Transmission of tuberculosis in high school students in Worcester, South Africa

By

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University of Cape Town

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Declaration

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1) Bunyasi, E.W., Schmidt, B., Abdullahi, L.H., *et al.* Prevalence of latent tuberculosis infection and tuberculosis disease among adolescents in high tuberculosis burden countries in Africa: a systematic review protocol. *BMJ Open* 2017;7.
2) Bunyasi, E.W., Geldenhuys H., Mulenga H., *et al.* Temporal trends in prevalence of *Mycobacterium tuberculosis* infection in South African adolescents. *Int J Tuberc Lung Dis* 23(5):571–578.
3) Bunyasi, E.W., Mulenga H., Luabeya A. K. K., *et al.* Regional Changes in tuberculosis disease burden among South African adolescents (2005–2015). *PLoS One* (in press).

Signature …… Signed by candidate ……………………..………. Date ………… 2nd December 2019.…….

Student name …… Erick Wekesa Bunyasi ……………… Student number …... BNYERI001 ………

Dedication

To my mom Mary for your earnest prayers,

my dad Joseph for support,

and my seven siblings for shared experiences.

Always there for me,

even when I wasn't.

Afar in pursuit of knowledge, wisdom and imagination.

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Thesis Abstract

Introduction

Although adolescents have the highest force of *Mycobacterium tuberculosis* infection[1] and rapidly increasing burden of tuberculosis (TB) disease through 10–19 years of age,[2] there are few studies on adolescent *Mycobacterium tuberculosis* infection, transmission, and TB disease in the WHO African region. Adolescents in the high TB burden countries of Africa are therefore an important, but neglected risk group for global TB control efforts. Adolescents spend a considerable amount of their time in school classrooms, but there is paucity of data on classroom risk of *Mycobacterium tuberculosis* transmission. To the best of our knowledge, no published study has conducted measurement of air quality and air sampling for *Mycobacterium tuberculosis* DNA in school classrooms, a novel approach that may support targeted TB disease case–finding strategies which may be more efficient than symptom–based TB screening in the congregate school setting.

The overall aims of this PhD project were:

1) To conduct a systematic review of adolescent latent TB infection (LTBI) and TB disease prevalence, and to examine the relationship between adolescent *Mycobacterium tuberculosis* infection and TB disease rates, in high TB burden African countries.
2) To describe temporal changes in prevalence of LTBI among adolescents living in a single TB endemic South African community, across two time periods spanning the decade 2005–2015.
3) To describe temporal changes in adolescent TB disease notification rates in the same community for the decade 2005–2015.
4) To determine classroom ventilation risk for *Mycobacterium tuberculosis* transmission in tandem with a pilot study of air sampling for *Mycobacterium tuberculosis* DNA; and to investigate the operational feasibility and yield of a pragmatic, symptom–based approach to TB disease surveillance in high schools.

Methods

To achieve Aim 1, we performed a bibliographic database search for studies conducted and published between 1990 and 2018 on prevalence of adolescent (10–19 years) LTBI and TB disease in high TB burden African countries. We calculated the ratio between the number of *Mycobacterium tuberculosis* infections based on Annual Risk of TB Infection (ARTI) estimates and the number of microbiologically–confirmed TB disease cases per year, and compared the observed ratio to the expected ratio of 8–12 published by Styblo *et al.*[3]

To achieve Aim 2, we collected adolescent LTBI (defined by positive QuantiFERON®–TB Gold In–Tube test) prevalence data from eight South African high schools, spanning the decade 2005–2015, from databases of an adolescent cohort study (2005–2007) and an adolescent vaccine trial (2014–2015). We used the two–sample test of equality of proportions to compare changes in LTBI prevalence over the two periods.

To achieve Aim 3, we collected adolescent TB disease notification data from the same community (using an electronic tuberculosis disease register) for the decade 2005–2015 and we used the Mann–Kendall test to explore temporal changes in notification rates.

To achieve Aim 4, we conducted a cross sectional study of 72 classrooms occupied by 1,836 high school learners, in addition to 7 comparator clinic spaces selected for high *a priori* risk of *Mycobacterium tuberculosis* transmission, and performed ventilation (carbon dioxide concentration) measurement to define spaces with high ventilation risk (>1,000 ppm) and ddPCR air sampling for *Mycobacterium tuberculosis* DNA, with active TB symptom screening among learners.

Results

1) There is paucity of data on adolescent LTBI and TB disease prevalence in high TB burden African countries (1990–2018). Based on the limited available data, both LTBI (16%–55%)[4–8] and TB disease

prevalence rates are high (180–679 cases per 100,000),[6–10] but corresponding infection–to–disease ratios are inconsistently low compared to that expected from Styblo's Rule.[3]

2) Overall adolescent LTBI prevalence remained high and relatively unchanged (44–49%) between 2005–2015.[11] However, although average LTBI prevalence was unchanged in lower socio–economic quintile schools, prevalence increased in highest socio–economic quintile schools (from 20% to 38%).[11]

3) Adolescent TB disease notification rates fell 45% (662 to 361 per 100,000) in the same community over the same period. Despite this decrease, recent TB disease prevalence remains high and is three–fold higher in older (15–19 years) than younger (10–14 years) adolescents (566 vs. 151 per 100,000 in 2015).

4) More than one–third of 72 high school classrooms were inadequately ventilated and one–fifth of classrooms had evidence of airborne *Mycobacterium tuberculosis* DNA detected by ddPCR air sampling. The average risk of inhaling 1 *Mycobacterium tuberculosis* DNA copy was similar between clinics and classrooms. Across all classrooms the average risk of a classroom occupant inhaling 1 *Mycobacterium tuberculosis* DNA copy over 1 lesson (35 minutes) was 0.71%; and the estimated risk over 1 academic year was 100%. However, yield from symptom–based TB screening was low, consistent with the presence of undiagnosed subclinical TB cases and risk of ongoing transmission in the school setting.

Conclusion

Despite the encouraging decline in adolescent TB disease notification rates observed between 2005–2015 in the study area, adolescent LTBI prevalence remains high, consistent with ongoing medium–term transmission. The relatively high proportion of inadequately ventilated classrooms would place learners at high risk of *Mycobacterium tuberculosis* transmission if exposed to an infectious occupant. This risk appears material, given the proportion of classrooms with a positive ddPCR air filtrate sample and the estimated cumulative risk of inhaling of at least one copy of *Mycobacterium tuberculosis* DNA. The presence of previously undiagnosed TB cases among learners is inferred from our classroom ddPCR air sampling data, which further suggest that pragmatic school–based TB symptom screening is an inefficient surveillance

strategy that likely missed learners with subclinical TB. Improved ventilation in school classrooms is a low–cost intervention that may reduce the risk of TB transmission in schools. New and more efficient targeted TB disease case–finding strategies are needed for congregate settings, including schools, in high TB burden countries. Based on our preliminary data, classroom ddPCR air sampling for *Mycobacterium tuberculosis* DNA appears feasible for this purpose, but requires further research to optimise diagnostic accuracy and demonstrate cost–effectiveness and public health value in high TB burden countries.

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Table of Contents

Declaration ii

Dedication iv

Acknowledgements v

Thesis Abstract vi

Table of Contents x

List of Tables xv

List of Figures xvi

List of Abbreviations xvii

1. Introduction 1

1.1. Overview 1

1.2. Rationale 1

1.3. Thesis structure 2

1.4. References 5

2. Systematic literature review of prevalence of latent *Mycobacterium tuberculosis* infection and tuberculosis disease among adolescents in high TB burden countries in Africa 7

2.1. Overview 7

2.2. Chapter abstract 9

Publication 11

2.3. Abstract 13

2.4. Introduction 14

2.5. Methods 17

2.5.1. Objectives 17

2.5.2. Definitions 17

2.5.3. Eligibility criteria 18

2.5.4. Search strategy 20

2.5.5. Selection of studies 20

2.5.6. Data management 21

2.5.7. Data extraction 21

2.5.8. Approach to missing data 22

2.5.9. Assessment of risk of bias of included studies 22
2.5.10. Data analysis 24
2.5.11. Data synthesis 24
2.5.12. Assessment of reporting biases 25
2.5.13. Assessment and management of heterogeneity 25
2.5.14. Subgroup analysis 25
2.5.15. Sensitivity analyses 26
2.5.16. Ethics 26
2.5.17. Dissemination (Reporting of this review) 26
2.5.18. Synthesis of evidence 26

