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Supplementary webappendix

This webappendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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National and sub-national cross sectional survey of drug resistant tuberculosis prevalence and imputed burden in South Africa

Appendix

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).

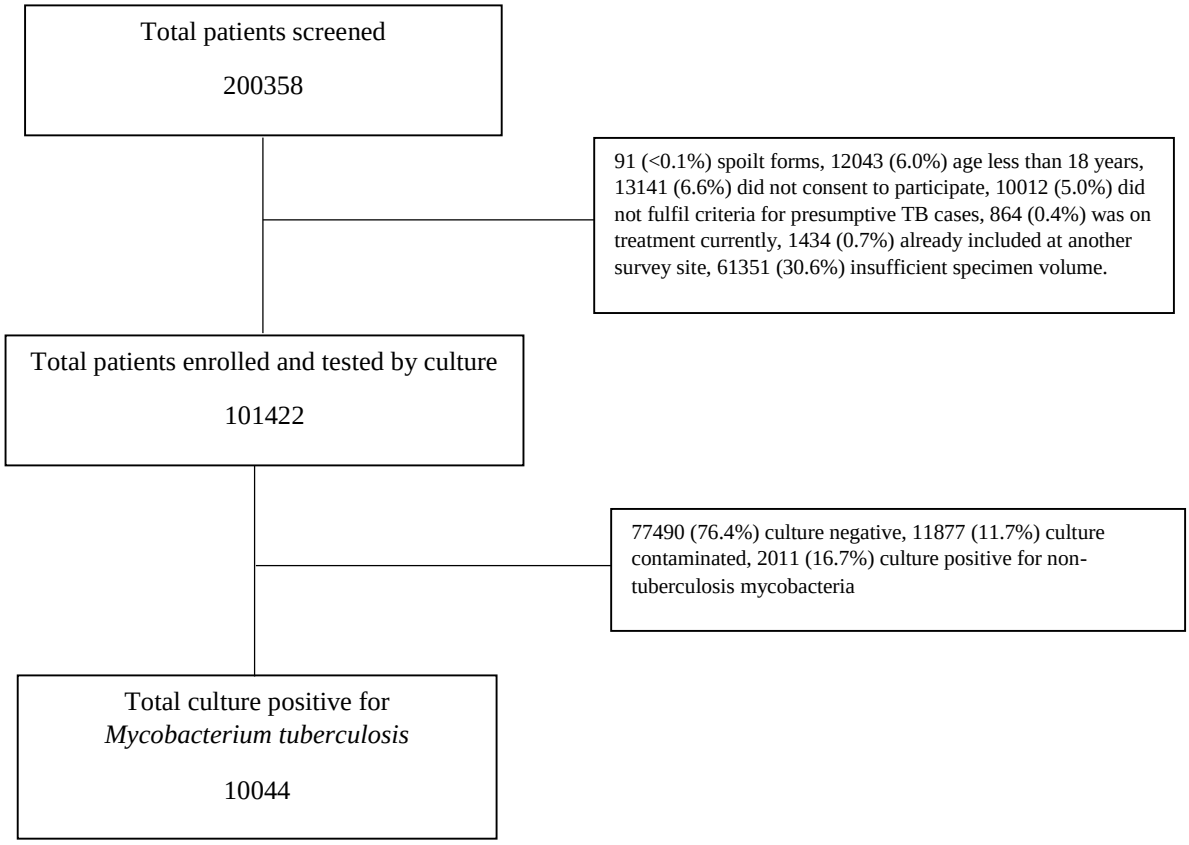
Additionally, patterns of missing data in key variables were tabulated by cluster and province. These variables included: cluster, province, age, sex, previous treatment history and an assessment made on the randomness of the missing values. After performing these tasks, a consultation was held with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

