless new strategies and drugs are developed—this should be a global priority.

WHO has endorsed the use of the line-probe assay for second-line resistance testing to rapidly identify pre-XDR and XDR tuberculosis cases,[28] constituting an important step in selecting rifampicin-resistant tuberculosis and MDR tuberculosis cases for the new seven-drug combination short-course regimen. The XDR cartridge on the GeneXpert platform, although not currently available, is an urgent need in light of the resistance levels observed and efforts to decentralise drug-resistant tuberculosis management. Although the background second-line resistance is a concern, rifampicin-resistant and RMR tuberculosis cases, which are on the increase, show lower levels of resistance and will be best suited for the short course regimen. Furthermore, isoniazid, even at a standard dosage for these RMR tuberculosis cases, would provide an effective oral agent. The inclusion of clofazimine, although not tested in the present survey, is likely to show low resistance prevalence in patients with rifampicin-resistant tuberculosis and

MDR tuberculosis since the drug was historically reserved for pre-XDR tuberculosis and XDR tuberculosis cases. Thus, at least three drugs are likely to be effective despite the worrying findings from this survey.

The situation for pre-XDR tuberculosis and XDR tuberculosis cases accounting for more than one in every eight rifampicin-resistant tuberculosis cases, is however less promising unless an aggressive approach is taken to consider all the new drugs (eg, bedaquiline and delamanid) and repurposed drugs (eg, linezolid) for the development of an effective combination regimen for these cases. One drug to which very low levels of resistance have been encountered is para-aminosalicylic acid, which is re-emerging as a therapeutic option,[29] although drug tolerability concerns have limited its use. Despite the excitement with the introduction of new drugs for tuberculosis, investment in drug discovery is still needed because these new drugs are arriving just in time to address a current and dire need but leave nothing for the future.

The XDR tuberculosis estimate among MDR tuberculosis cases in this survey of 4·9% was lower than that reported globally at 9·7%,[1] but the difference was not significant. This finding suggests that the XDR tuberculosis problem that has seen two outbreaks during the period between the two drug resistance surveys has not become widespread across the country. A contributory factor could have been the high mortality associated with these cases. Additionally, XDR tuberculosis cases could have been concentrated in certain provinces or districts and this survey might not have been powered to assess the distribution of such cases.

The findings of this survey are important but should be seen in the context of certain limitations. This large nationwide survey was done using existing health services in a resource constrained setting. The recording of previous treatment history was by self-report and prone to recall bias; however, the retreatment rates reported here are comparable to findings observed in the 2001–02 survey. Patient screening was not consistently consecutive and a large proportion of cases were not included. Non-consecutive recruitment is unlikely to have an impact on the survey outcomes because the study population included confirmed tuberculosis cases only, and predicting tuberculosis in patients would be difficult, even for experienced clinicians. Non-inclusion did not show specific geographical localisation and was therefore likely to be random. To address these concerns, imputation and inverse probability weighting were applied, and although the estimates were similar,[30] we have reported on the adjusted data. Lastly, drug susceptibility testing has inherent limitations and is less reliable for second-line drugs. However, the survey used established procedures at an International Organization for Standardization-accredited reference laboratory, which is part of the WHO supranational reference laboratory network, and showed good performance in the external quality assurance programme for both first-line and second-line drug testing. In addition, and where available, sequencing was done to cross-check resistance profiles.

Contributors

NAI, LM, CI and SAM were involved in the conception and design of the study. NAI, LM, CI, AN SV and AD were involved in study implementation. NAI, AN and AD did the data analysis. NAI, LM, AN, AD, SV, SB,

TM, MvdW, AA, VD, CI and SAM interpreted the data and provided important intellectual input. NAI, AN, SV, CI and SAM wrote the first draft.