2.6. Results 29

2.6.1. Bibliographic database search and screening 29
2.6.2. Main reason for exclusion of eligible articles 29
2.6.3. Description of included studies 31
2.6.4. Adolescent LTBI and TB prevalence 31
2.6.5. Benchmarking against 2010 TB prevalence 35
2.6.6. Infection–to–disease ratios 35
2.6.7. Assessment of quality of included studies 36

2.7. Discussion 36

2.8. References 40

3. Temporal trends in prevalence of *Mycobacterium tuberculosis* infection in South African adolescents 44

3.1. Overview 44

Publication 46

3.2. Abstract 48

3.3. Introduction 49

3.4. Methods 50

3.5. Results 51

3.5.1. Socio–demographic characteristics 51
3.5.2. Prevalence and risk factors for LTBI 54
3.5.3. Prevalence of LTBI across time periods 57
3.5.4. Annual risk of tuberculosis infection (ARTI) 61

3.6. Discussion 62

3.7. References 67

4. **Regional changes in tuberculosis disease burden among South African adolescents (2005–2015)** 70

4.1. Overview 70

Publication 71

4.2. Abstract 72

4.3. Background 73

4.4. Methods 74

4.4.1. Study design, population and setting 74
4.4.2. Definitions 74
4.4.3. Statistical analysis 75
4.4.4. Ethical approval 76
4.4.5. Role of the funding source 76

4.5. Results 76

4.5.1. Demographic and clinical characteristics 76
4.5.2. General population TB rates by age and year 80
4.5.3. Trends in childhood, adolescent and adult TB disease 80
4.5.4. Adolescent TB trends by age 83
4.5.5. Adolescent TB trends by sex 85

4.6. Discussion 85

4.7. References 91

5. **Indoor Air Quality and tuberculosis risk in South African schools** 94

5.1. Overview 94

5.2. Chapter abstract 96

5.3. Background and Rationale 99

5.3.1. Burden of adolescent tuberculosis 100
5.3.2. Measurement of ventilation in classrooms 101
5.3.3. Classroom air sampling for RD9 *Mycobacterium tuberculosis* DNA 103
5.3.4. Droplet digital polymerase chain reaction (ddPCR) 105
5.3.5. Classroom–based Symptom Screening for tuberculosis disease 105

5.4. Objectives 106

5.5. Methods 107

5.5.1. Study design 107
5.5.2. Regulatory and administrative process and approval 109
5.5.3. Training 110

5.5.4. Study procedures 110
5.5.5. Statistical analysis 116

5.6. Results 117

5.6.1. Characteristics of clinic and classrooms 117
5.6.2. ddPCR assay results 122
5.6.3. Sociodemographic characteristics of students screened for tuberculosis disease 123
5.6.4. Symptom screening for tuberculosis disease 124
5.6.5. Relationship between classroom ambient carbon dioxide concentration, ddPCR air sampling, and tuberculosis disease symptoms 125
5.6.6. Profile of classroom occupied by a student with undiagnosed tuberculosis disease 127

5.7. Discussion 129

5.8. References 134

6. **Discussion 140**

References 146

7. **Appendices** 148

7.1. Appendix 2.1 148
7.2. Appendix 2.2 150
7.3. Appendix 2.3 151
7.4. Appendix 2.4 152
7.5. Appendix 2.5 156
7.6. Appendix 2.6 158
7.7. Appendix 3.1 161
7.8. Appendix 3.2 162
7.9. Appendix 3.3 164
7.10. Appendix 3.4 165
7.11. Appendix 3.5 166
7.12. Appendix 4.1 168
7.13. Appendix 4.2 171
7.14. Appendix 4.3 172
7.15. Appendix 4.4 173
7.16. Appendix 4.5 176

7.17. Appendix 4.6 178
7.18. Appendix 5.1 179
7.19. Appendix 5.2 180
7.20. Appendix 5.3 181
7.21. Appendix 5.4 182
7.22. Appendix 5.5 183
7.23. Appendix 5.6 186
7.24. Appendix 5.7 187
7.25. Appendix 5.8 187
7.26. Appendix 5.9 188
7.27. Appendix 5.10 189
7.28. Appendix 5.11 190
7.29. Appendix 5.12 191
7.30. Appendix 5.13 192
7.31. Appendix 5.14 196
7.32. Appendix 5.15 197

List of Tables

Table 2.1: Risk of bias and quality assessment criteria for prevalence studies 23

Table 2.2: Adolescent LTBI prevalence in high TB burden countries in Africa 32

Table 2.3: Adolescent TB prevalence in high TB burden countries in Africa 33

Table 3.1: Socio–demographic characteristics of study population 52

Table 3.2: Factors associated with risk of latent tuberculosis infection 56

Table 3.3: Prevalence of latent tuberculosis infection by socio–demographic variables 59

Table 3.4: Annual risk of tuberculosis infection 62

Table 4.1: Demographic and clinical characteristics of adolescent TB disease cases 77

Table 4.2: TB rate by year, age and diagnostic approach 81

Table 5.1: Classification of ventilation risk status in clinics and classrooms 122

Table 5.2: Sociodemographic characteristics of enrolled students 124

Table 5.3: Screening for self–reported symptoms 125

Table 5.4: Screening for self–reported tuberculosis disease symptoms among students by ddPCR assay result 126

Table 5.5: Self–reported tuberculosis disease symptoms among students by ventilation risk status of classrooms 127

List of Figures

Figure 2.1: PRISMA flowchart of study selection process 30

Figure 2.2: Estimated TB disease prevalence for 2010 35

Figure 3.1: Prevalence of LTBI by age and socioeconomic quintiles........ 55

Figure 3.2: Prevalence of LTBI by school and time period........ 57

Figure 3.3: Prevalence of LTBI across two time periods, 2005–2007 and 2014–2015 58

Figure 4.1: General population TB rates by age in 2005, 2010 and 2015. 80

Figure 4.2: Comparison of adult and adolescent TB trends 2005–2015 83

Figure 4.3: Adolescent TB temporal trends by age and year........ 84

Figure 4.4: Adolescent TB temporal trends by sex........ 85

Figure 5.1: Sampling schema of spaces included in the study 108

Figure 5.2: Proportion of positive ddPCR assay by fluorescence amplitude intensity threshold 114

Figure 5.3(a): Representative examples of clinic ambient carbon dioxide concentration over time 119

Figure 5.3(b): Representative examples of classroom ambient carbon dioxide concentration over time 120

Figure 5.4: Comparison of ambient carbon dioxide concentration in clinics and classrooms 121

List of Abbreviations

ART	Antiretroviral Therapy
CO_2	Carbon Dioxide
ddPCR	Digital Droplet Polymerase Chain Reaction
DFU	Dry Filter Unit
DOTS	Directly Observed Treatment, Short course
HAART	Highly Active Antiretroviral Therapy
IAQ	Indoor Air Quality
LMIC	Low and Middle Income countries
LTBI	Latent Tuberculosis Infection
QFT	QuantiFERON TB Gold test
QFT–IT	QuantiFERON TB Gold In–Tube test
RNA	Ribonucleic Acid
SA	Republic of South Africa
SATVI	The South African Tuberculosis Vaccine Initiative
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organisation
Xpert/MTB RIF	Xpert *Mycobacterium Tuberculosis* & Rifampicin resistance assay

Chapter 1

1. Introduction

1.1. Overview

This thesis examines the epidemiological burden of tuberculosis (TB) among adolescents in a high TB burden community in South Africa (Worcester) over the decade 2005–2015 to determine epidemiological trends and impact of TB control efforts, describe high school classroom ventilation risk for *Mycobacterium tuberculosis* transmission and investigate air sampling for *Mycobacterium tuberculosis* DNA detection as a novel strategy for TB disease screening in congregate spaces.