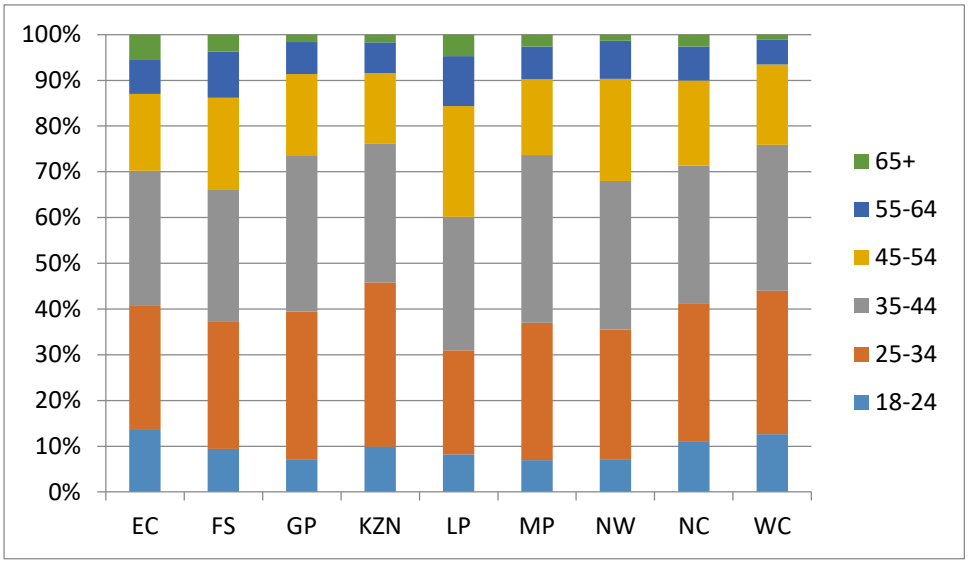
The estimates were then tabulated for resistance among new and retreatment cases, as well as overall and compared to the individual level crude analysis and cluster level analysis for each province. These were then also compared during analysis using logistic regression with robust standard errors (RSE)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

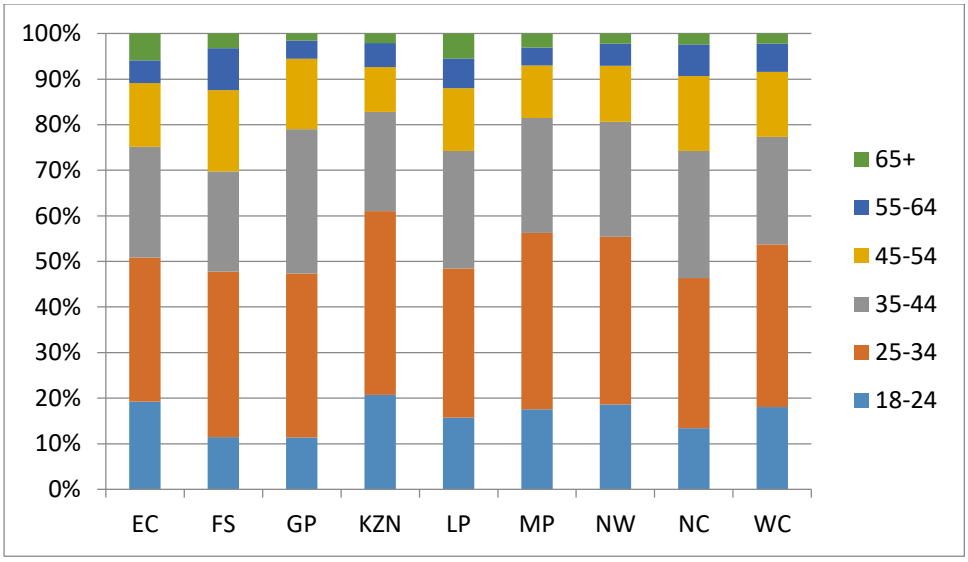


Supplementary Figure 1: Consort diagram of patients screened, enrolled and culture positive for *Mycobacterium tuberculosis*

*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