Conflict of interest

We declare we have no conflicts of interest

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Research in context

Evidence before this study

We searched PubMed for original research that presented results for national and provincial prevalence rates of drug resistant tuberculosis in South Africa published in English between Jan 1, 2000, and December 31, 2017. We combined search terms for tuberculosis/TB, prevalence, drug resistance, survey/DRS and South Africa and looked for studies indicating population level prevalence estimates for first line and second line resistance ("rifampicin", "isoniazid", "MDR", "XDR"). Only one prior national drug resistance survey was conducted in South Africa and this was in 2001-2. Although these surveys are recommended to be conducted every 5 years this was not done. The previous survey showed low levels of MDR-TB and showed a relatively low MDR-TB rate of 1·6% (95% CI: 1·1%-2·1%) in new cases and 6·6% (95% CI: 4·9%-8·2%) in retreatment cases. Several publications have highlighted the emergence of XDR-TB in Kwa-Zulu Natal and Eastern Cape provinces and more recently the emergence of RMR-TB in South Africa. Studies have also highlighted person-to-person transmission as an important driver of drug resistant TB in South Africa. However, these have been either small studies or geographically restricted and not designed to provide population level estimates at a national or provincial level for the different types of drug resistance.

Added value of this study

The current survey provides updated first line TB drug resistance prevalence estimates for South Africa that have been long overdue. Increase in rifampicin resistance nationally among new cases suggests ongoing transmission as a primary reason and is widespread requiring universal testing for drug resistance. Isoniazid resistance was above 10% nationally and has implications for TB preventative therapy. Both ofloxacin and pyrazinamide were also tested among TB cases and provides country level information on these widely used companion drugs in new regimens being trialed. For the first time population level second line resistance is reported for South Africa and is worrying. Commonly used second line agents have shown high prevalence of resistance with almost half being resistant to pyrazinamide and more than a quarter resistant to ethionamide. However, the national population prevalence of XDR-TB was similar to the global average.

Implications of all the available evidence

Our findings support the universal roll-out of the Xpert MTB/Rif assay for the early detection of drug resistant TB irrespective of prior treatment history. The endorsement of the first and now second line molecular assays is a timely improvement to ensure that patients on the new short MDR-TB regimen are appropriately managed and second line resistance excluded early. South Africa uses isoniazid mono-therapy for prevention and an evaluation of the impact of the intervention on emerging resistance is needed. In addition, combination therapies for prevention (e.g. rifapentine and isoniazid) should be considered in light of the findings.

References

1. WHO. Global TB Report 2015. 2015. http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf?ua=1 (accessed 11 Jan 2017.
2. Weyer K, Brand J, Lancaster J, Levin J, van der Walt M. Determinants of multidrug-resistant tuberculosis in South Africa: results from a national survey. *S Afr Med J* 2007; **97**(11 Pt 3): 1120-8.
3. Marisa K, Robin Mark W, Cindy H, et al. Emergence and Spread of Extensively and Totally Drug-Resistant Tuberculosis, South Africa. *Emerging Infectious Disease journal* 2013; **19**(3): 449.
4. Ndjeka N. Strategic overview of MDR-TB care in South Africa. 2014. http://www.health-e.org.za/wp-content/uploads/2014/03/Strategic_overview_of_MDR_TB_RSA.pdf (accessed 15 April 2016.
5. Ndjeka N, Conradie F, Schnippel K, et al. Treatment of drug-resistant tuberculosis with bedaquiline in a high HIV prevalence setting: an interim cohort analysis. *Int J Tuberc Lung Dis* 2015; **19**(8): 979-85.
6. WHO. Guidelines for surveillance of drug resistance in tuberculosis: World Health Organization; 2015.
7. Salman Siddiqi SR-G, Heather Alexander, Francis Drobniewski, Knut Feldman. MGIT Procedure Manual for Bactec MGIT 960 TB system. 2006. http://www.finddiagnostics.org/export/sites/default/resource-centre/find_documentation/pdfs/mgit_manual_nov_2007.pdf (accessed 3 January 2017.
8. NICD. Microbiologically confirmed tuberculosis 2004-2015: South Africa. 2017. http://www.nicd.ac.za/index.php/centres/centre-for-tuberculosis/public-health-surveillance/surveillance-of-microbiologically-confirmed-tb/ (accessed 5 April 2017.
9. STATSSA. MDG Goal 6: Combat HIV, Malaria and other diseases. 2015. http://www.statssa.gov.za/MDG/MDG_Goal6_report_2015_.pdf (accessed 01 June 2017.
10. Sanchez-Padilla E, Dlamini T, Ascorra A, et al. High prevalence of multidrug-resistant tuberculosis, Swaziland, 2009-2010. *Emerging infectious diseases* 2012; **18**(1): 29-37.
11. Rigouts L, Gumusboga M, de Rijk WB, et al. Rifampin Resistance Missed in Automated Liquid Culture System for Mycobacterium tuberculosis Isolates with Specific rpoB Mutations. *Journal of Clinical Microbiology* 2013; **51**(8): 2641-5.
12. Shah NS, Auld SC, Brust JC, et al. Transmission of Extensively Drug-Resistant Tuberculosis in South Africa. *N Engl J Med* 2017; **376**(3): 243-53.
13. Dheda K, Limberis JD, Pietersen E, et al. Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *The Lancet Respiratory Medicine*.
14. Yoshida S, Tsuyuguchi K, Suzuki K, et al. [Molecular epidemiology of rifampicin mono-resistant Mycobacterium tuberculosis]. *Kekkaku* 2012; **87**(2): 41-5.
15. Coovadia YM, Mahomed S, Pillay M, Werner L, Mlisana K. Rifampicin mono-resistance in Mycobacterium tuberculosis in KwaZulu-Natal, South Africa: a significant phenomenon in a high prevalence TB-HIV region. *PLoS One* 2013; **8**(11): e77712.
16. van Ingen J, Aarnoutse RE, Donald PR, et al. Why Do We Use 600 mg of Rifampicin in Tuberculosis Treatment? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011; **52**(9): e194-9.
17. Meyssonnier V, Bui TV, Veziris N, Jarlier V, Robert J. Rifampicin mono-resistant tuberculosis in France: a 2005-2010 retrospective cohort analysis. *BMC Infect Dis* 2014; **14**: 18.