1.2. Rationale

A mathematical modelling study estimated that 220,000 (30%) of the 727,000 adolescent tuberculosis (TB) disease cases occurring globally in 2012 were in the WHO African region, resulting in an estimated African regional adolescent TB prevalence of 100 per 100,000.[12] Although adolescents have the highest force of *Mycobacterium tuberculosis* infection[1] and increasing burden of TB disease through 10–19 years in this region,[2] there are few studies on adolescent TB burden making this an important but neglected group in TB control. Data on the burden and temporal trends of adolescent TB disease and LTBI is vital for re–appraisal of TB control interventions in line with the 2030 global "End TB Strategy" targets.[13] Furthermore, few studies describe where and how adolescents acquire TB infection.[4] South African studies show that a significant proportion of adolescents, unlike younger children, acquire TB infection from non–household settings.[4, 14–16] The lack of implementation of TB disease screening in schools,[17] where adolescents spend a considerable amount of their time, and the attendant risk of *Mycobacterium tuberculosis* transmission if exposed to an infectious learner or staff member, makes school–based TB disease screening, potentially including air sampling for *Mycobacterium tuberculosis* DNA detection, an important

research priority. Studies of novel TB screening tools could inform focused screening strategies that allow timely and effective treatment of undiagnosed cases to ensure schools are safe places for learning.[20, 21]

The objectives of this thesis were:

1) To conduct a systematic review of adolescent latent TB infection (LTBI) and TB disease prevalence, and to examine the relationship between adolescent *Mycobacterium tuberculosis* infection and TB disease rates, in high TB burden African countries
2) To describe temporal changes in LTBI prevalence among adolescents living in a single TB endemic South African community over the decade 2005–2015
3) To describe temporal changes in adolescent TB disease notification rates in the same community over the decade 2005–2015
4) To determine classroom ventilation risk for *Mycobacterium tuberculosis* transmission in tandem with a pilot study of air sampling for *Mycobacterium tuberculosis* DNA; and to investigate the operational feasibility and yield of a pragmatic, symptom–based approach to TB disease surveillance in high schools.

1.3. Thesis structure

The systematic review (Chapter 2) provides a background perspective against which to view subsequent chapters that describe temporal trends in the burden of LTBI (Chapter 3) and TB disease (Chapter 4) among adolescents in the study region. Chapter 5 focuses on risk for TB transmission in classrooms, where adolescents spend a considerable part of the school year, and evaluates classroom ventilation as a risk factor for exposure to airborne *Mycobacterium tuberculosis* and investigates the operational feasibility and yield of TB disease surveillance in high schools. The individual chapters are further described below:

Chapter 2: Systematic review of prevalence of latent *Mycobacterium tuberculosis* infection and tuberculosis disease among adolescents in high TB burden countries in Africa

This chapter presents a systematic literature review of the prevalence and relationships between adolescent LTBI and TB disease in high TB burden African countries, benchmarked against expected infection–to–disease ratios (8–12) reported by Styblo *et al.*, and against national TB disease data reported for the year of study.

Chapter 3: Temporal trends in prevalence of *Mycobacterium tuberculosis* infection in South African adolescents

This chapter presents an analysis of temporal trends in adolescent prevalence of LTBI in the study region (2005–2015) to monitor the impact of TB control interventions on medium–term TB transmission. These data informed school selection for the field work (classroom air quality testing, symptom–based TB case–finding, and air sampling for *Mycobacterium tuberculosis DNA)* that is presented in Chapter 5.

Chapter 4: Regional changes in tuberculosis disease burden among South African adolescents (2005–2015)

This chapter describes temporal changes in prevalence of TB disease in the study region over the decade 2005–2015, in the context of the LTBI prevalence data presented in Chapter 2.

Chapter 5: Indoor air quality and tuberculosis risk in South African schools

This chapter examines classroom ventilation as a risk factor for *Mycobacterium tuberculosis* transmission; presents proof of concept for classroom air sampling of *Mycobacterium tuberculosis* DNA as a novel TB screening tool in congregate settings; and examines the feasibility and yield of a pragmatic school–based TB disease screening strategy.

Chapter 6: Discussion

This chapter critically reviews overarching themes, conclusions, outstanding research questions and public health implications of the findings presented in the preceding chapters.

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Chapter 2

2. Systematic literature review of prevalence of latent *Mycobacterium tuberculosis* infection and tuberculosis disease among adolescents in high TB burden countries in Africa

2.1. Overview

Summary

This chapter provides a systematic literature review of published adolescent latent *Mycobacterium tuberculosis* infection (LTBI) and tuberculosis (TB) disease prevalence from high TB burden countries in Africa for the period 1990–2018. The chapter is organised into four main sections: (1) Chapter abstract; (2) Systematic review protocol, provided as published (**Bunyasi E.W.**, Schmidt B–M, Abdullahi L.H., *et al.* Prevalence of latent TB infection and TB disease among adolescents in high TB burden countries in Africa: a systematic review protocol. *BMJ Open* 2017;7: e014609. doi:10.1136/*BMJ*open–2016–014609); (3) Systematic review findings; (4) Discussion.

Contribution to thesis and novelty

This systematic review provides a background perspective against which to view subsequent chapters that deal with the burden of latent *Mycobacterium tuberculosis* infection and tuberculosis disease in adolescents in the study region; and may inform TB control policies in high TB burden countries in Africa, including new preventive therapy and vaccination strategies, by providing a reference for monitoring of trends in adolescent TB transmission and TB disease prevalence.

Contributions of candidate

The candidate conceived and wrote the protocol, reviewed the literature and wrote the findings with editorial input and guidance from supervisors. Co–authors contributed to research group discussions and commented on the final draft manuscript.

Publication status

The systematic review protocol is presented in the form of a peer–reviewed paper published by an international journal (Citation: **Bunyasi E.W.**, Schmidt B–M, Abdullahi L.H., *et al.* Prevalence of latent TB infection and TB disease among adolescents in high TB burden countries in Africa: a systematic review protocol. *BMJ Open* 2017;7). Approval was obtained from co–authors, journal editors and the Doctoral Degrees Board for inclusion of the published protocol. Minor protocol amendments are provided as Appendix 2.5.

2.2. Chapter abstract

Introduction

Adolescents in TB endemic countries have a high force of *Mycobacterium tuberculosis* infection, but a lower TB disease incidence, compared to adults. We performed a systematic review to examine the relationships between adolescent LTBI and TB disease, benchmarked against expected infection–to–disease ratios (8–12) reported by Styblo *et al.*, and against national TB disease data for the year of study.

Methods

We performed a bibliographic database search for studies conducted and published in English between 1st January 1990 and 11th August 2018 on prevalence of adolescent (aged 10 to 19 years) latent *Mycobacterium tuberculosis* infection (LTBI) and TB disease in high TB burden countries in Africa in the following databases; *PubMed*, *Scopus*, *Cochrane* library, *Web of Science, Africa Wide, CINAHL* and the *Africa Index Medicus*; supplemented by a search of references of obtained articles. We calculated the ratio between the number of *Mycobacterium tuberculosis* infections based on Annual Risk of TB Infection (ARTI) estimates and the number of smear– and culture–positive TB disease cases per year for the same study population; and compared the observed ratio to the expected ratio of 8–12 published by Styblo *et al.*[1]

Results

Our search retrieved 14,724 articles, 9,120 of which were duplicates and thus excluded. 5,505 were excluded based on review of the title and abstract due to not being LTBI or TB prevalence studies. From full text review of 105 articles, 5 adolescent LTBI and 5 adolescent TB disease prevalence studies qualified for inclusion. Reported adolescent LTBI prevalence was between 42–55% in the three studies conducted in South Africa (2002–2009); 32% in one study conducted in Kenya (2010); and 16% in one study conducted in Uganda (2009–2011). Reported adolescent TB disease (i.e. all cases started on TB treatment) prevalence was between 210–341 cases per 100,000 in the three South African studies (2002–2013); 180

cases per 100,000 in one Ugandan study (2009–2011); and 679 cases per 100,000 in one Kenyan study (2010). However, when limited to culture–confirmed TB disease, TB rates were 320 per 100,000 in Kenya, 308 per 100,000 in South Africa, 160 per 100,000 in Uganda. ARTI data were available for one South African study (Mahomed *et al.*)[2] with a low infection–to–disease ratio (6.3) and one Kenyan study (Nduba *et al.*)[3] with a very high infection–to–disease ratio (40.1) on sputum smear, compared to the 8–12 ratio expected from the Styblo Rule. However, when restricted to culture–confirmed TB, infection–to–disease ratio was 5.7 and 2.5 for the Kenyan and South African studies respectively, which corrects for unconfirmed clinical cases in the Kenyan study.

Conclusion

This systematic review highlights the paucity of data on adolescent LTBI and TB disease prevalence in high TB burden countries in Africa and the high prevalence of both LTBI and TB disease in adolescents. Calculated infection–to–disease ratios in adolescents using sputum smear or culture confirmation were inconsistent (lower or higher) than expected from the Styblo Rule. The variability of prevalence estimates for the period 1990–2018 illustrates the need to benchmark comparisons across countries against microbiologically–confirmed TB disease. Improved age disaggregation of adolescent data in research studies and WHO reporting would facilitate evaluation and future monitoring of trends in adolescent TB transmission in response to TB control interventions.

Publication

Prevalence of latent TB infection and TB disease among adolescents in high TB burden countries in Africa: a systematic review protocol

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2.3. Abstract

Introduction

Almost a third of the world population has latent TB infection (LTBI), approximately 10 million of whom develop TB disease annually, despite existence of effective, but lengthy, preventive and curative drug regimens. Although adolescents appear to have a very high force of *Mycobacterium tuberculosis* infection, their reported incidence of TB disease is less than that of their corresponding general population. The few available studies on adolescent TB infection and disease prevalence are not sufficient to address the apparent discordance between rates of infection and disease in high TB burden countries in Africa. Therefore, we aim to perform a systematic review to examine the relationship between adolescent LTBI and TB disease, benchmarked against national TB disease burden data.

Methods and analysis

A comprehensive literature search will be performed for cross–sectional studies and screening data in cohort studies to determine prevalence of LTBI and TB disease among adolescents in high TB burden countries in Africa in the following databases; *Pubmed*, *Scopus*, *Cochrane* library, *Web of Science, Africa Wide, CINAHL and the Africa Index Medicus*. This will be supplemented by a search of reference lists of selected articles for potentially relevant articles. We will restrict our search to articles published in English language between 1990 and 2016 among adolescents in order to obtain estimates reflective of the mature HIV epidemic in most high TB burden countries in Africa that occurred over this critical period. Primary endpoints are; prevalence of LTBI and TB disease. We will use the random–effects or fixed effects modelling for our meta–analysis based on heterogeneity estimates.

Ethics and dissemination

No ethics approval is required given this is a systematic review. Findings will be disseminated in a peer–reviewed journal in line with the *Preferred Reporting Items for Systematic reviews and Meta–Analyses* (PRISMA).

Registration details

This protocol is registered with the *International Prospective Register of Ongoing Systematic Reviews* (PROSPERO), registration number CRD42015023495.

Key words

Prevalence; latent TB infection; TB disease; adolescents; protocol; systematic review.

Strengths and limitations of this study

- To the best of our knowledge, this is the first systematic review to conduct and compare adolescent LTBI and TB disease prevalence in high TB burden countries in Africa.
- By examining the relationship between adolescent LTBI and TB disease benchmarked against national TB disease burden data, our study will provide key insights into this relationship.
- Data reporting adheres to the Preferred Reporting Items for Systematic reviews and Meta–Analyses (PRISMA) guidelines for reviews (PRISMA) and protocols (PRISMA–P).
- Our choice of period for review is primarily driven by the need to provide findings reflective of the mature HIV epidemic in high TB burden countries in Africa both before and after the advent of wide ART availability, thus we appreciate that our estimates will not provide old or historical trends in TB burden.
- Our restriction of analysis to articles published in English language may introduce publication and language bias, respectively.

2.4. Introduction

TB remains a key public health problem especially in Africa which reported almost a third of the 10.4 million incident Tuberculosis (TB) disease cases globally in 2015. [4] The estimated incidence rate of TB disease in Africa in 2015, of roughly 237 cases per 100,000 people, was almost double the global average of 133 cases per 100,000 people.[4] In 2015, TB caused 1.4 million deaths worldwide and was the leading

cause of death by an infectious agent. A relatively small proportion (5–15%) of an estimated 2–3 billion people worldwide who are latently infected with *Mycobacterium tuberculosis* will develop TB disease in their lifetime. The probability of developing TB disease is much higher among people living with HIV.[4]

The force of *Mycobacterium tuberculosis* infection, defined as the proportion of susceptible individuals (i.e. individuals without latent TB infection (LTBI)) who become latently infected with *Mycobacterium tuberculosis* per annum, is a key measure of TB transmission in a defined population. Unfortunately, very few longitudinal cohort studies of child or adolescent LTBI exist across high TB burden countries in Africa. A South African longitudinal study reported a high annual force of *Mycobacterium tuberculosis* infection among adolescents of 14.0%.[5] Similarly, there is paucity of data on prevalence of LTBI among adolescents in high TB burden countries in Africa, with most of the few available studies having been conducted in South Africa. A cross–sectional South African study reported an increase in prevalence of LTBI from 26% at 5–8 years to 53% at 14–17 years to 75% at 25 years.[6-10] A nationally representative Kenyan survey of children aged 6–14 years reported prevalence of LTBI of 10.2%, a figure that did not significantly change over 2 decades, between 1986 and 2006.[11] Although adolescents in Cape Town, South Africa, appear to have a very high force of *Mycobacterium tuberculosis* infection(14%)[5], their reported incidence of TB disease (approximately 710/100,000) is less than the incidence in young adults (1,400/100,000) and less than the incidence in the general population (834/100,000).[4, 5, 10, 12]

A new TB infection in an infant or young child is a sentinel signal of active transmission from a person, usually an adult within their household, with active pulmonary TB disease. Thus, we would expect high rates of childhood LTBI to be associated with high prevalence of adult TB disease in the same community. There is little research that describes settings from which adolescents acquire TB infection [13] which makes it difficult to explain the apparent discordance between very high rates of adolescent force of *Mycobacterium tuberculosis* infection and low rates of notified adolescent TB disease in the same community. In a South African township, prevalent TB infection among children aged 5 to 14 years was directly and significantly associated with residential (i.e. within their residential plot) exposure to an adult case of TB disease.

However, a non–significant association was observed for individuals aged 15–22 years despite their high force of *Mycobacterium tuberculosis* infection.[13] This finding suggests increasing significance of settings other than residential plot as a determinant of TB infection and subsequent disease from mid–adolescence onwards.[14, 15] Glynn *et al.* recently (2015) demonstrated via whole genome sequencing that, overall, known smear positive prior contacts accounted for less than 10% of tuberculosis cases in a Malawian community, and that even for those with a prior contact with smear positive tuberculosis in their family, there was a higher than 50% chance that they acquired their tuberculosis elsewhere, similar to our own previous finding in Cape Town, South Africa.[13, 16, 17] Andrews *et al.* used statistical modelling techniques to estimate that up to a half of TB transmission among individuals aged 15–19 years occurs in the school setting, with this figure being 25% in individuals aged 0–14 years.[9] If this hypothesis were true, we would expect to observe high prevalence of TB disease in parallel with high force of *Mycobacterium tuberculosis* infection among high school–aged adolescents in the same high burden communities. The fact that this apparently reasonable observation does not appear to hold true deserves further investigation. Our study will quantify prevalence of LTBI and TB disease among adolescents in high TB burden countries in Africa and highlight this pattern across these countries. However, we appreciate that the design of this systematic review may not provide definitive reasons for this paradoxical yet persistent observation across many countries and settings. Due to lack of a systematic review on prevalence of LTBI and TB disease among adolescents, this systematic review will provide useful data for policy by consolidating and synthesising available data regarding a key sub–population with the highest force of *Mycobacterium tuberculosis* infection[5] but a relatively low reported notification rate of TB disease as compared to their corresponding general population. Our findings will not only contribute to our better understanding of TB transmission among adolescents, but will also inform TB policies in high TB burden countries in Africa by providing a reference for monitoring future TB transmission trends in the wake of global efforts to end the TB epidemic whose targets are defined in sustainable development goals for 2035. [4] Our findings will also be useful in planning of novel TB vaccine research studies among adolescents who are increasingly becoming a key focus subpopulation for global TB vaccine research efforts.

2.5. Methods

This protocol was developed in line with the *Preferred Reporting Items for Systematic reviews and Meta–Analyses guidelines for protocols* (PRISMA–P),[18, 19] see Appendix 1 for a PRISMA–P checklist of the recommended bare minimum items to be included.

2.5.1. Objectives

Primary objectives

- To determine prevalence of latent TB infection in adolescents in the 25 high TB burden countries in Africa as defined by the WHO in the 2016 Global TB report.
- To determine prevalence of TB disease among adolescents in the 25 high TB burden countries in Africa, as defined by the WHO in the 2016 Global TB report.

Secondary objective

- To explore the relationship between age–specific risk of LTBI and age–specific prevalence of TB disease, benchmarked against published estimates of national TB disease incidence and notification rates.

2.5.2. Definitions

Prevalence of LTBI is defined as the number of individuals with LTBI divided by total number of individuals in a cross–sectional, population–based study or screening database in cohort studies with a positive or negative result from a diagnostic test for LTBI. We will consider LTBI diagnosed by the Tuberculin Skin Test (TST) and/or the Interferon Gamma Release Assay.