18. Sanders M, Van Deun A, Ntakirutimana D, et al. Rifampicin mono-resistant Mycobacterium tuberculosis in Bujumbura, Burundi: results of a drug resistance survey. *Int J Tuberc Lung Dis* 2006; **10**(2): 178-83.
19. Menzies D, Benedetti A, Paydar A, et al. Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS medicine* 2009; **6**(9): e1000150.
20. Cattamanchi A, Dantes RB, Metcalfe JZ, et al. Clinical Characteristics and Treatment Outcomes of Isoniazid Mono-Resistant Tuberculosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009; **48**(2): 179-85.
21. WHO. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resourceconstrained settings. 2011. http://apps.who.int/iris/bitstream/10665/44472/1/9789241500708_eng.pdf (accessed 18 January 2017.
22. Mills HL, Cohen T, Colijn C. Community-wide isoniazid preventive therapy drives drug-resistant tuberculosis: a model-based analysis. *Science translational medicine* 2013; **5**(180): 180ra49.
23. Jacobson KR, Theron D, Victor TC, Streicher EM, Warren RM, Murray MB. Treatment Outcomes of Isoniazid-Resistant Tuberculosis Patients, Western Cape Province, South Africa. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 2011; **53**(4): 369-72.
24. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. *The International Journal of Tuberculosis and Lung Disease* 2015; **19**(11): 1276-89.
25. Mphahlele M, Syre H, Valvatne H, et al. Pyrazinamide Resistance among South African Multidrug-Resistant Mycobacterium tuberculosis Isolates. *Journal of Clinical Microbiology* 2008; **46**(10): 3459-64.
26. Alame-Emane AK, Xu P, Pierre-Audigier C, et al. Pyrazinamide resistance in Mycobacterium tuberculosis arises after rifampicin and fluoroquinolone resistance. *The International Journal of Tuberculosis and Lung Disease* 2015; **19**(6): 679-84.
27. Louw G, Warren R, Donald P, et al. Frequency and implications of pyrazinamide resistance in managing previously treated tuberculosis patients. *The International Journal of Tuberculosis and Lung Disease* 2006; **10**(7): 802-7.
28. WHO. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs. 2016. http://apps.who.int/iris/bitstream/10665/246131/1/9789241510561-eng.pdf?ua=1 (accessed 07 February 2017.
29. Donald PR, Diacon AH. Para-aminosalicylic acid: the return of an old friend. *Lancet Infect Dis* 2015; **15**(9): 1091-9.
30. NICD. South African Tuberculosis Drug Resistance Survey 2012-14. 2016. http://www.nicd.ac.za/?page=south_african_tuberculosis_drug_resistance_survey&id=232 (accessed 3 February 2017.