Prevalence of TB disease is defined as the number of individuals with TB disease divided by total number of individuals in a cross–sectional, population–based study, or screening database in cohort studies. We will consider the following diagnostic modalities for TB disease: solid and liquid mycobacterial culture, Xpert MTB/RIF assay, sputum smear for acid fast bacilli and clinical diagnosis. Studies restricted to one

or more forms of non–pulmonary TB disease only e.g. Koch's disease, TB lymphadenitis or disseminated TB, will not be included. Studies reporting on respiratory diseases in general and not clearly defining the prevalence of LTBI or TB disease will not be eligible.

Adolescents will be defined as individuals aged between 10 to 19 years, as defined by the WHO.[20] In 2016, the WHO defined 'high TB burden countries' along three broad categories that included: (1) countries with the highest burden of TB/HIV coinfection, (2) countries with the highest burden of multi–drug resistant TB and (3) countries with the highest burden of TB. This classification takes consideration of both the absolute number of cases of TB disease and the relative burden of TB disease after factoring the population size or denominator. In this study, we will restrict our review to the 25 countries from across these three WHO high TB disease burden categories that are found on the African continent.[4] These include: (a) The Democratic Republic of Congo, (b) Ethiopia, (c) Kenya, (d) Uganda, (e) United Republic of Tanzania, (f) Zimbabwe, (g) South Africa, (h) Mozambique, (i) Angola, (j) Sierra Leone, (k) Central African Republic, (l) Congo, (m) Lesotho, (n) Liberia, (o) Namibia, (p) Zambia, (q) Botswana, (r) Cameroon, (s) Chad, (t) Ghana, (u) Guinea–Bissau, (v) Malawi, (w) Swaziland, (x) Somalia and (y) Nigeria.

2.5.3. Eligibility criteria

This is the criteria for consideration of studies for this review.

(i) Study designs

We will consider cross–sectional or prevalence study designs and screening data in cohort studies that report primary data on prevalence of LTBI or TB disease. Statistical or mathematical modelling articles, cost–effectiveness studies, opinion pieces, narrative reviews, case studies, case series and letters to editors will not be considered. Grey/unpublished literature will also be excluded.

(ii) Participants

Adolescent participants should be representative of the general adolescent population in the setting in which the study was conducted. Studies conducted among the general school–going population will also

be considered provided that age is reported. For studies that report on age ranges that extend beyond the 10–to–19–year age bracket, data on individuals aged 10 to 19 years will be extracted, if possible. Otherwise, these data will be sought from corresponding authors. If extraction is not possible and these data are not obtainable from corresponding authors, at least 75% of participants should fall between the ages of 10 to 19 years. Studies reporting prevalence of TB infection or TB disease in subpopulations that are not representative of the general adolescent or school–going population in a specific study setting will be excluded e.g. studies reporting prevalence of TB restricted to HIV positive adolescents only.

(iii) Outcome measures

Outcome measures of interest will include; prevalence of LTBI and TB disease. Studies which do not measure any of our primary outcomes; do not clearly state the case definition of LTBI or TB disease; do not report primary data; or lack explicit description of methodology, will be excluded.

(iv) Time frame

We will consider studies reported between 1st January 1990 and 1st July 2016 because this period will also reflect the TB burden in mature or generalised Human Immunodeficiency Virus (HIV) epidemics across the high TB burden countries in Africa.

(v) Study setting

Studies should have been performed in at least one of the 25 high TB burden countries in Africa as defined above. [4] Studies not conducted in one of these countries or, for multi–country studies, if data pertaining to the listed high TB burden countries in Africa is not obtainable, they will be excluded.

(vi) Language

We will only consider articles published in English language because of limited time and financial resources available to this study.

2.5.4. Search strategy

We will systematically search for articles published between 1990 and 2016 using a combination of database specific medical subject headings (MeSH terms) and a range of free text or key words that will include the following, among others: adolescents, persons, latent, tuberculosis, LTBI, epidemiology, prevalence, morbidity and burden. Our draft *PubMed* search–term is provided in Appendix 2. The specific search strategies will be finalised with guidance from a health sciences librarian with expertise in systematic review searching with input from the project team. After the *PubMed* strategy is finalized, it will be adapted to the syntax and subject headings of the other targeted databases. We will review reference lists of selected articles to identify potentially relevant articles to our research questions that would have been missed by our search term in specified bibliographic databases. Our search will be limited to the following electronic databases due to limited time and financial resources; *PubMed, Scopus, Web of Science, Cochrane library, Africa Wide, Africa Index Medicus* and *CINAHL*. This review will not include grey/unpublished reports due to the low likelihood of peer–review and potential practical difficulties of obtaining supplementary or missing data. We appreciate that this may lead to publication bias and acknowledge this as a limitation of our planned review.

2.5.5. Selection of studies

The first author (EB) will perform a systematic search for articles by employing the search strategy. For duplicate articles or publications reporting the same data in multiple articles, only the more recent and/or complete version of the publication will be considered. EB will review references of selected articles to identify articles relevant to our review which would have been missed by the search strategy. EB and BS will independently classify articles as either: (i) 'included', (ii) 'excluded' or (iii) 'pending'. A 'pending' status shall imply the reviewer is unsure on whether to include or exclude an article. This classification will be done by applying the inclusion and exclusion criteria, and will initially be based on the title and abstract, and then a quick scan, assessment or reading of the full text of the articles. Articles that both reviewers classify as 'excluded' will be excluded from further consideration whereas those that both reviewers classify

as 'included' will be included in the review. We will obtain full reports for all 'included' titles and those with contradictories in classification between the two reviewers. We will seek additional information from study authors where necessary to resolve questions about eligibility. A discussion will be held between EB and BS to resolve differences or contradictories in classification of articles by reviewing full text. A third reviewer (LA) will be consulted to resolve persistent disagreements following discussion. We will present a flow chart, in keeping with PRISMA guidelines as much as practicable, to summarise the search process and selection of studies for the review and document reasons for exclusion of studies (see Appendix 3). We will include a table of all selected studies in the final review and document reasons for exclusion of articles.

2.5.6. Data management

Data management will be done by the first author (EB) in liaison with the second author (BS). A google drive electronic folder will be maintained for the review and will contain; the protocol, a record of obtained articles and documentation of steps in data synthesis and analysis (including records included and excluded), risk of bias and quality scoring, among others. A back–up of the electronic records will be stored on a laptop and on a memory flash drive. '*Refworks'* bibliographic management software[21] will be used to manage references.

2.5.7. Data extraction

EB will read, extract and collate data from selected articles on to a standardised Data Extraction Form (see Appendix 4). This form will be piloted on at least 4 randomly selected studies meeting the criteria for consideration. BS will verify abstracted data in order to reduce bias and reduce errors in data extraction. Data to be abstracted will include: study characteristics– title, year of publication, authors, study design; study setting and population– country, socio–demographics (age and gender); study conduct– number of study participants (total in the study and those participants with TB, by diagnostic approach and number

with LTBI). Reviewers will resolve disagreements by discussion, with arbitration by LA for unresolved disagreements. We will contact study authors for data that may resolve any uncertainties.

2.5.8. Approach to missing data

In the event of missing data that are key, we will attempt to contact the corresponding authors of the studies to obtain the relevant missing data via email. A second email will be sent after one week of the first email in the event of no response to the first email. A two–week wait period from the date of submission of the second email will be allowed for responses, failing which these studies will be excluded, if no communication or response is established.

2.5.9. Assessment of risk of bias of included studies

Risk of bias and assessment of quality will be evaluated using an assessment tool adapted from Hoy *et al.*[22] by Werfalli *et al.* who included a scoring system for evaluation of prevalence studies.[23] The tool helps evaluate internal and external validity (see table 2.1). This tool was preferred over others because it was designed via an expert consensus exercise then tested, retested, validated and thus optimised for evaluation of quality of prevalence studies via a rigorous published process that included a review of limitations of existing tools.[22, 24] The tool was shown to have a high inter–rater agreement.[22] Two authors (EB and BS) will independently score the risk of bias using this tool and the mean score calculated. Agreement between the two raters will be assessed for each item in the tool and overall using proportion of agreement (P_0) and the Kappa ($\varkappa$) statistic. For the Kappa statistic, its values range from –1 to +1. Values of 0 or less will be regarded as poor agreement, 0.01 to 0.20 slight, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 substantial, and 0.81 to 0.99 almost perfect agreement.[25] Raw agreement and Kappa values (including their 95% confidence intervals) will be calculated using *STATA* version 14.0 for windows.[26] Neither of the review authors will be blinded to the journal titles or to the study authors or institutions.

Table 2.1: Risk of bias and quality assessment criteria for prevalence studies

Item under review	Quality score (Points)
External Validity	
Was the study's target population a close representation of the national population in relation to relevant variables?	1
Was the sampling frame a true or close representation of the target population?	1
Was some form of random selection used to select the sample, OR was a census undertaken?	1
Was the likelihood of non–response bias minimal?	1
Total	**4 points**
Internal validity	
Were data collected directly from the participants (as opposed to a proxy)?	1
Was an acceptable case definition used in the study?	1
Was the study instrument that measured the parameter of interest shown to have validity and reliability?	1
Was the same mode of data collection used for all subjects?	1
Was the length of the shortest prevalence period for the parameter of interest appropriate?	1
Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	1
Total	**6 points**
Summary item on the overall risk of study bias (low, moderate or high)	

Legend: As described by Hoy *et al.*, the summary assessment evaluates the overall risk of study bias and is based on the rater's subjective judgment given responses to the preceding 10 items. This approach is consistent with the Cochrane and GRADE (GRADE=Grading of Recommendation, Assessment, Development and Evaluation) working group[27] recommendation or approaches. Furthermore, as summarized in the PRISMA (PRISMA= The Preferred Reporting Items for Systematic reviews and Meta–Analyses) elaboration document, summative scales that numerically summarize multiple components into a single number are misleading and unhelpful,[28] hence our choice of an overall ordinal scale for risk of bias. Response options for individual items are either low (1) or high risk of bias (0). If there is insufficient information in the article to permit judgment of a particular item, then the article is deemed to be at high risk of bias with respect to that item.[22, 29, 30]

2.5.10. Data analysis

We hypothesise that there will be substantial statistical heterogeneity in study results because prevalence of LTBI and TB disease varies by distribution of socioeconomic determinants of health and HIV prevalence within and across settings, among other factors. *A priori*, random effects meta–analysis will be preferred due to the anticipated heterogeneity. However, choice of random–effects or fixed effects modelling will be based on observed statistical heterogeneity. For the latter, we will not pool the results but summarise findings in a narrative format. Additionally, we will derive Annual Risk of LTBI using the formulae: $1-(1-\text{Prevalence})^{1/(\text{mean age})}$ for every year of adolescence. We will then describe the relationship between the annual risk of TB infection and observed TB prevalence from our review. Alternatively, for countries with insufficient data, we will describe the relationship between the Annual Risk of TB Infection and reported TB notification (or incidence rates estimates) by National TB Programs or estimates from the WHO.

In random effects modelling, effect measures are assumed to vary between studies and the summary effect is the weighted average of the effects reported in different studies.[31] This model directly adjusts for inverse of the standard error, and thus indirectly for the sample size reported in studies. Thus, studies with smaller standard error and larger sample sizes will be given more weight in the calculation of the pooled prevalence and 95% confidence intervals.

2.5.11. Data synthesis

Our outcome will be combined and calculated using the Cochrane Review Manager (*RevMan*) statistical software,[32] according to the statistical guidelines in the *Cochrane Handbook for Systematic Reviews of Interventions*.[29] If statistical heterogeneity is observed, the random effects model will be chosen over the fixed effects model. If there is substantial statistical heterogeneity, we will not perform a meta–analysis; a narrative, qualitative summary will be done supported by a table (Appendix 2.5) and figures, where appropriate. This will be done by the first reviewer and checked by the second reviewer for accuracy.

2.5.12. Assessment of reporting biases

The potential for publication or reporting bias will be explored by funnel plots if we obtain at least 10 articles. This will be done by visually assessing asymmetry of funnel plots. As suggested by Egger *et al.*, asymmetry of funnel plots will indicate presence of publication bias.[33] We appreciate that our choice of considering articles reported in English only (language bias) and the fact that we are only searching in a sample of bibliographic databases may be a source of reporting bias.

2.5.13. Assessment and management of heterogeneity

We anticipate clinical and statistical heterogeneity in prevalence estimates within and across settings and countries. Statistical heterogeneity will be quantified using the I^2 test statistic to determine the extent of variation in effect estimates that is due to heterogeneity rather than chance. Statistical heterogeneity will be explored graphically by inspection of forest plots (i.e. the 'eyeball test'). Non–overlap of 95% confidence intervals will suggest remarkable heterogeneity. A formal test for statistical homogeneity, the Cochran's χ^2 Q test statistic, will be performed using an alpha cut–off level of 10% as suggested by Higgin's *et al.*[34] and Cochrane[35], due to the test statistic's low power in detecting heterogeneity, particularly when the number of studies is low. The I^2 test statistic will be used to quantify statistical heterogeneity between studies i.e. provide percentage of observed total variation across studies that is due to real heterogeneity rather than chance. This will provide a quantitative measure of heterogeneity. Cochrane provides the following rough guide to interpretation of heterogeneity: 0% to 40%: might not be important; 30% to 60%: may represent moderate heterogeneity; 50% to 90%: may represent substantial heterogeneity; 75% to 100%: considerable heterogeneity.[36] If substantial heterogeneity is observed, we will try to explain the source of heterogeneity by subgroup analysis and/or sensitivity analysis.

2.5.14. Subgroup analysis

Subgroup analysis will be done in order to obtain estimates that are reflective, and thus potentially more useful and applicable, for specific sub–population groups or settings, and will be conducted along the

following strata, subject to availability of sufficient data: (1) schooling status– adolescents in school vs those not in school; (2) country of study participants; (3) age (4) gender; (5) years of data collection, i.e. 1990–1999, 2000–2016 and 1990–2016; and (6) diagnostic modality of LTBI and TB disease. The analysis along the strata of years of data collection i.e. 1990–1999 and 2000–2016 will be done in order to account for differences attributable to the advent of wide and free availability of anti–retroviral therapy, although we appreciate that HIV prevalence is generally very low among adolescents as compared to adults.

2.5.15. Sensitivity analyses

Sensitivity analyses will be conducted to explore the source of heterogeneity i.e. determine impact of specific studies on pooled prevalence estimate, by exclusion of studies with low quality scores and thus higher risk of bias. We will also explore exclusion of studies with deficiency in specific items on the 10–point modified Hoy *et al.* quality assessment tool, in order to evaluate impact of this exclusion on pooled prevalence estimates.

2.5.16. Ethics

Given that we will utilise published anonymised data, which is publicly available and peer–reviewed, ethical approval is not required for this study.

2.5.17. Dissemination (Reporting of this review)

Our review will be reported, as much as possible, in keeping with the Preferred Reporting Items for Systematic reviews and Meta–Analyses (*PRISMA*) Statement,[37] and will include the PRISMA check–list (or adapted as practicable). Our findings will be published in a peer–reviewed journal and as part of a doctoral thesis at the University of Cape Town.

2.5.18. Synthesis of evidence

The *Preferred Reporting Items for Systematic reviews and Meta–Analyses guidelines for protocols* (PRISMA–P)[18, 19] recommends gauging of overall judgement of quality of evidence from obtained articles and indicates

increasing support and use of *the Grading of Recommendations Assessment, Development and Evaluation* (GRADE) working group[27] methodology. We will consider methodological quality of included studies and strength of evidence and adapt the basic principles of the GRADE approach.

Competing interest statement

None. All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

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Author contributorship statement

Erick Wekesa Bunyasi conceptualised and designed the study. Erick Wekesa Bunyasi, Bey–Marrie Schmidt, Leila Hussein Abdullahi, Humphrey Mulenga, Michele Tameris, Angelique Luabeya, Justin Shenje,

Thomas Scriba, Hennie Geldenhuys, Robin Wood and Mark Hatherill were involved in development of the study protocol. Erick Wekesa Bunyasi prepared the first draft of the manuscript with supervision from Hennie Geldenhuys, Robin Wood and Mark Hatherill. Erick Wekesa Bunyasi, Bey–Marrie Schmidt, Leila Hussein Abdullahi, Humphrey Mulenga, Michele Tameris, Angelique Luabeya, Justin Shenje, Thomas Scriba, Hennie Geldenhuys, Robin Wood and Mark Hatherill critically reviewed, revised and approved the subsequent and final version of the protocol. Erick Wekesa Bunyasi is the guarantor. Erick Wekesa Bunyasi and Bey–Marrie Schmidt will perform the study search, screening and extraction of data under the guidance of Hennie Geldenhuys, Robin Wood and Mark Hatherill.

Provenance and peer review

Not commissioned; externally peer reviewed.

Amendment procedure

In the event that amendment to this protocol is required, we will describe the change and give the rationale in the methods section of the published review. EB will ultimately be responsible for approving, documenting, and implementing any amendments.

Data sharing

The authors declare that this research protocol is an original work. Results from the study completed using this protocol will be published in a peer–reviewed journal.