Supplementary Methods

Definitions

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 smear-positive 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])

Survey patient enrolments

A standardised case report form (CRF) was used at all survey facilities collecting demographic, clinical, enrolment criteria and risk factor information and was administered by the existing routine healthcare workers in the selected facilities, no additional staff were employed for this activity. The CRF was accompanied by an informed consent form 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.

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.

Completed DRS case report forms received from the facilities, including the printed unique laboratory number, were manually double-captured in provincial batches 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.

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.

Data analysis

Both descriptive and statistical analysis accounting for the complex multistage sampling strategy and clustering of patients within primary sampling units were performed. The consort diagram is shown in Supplementary Figure 1. 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).

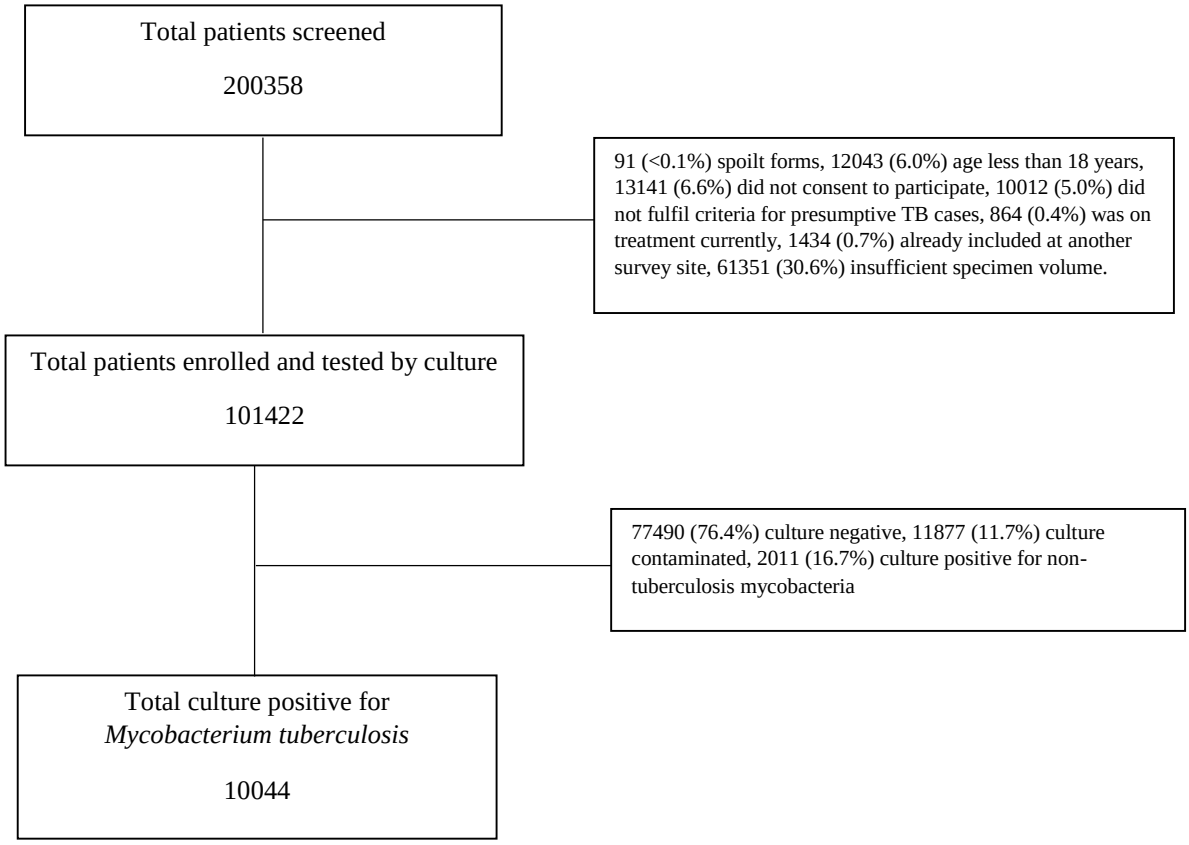
Additionally, patterns of missing data in key variables were tabulated by cluster and province. These variables included: cluster, province, age, sex, previous treatment history and an assessment made on the randomness of the missing values. After performing these tasks, a consultation was held with 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 (Figure 1). 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.

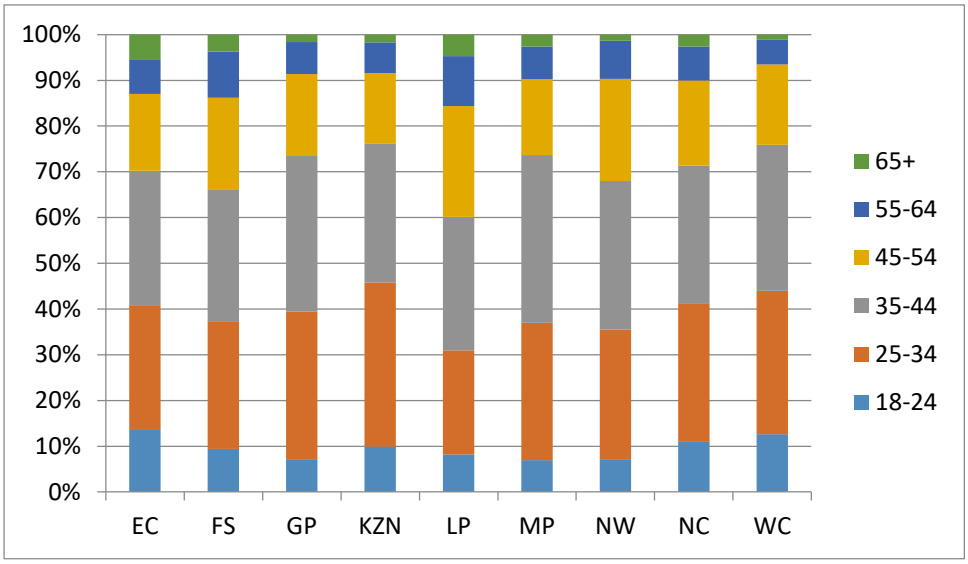
The estimates were then tabulated for resistance among new and retreatment cases, as well as overall and compared to the individual level crude analysis and cluster level analysis for each province. These were then also compared during analysis using logistic regression with robust standard errors (RSE) 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 appreciable 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.

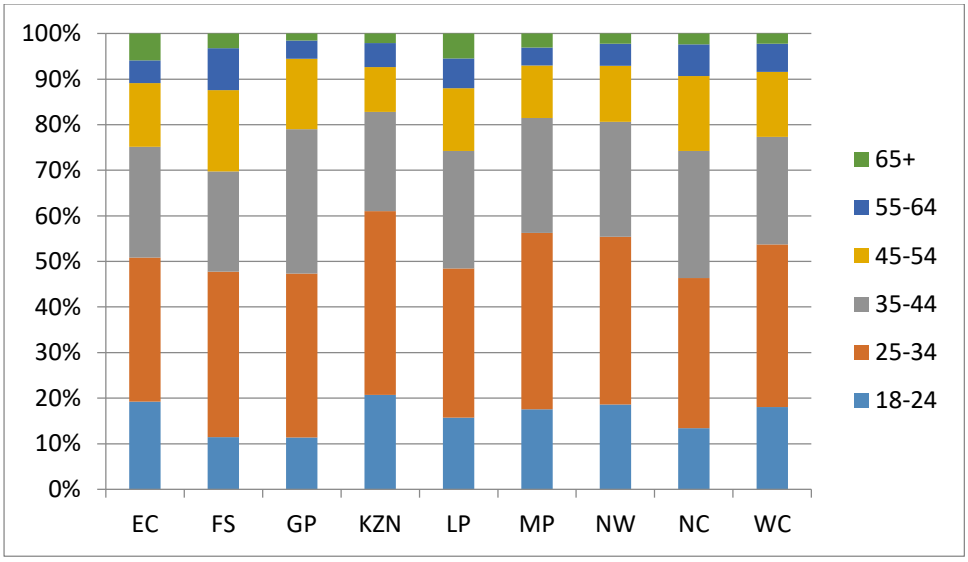


Supplementary Figure 1: Consort diagram of patients screened, enrolled and culture positive for *Mycobacterium tuberculosis*

*missing data imputed: age group (1.5%), sex (1.8%), previous treatment history (16.6%), drug susceptibilities [rifampicin (5.8%), isoniazid (5.7%), ethambutol (15.2%), streptomycin (15.1%), pyrazinamide (15.1%), second line injectable (19.2%) and fluoroquinolones (19.1%)