2.6. Results

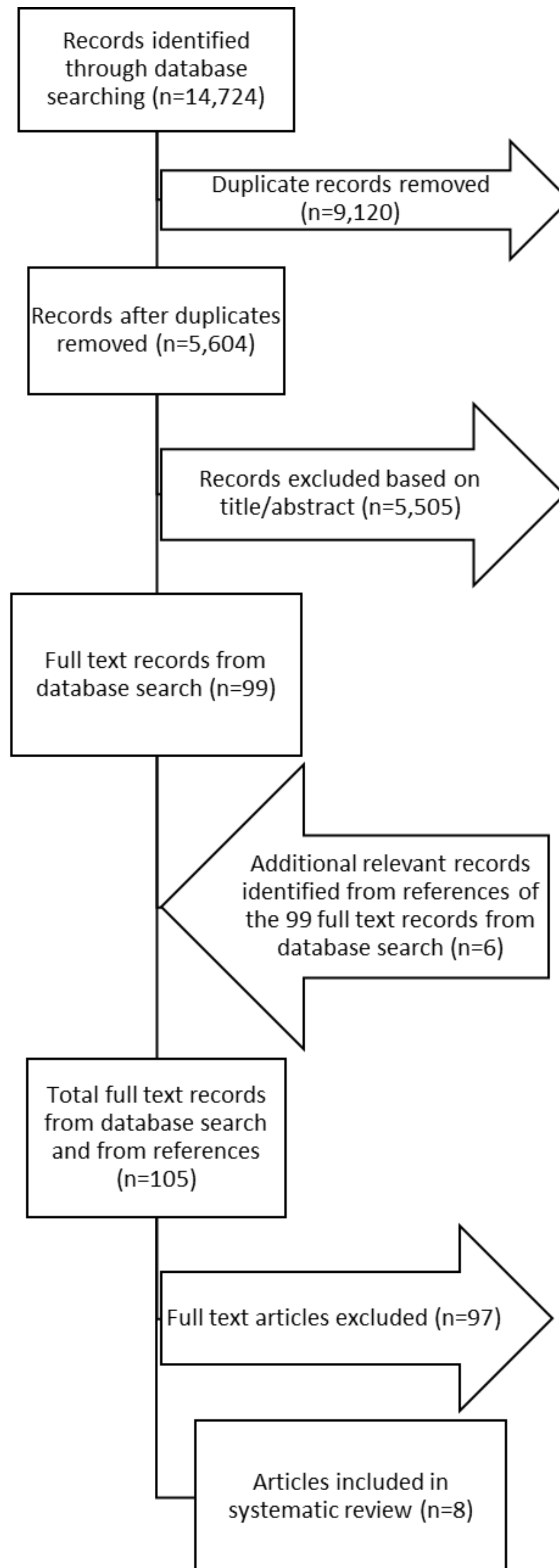
2.6.1. Bibliographic database search and screening

The bibliographic database search for prevalence studies on adolescent LTBI and TB disease in high TB burden countries in Africa conducted and published between 1st January 1990 and 11th August 2018 retrieved 14,724 articles, 9,120 of which were excluded because of duplication. Of 5,604 articles obtained after exclusion of duplicates, 5,505 were excluded based on review of the title and abstract because they were not LTBI or TB disease prevalence studies, or they contained an exclusion criterion within the title or abstract that made them ineligible.

2.6.2. Main reason for exclusion of eligible articles

105 articles were advanced to full text screening against the full inclusion criteria. The main reason (22/105 (21%)) for exclusion of articles during full text screening (Figure 2.1) was the inability to extract adolescent data due to lack of adolescent age disaggregation in childhood TB studies (with age cut–offs of 12 or 15 years) or adult TB studies (with age cut–offs of 15 or 18 years); and the inability to obtain these data from authors on multiple written attempts. For example, two authors (2/22 (9%)) who replied to supplementary data enquiries were not able to provide disaggregated adolescent data for 10–15–year olds, or 18–19–year olds, respectively. Most of the excluded adult TB prevalence studies presented data for the subpopulation aged 15–24 years old without disaggregation of adolescent from adult data.

Figure 2.1: PRISMA flowchart of study selection process



Legend: PRISMA=Preferred Reporting Items for Systematic Reviews and Meta–analyses guidelines for reviews; TB=Tuberculosis; LTBI=Latent Tuberculosis Infection. Reasons for exclusion of full text articles were: Age range ineligible (n=1); HIV or other patient groups only (n=1); Case definition or methods unclear (n=2); Study design ineligible (n=2); TB contacts only (n=2); Country ineligible (n=3); Duplicate study (n=3); Language ineligible (n=3); Patients only (n=3); TB symptomatic individuals only (n=4); Unpublished/not a peer–reviewed article (n=4); Adolescent population less than 75% of study population and adolescent data unobtainable (n=6); Year ineligible (n=10); TB suspects only (n=11); Not a TB/LTBI prevalence study (n=20); Adolescent data (cases or denominator) not obtainable (n=22).

2.6.3. Description of included studies

A total of 8 articles qualified for inclusion in this review. These studies were conducted in only three of the 25 countries eligible for review (South Africa, Kenya and Uganda). Three studies exclusively reported LTBI prevalence; three studies exclusively reported TB disease prevalence; and two studies reported both LTBI and TB prevalence (see Table 2.2 and Table 2.3). All studies were conducted between 2002–2013. None of the included TB prevalence studies utilised the Xpert MTB/RIF® assay to diagnose TB disease. All but one LTBI prevalence study used the Tuberculin Skin Test (TST) to diagnose LTBI. Criteria for TST positivity varied across and within included studies with TST induration cut–offs of 5mm (for HIV positive adolescents), 10 mm and 15 mm.

2.6.4. Adolescent LTBI and TB prevalence

Reported adolescent LTBI prevalence ranged between 16%–55% (2002–2011) across the three countries; and was between 42–55% in the three studies conducted in South Africa (2002–2009);[13, 38, 39] 32% in the one study conducted in Kenya (2010);[3] and 16% in the one study conducted in Uganda (2009–2011)[2] (Table 2.2).

Table 2.2: Adolescent LTBI prevalence in high TB burden countries in Africa

First author, year (ref)	Country	Study period	Age (yrs)	Test	TST cut–off	LTBI cases	Denominator	LTBI prevalence
Mahomed, 2011[38]	South Africa	2005 – 2007	12–18	TST	5 mm	2894	5244	55%
				TST	10 mm	2213	5244	42%
				QFT	NA	2669	5244	51%
Marais, 2005[39]	South Africa	2002	10–14	TST	15 mm	210	476	44%
Middelkoop, 2014[13]	South Africa	2006 – 2009	10–22	TST	10 mm	406	842	48%
Mumpe–Mwanja, 2015[2]	Uganda	2009 –2011	12–18	TST	5 mm & 10 mm	803	4981	16%
Nduba, 2015[3]	Kenya	2010	12–18	TST	5 mm & 10 mm	1544	4808	32%

Legend: ref=Reference; mm=millimetres; NR=Not reported; TST= Tuberculin Skin Test; QFT=QuantiFERON TB Gold In–Tube; * Induration diameter ≥5 mm for HIV positive, ≥10 mm for HIV negative or unknown HIV status; LTBI=Latent Tuberculosis Infection

Reported adolescent all–TB disease rates (both microbiologically–confirmed and clinically diagnosed) ranged between 180–679 per 100,000 across included studies (Table 2.3). Culture–confirmed adolescent TB disease rates in Kenya and South Africa were comparable (320 vs. 308 cases per 100,000), but almost double the estimate from the Ugandan study (160 cases per 100,000). Prevalence of microbiologically–confirmed TB among adolescents was lower than the estimated country–level general population TB prevalence in South Africa (734 vs. 308 cases per 100,000) and Uganda (240 vs. 160 cases per 100,000) but comparable for Kenya (283 vs. 320 cases per 100,000).