Supplementary Figure 2: Age distribution among males by province among confirmed TB cases in the survey



Supplementary Figure 3: Age distribution among females by province among confirmed TB cases in the survey

Supplementary Table 1: Provincial IR and IMR prevalence among TB cases, South Africa – 2012-14

Province	New Cases				Previously Treated Cases				Overall			
	IR		IMR		IR		IMR		IR		IMR	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Eastern Cape	7·1	4·9-9·3	5·4	3·3-7·5	10	6·1-13·9	7·2	4·1-10·3	8·9	6·6-12	6·4	4·6-9
Free State	8·8	6·4-11·1	7	4·9-9·1	10·1	5·2-15	6·1	2·2-10	10	7·8-12·9	7·3	5·6-9·6
Gauteng	7·5	5·4-9·5	4·8	3·3-6·3	12·8	7·3-18·3	6·3	2·7-10	9·2	7·2-11·7	5·3	4·1-6·9
KwaZulu Natal	6·6	3·5-9·7	4·8	2·1-7·4	12·5	6·4-18·5	6	2·3-9·8	8·5	5·9-12·4	5·3	3·3-8·5
Limpopo	6·6	4·7-8·4	5·1	3·8-6·5	7·1	3·2-11	4·5	1·3-7·6	7·1	5·5-9·1	5·3	4·1-6·9
Mpumalanga	10·5	8-13·1	6·3	4-8·7	14·6	7·6-21·6	6·9	2·6-11·2	12·7	9·8-16·5	6·9	4·8-9·9
North West	7·7	6-9·5	5·8	4·3-7·2	9·4	5·6-13·2	5·1	2·1-8·1	8·9	7·2-11	6	4·6-7·7
Northern Cape	8·5	7·2-14·1	7·2	5·4-9·2	10·7	7·2-14·1	8·1	4·8-11·4	10·1	8·2-12·5	8·1	6·4-10·3
Western Cape	8·9	6·5-11·3	6·9	5·1-8·7	11·1	7-15·3	6·6	3·7-9·5	10·8	8·5-13·7	7·3	5·5-9·7

IR: Isoniazid resistant (H_R), IMR: Isoniazid mono-resistant (R_SH_R)

Supplementary Table 2: Cross-resistance between selected drugs among MDR-TB cases, South Africa – 2012-14

Cross Resistance	MDR		
	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%

R: number of isolates resistant, N=Number of isolates tested

Supplementary Table 3: National and provincial prevalence of RR- and MDR-TB stratified by HIV status, South Africa – 2012-14

	RR-TB				MDR-TB			
	HIV negative		HIV positive		HIV negative		HIV positive	
Province	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Eastern Cape	1·3	0·1-2·5	4·6	2·4-6·8	0·6	0-1·4	3·2	1·4-5·0
Free State	2·9	0·7-5·1	4·5	2·5-6·5	1·1	0-2·5	2·4	1·2-3·6
Gauteng	2·7	0·4-5·1	5·3	3·5-7·1	2·5	0·3-4·7	3·7	2·3-5·1
KwaZulu Natal	4·5	1·2-7·9	4·5	2·5-6·5	2·3	0·1-4·5	2·8	1·2-4·4
Limpopo	2·4	0·9-4·0	4·6	2·8-6·4	1·3	0-2·7	1·8	0·6-3·0
Mpumalanga	7·2	3·5-10·9	7·8	5·8-9·8	4·9	2·4-7·5	4·7	3·1-6·3
North West	3·5	1·2-5·9	5·0	3·2-6·8	1·6	0-3·6	2·8	1·6-4·0
Northern Cape	2·7	1·3-4·1	3·2	2·0-4·4	1·7	0·7-2·7	1·7	0·7-2·7
Western Cape	3·3	1·9-4·7	5·3	3·3-7·3	2·5	1·5-3·5	3·7	1·9-5·5
South Africa	**3·2**	**2·1-4·3**	**4·9**	**3·8-6·1**	**2·0**	**1·1-2·8**	**3·1**	**2·2-4·0**

Supplementary Table 4: Ratio of MDR-TB to RMR-TB point prevalence estimate stratified by province, South Africa – 2012-14

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