Table 2.3: Adolescent TB prevalence in high TB burden countries in Africa

Author, year (ref)	Country	Study period	Age (yrs)	Diagnostic approach	TB disease cases	Denominator	Adolescent TB prevalence	TB incidence in general country population[40]	TB prevalence in general country population[40]	HIV prevalence in incident TB disease cases[40] in country (%)
Mahomed, 2013[8]	South Africa	2005–2007	12 – 18	All TB	21	6,163	341	829	734	58
				Culture confirmed	19	6,163	308	NR	NR	NR
				Sputum smear	17	6,163	276	328	NR	NR
Marais, 2005[39]	South Africa	2002	10 – 14	All TB	NR	NR	NR	558	NR	NR
				Sputum smear	1	476	210	227	NR	NR
Nduba, 2015[3]	Kenya	2010	12–18	All TB	34	5,004	679	298	283	41
				Culture confirmed	16	5,004	320	NR	NR	NR
				Sputum smear	1	5004	20	NR	NR	NR
Snow, 2017[41]	South Africa	2013	10–19	All TB	2,616	955,851	274*	860	715	61
				Sputum smear	NR	NR	NR	NR	NR	NR
	Uganda		12–18	All TB	9	5,000	180	238	240	53

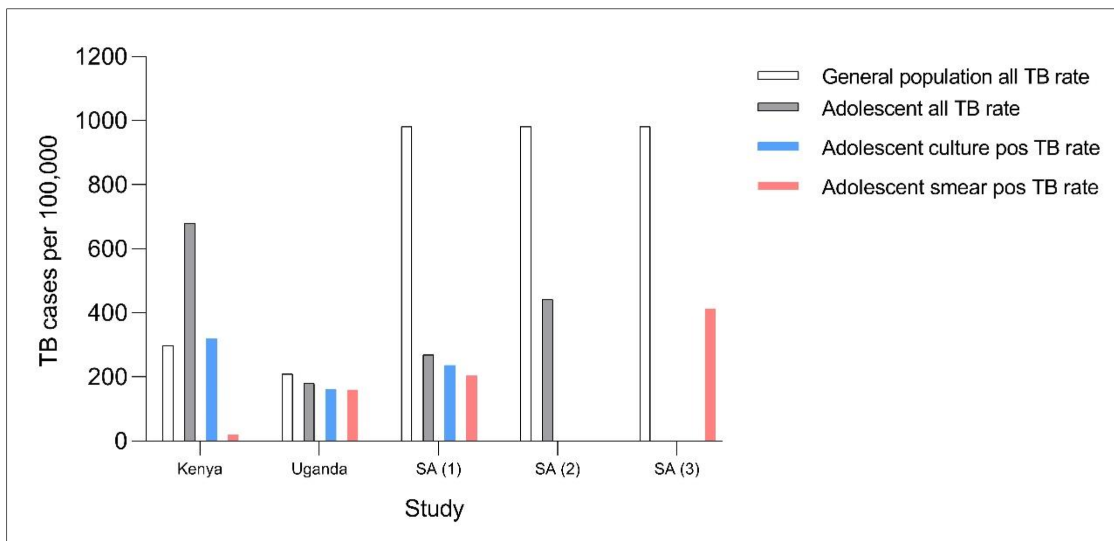
Waako, 2013[2]		2009–2011		Culture confirmed	8	5,000	160	NR	NR	NR
				Sputum smear	8	5,000	160	NR	NR	NR

Legend: ref=reference; TB=Tuberculosis; NR=Not reported. Adolescent TB incidence and prevalence rates are not routinely reported by the World Health Organization. Incidence and prevalence are for the year of study conduct, or the average for the period of study conduct. Prevalence and incidence rates are per 100,000 population. [40] The population prevalence estimates by the World Health Organization are only provided for context. It should be noted that WHO prevalence estimates are indirectly derived from country notification data following extrapolations. [41] This study used routine health system TB notification data and thus presents TB notification rate and not prevalence

2.6.5. Benchmarking against 2010 TB prevalence

We compared estimates of time–adjusted adolescent TB disease prevalence rates for 2010 for the South African studies to the published adolescent TB prevalence rates for the Kenyan and Ugandan studies conducted in the same year. Time–adjusted adolescent all–TB prevalence was highest in Kenya, followed by South Africa and Uganda (679 vs. 269–442 vs 180 per 100,000), a pattern also observed for culture–positive TB (320 vs. 236 vs. 160 per 100,000), but not for sputum smear–positive TB for which the rates in Kenya were very low (20 vs. 204 vs. 160), respectively (Figure 2.2).

Figure 2.2: Estimated TB disease prevalence for 2010



Legend: Estimated time–adjusted TB disease prevalence for 2010 for South African studies compared with published adolescent TB prevalence for Kenya and Uganda in that year. SA (1) = Mahomed *et al.*[8] SA (2) = Snow *et al.*[41] SA (3) = Marais *et al.*[39] TB=Tuberculosis. Missing bars are due to non-reporting of TB prevalence in primary studies so that the 2010 estimate could not be generated. General population all–TB rates were obtained from the 2011 WHO tuberculosis report for 2010.[42]

2.6.6. Infection–to–disease ratios

Styblo *et al.*[1] did seminal work on infection–to–disease ratios by calculating the ratio between the number of tuberculous infections (based on ARTI estimates) and the number of smear–positive TB disease cases per year and obtained a ratio of 8–12 (Appendix 5). In the present study, the annual risk of TB infection

(ARTI) was only available for one South African study[2] and one Kenyan study[3], due to lack of data presented in the other studies[2, 39, 41] (see Appendix 5). For Kenya and South Africa, the infection–to–disease ratio was 6.3 for Mahomed *et al.* (South Africa; ARTI 3.7%);[8] and 40.1 for Nduba *et al.*[3](Kenya; ARTI=2.6%)[43] based on sputum smear results. However, using mycobacterial culture results TB infection–to–disease ratio was 5.7 and 2.5 for the Kenyan and South African studies, respectively. Both ratios are lower than the 8–12 range expected from the Styblo rule.

2.6.7. Assessment of quality of included studies

All included studies scored poorly on external validity, primarily because the tool used for assessment depended on national surveys, with samples randomly selected from national subpopulation or censuses. Such studies are costly and resource–intense and are rarely conducted. The summary of quality assessment is attached as Appendix 6.

2.7. Discussion

We identified five adolescent LTBI and five adolescent TB disease prevalence studies from three of the 25 high TB burden countries in Africa for the period 1990–2018. Adolescent LTBI prevalence varied widely (16%–55%), as did TB disease prevalence (180–679 per 100,000). If adjusted for the high rate of clinically diagnosed, but unconfirmed TB disease in the Kenyan study, the infection–to–disease ratio based only on microbiologically–confirmed TB disease was 5.7 for the Nduba *et al.*[3] Kenyan study and 2.5 for the Mahomed *et al.*[8] South African study respectively. These calculated adolescent TB infection–to–disease ratios were lower than expected from the Styblo Rule (8–12). The remaining studies reported insufficient data for the infection–to–disease ratio to be estimated. Therefore, although it appears that adolescents in South Africa, Uganda and Kenya bear a high burden of both LTBI and TB disease, there are insufficient data to draw any conclusions about adolescent LTBI and TB disease prevalence in the majority of high TB burden countries in Africa. The disparity in infection–to–disease ratios between the South African and Kenyan studies also suggests that additional adolescent TB research is needed to

understand the epidemic in this age group in these countries. However, the apparent disparity in infection–to–disease ratios is mitigated when only culture–confirmed TB disease cases are evaluated, suggesting that microbiological confirmation should be a requirement for comparison of study data across different research settings and populations.

We report adolescent LTBI prevalence of 42–55% in three South African studies (2002–2009),[13, 38, 39] 32% in one Kenyan study (2010)[3] and 16% in one Ugandan study (2009–2011).[2] These data are consistent with an earlier Kenyan LTBI survey conducted between 1986–1990, which reported adolescent LTBI prevalence of 27%.[44] By contrast, reported adolescent LTBI prevalence was low in Europe, USA and Australia, i.e. 0.4% in Italy (2002),[45] 0.8% in USA (2011)[46], and 2.5% in Australia (1995).[47]

We report adolescent culture–confirmed TB disease prevalence of 308 per 100,000 in South Africa, 160 cases per 100,000 in Uganda and 320 cases per 100,000 in Kenya. These findings are consistent with other African studies that reported aggregate data for young adults aged 15–24, which includes adolescents. TB disease prevalence (smear– or culture–positive, per 100,000) in these studies was reported as 129 (Zimbabwe, 2014), 184 (Ghana, 2013), 274 (Nigeria, 2012), 285 (Zambia, 2003–2014) and 360 (Kenya, 2015).[48] Reported TB prevalence (smear– or culture–positive, per 100,000) was also highly variable among young people aged 15–24 years in Asia, i.e. 45 in China (2010),[48] 92 in Myanmar (2010),[48] 103 in Bangladesh (2015),[48] 130 in Cambodia (2010),[48] 142 in Laos (2010),[48] 155 in Thailand (2012),[48] 241 in Pakistan (2011),[48] 799 in Philippines (2016)[48] and 63 in Russia (1995).[49]

The relatively lower adolescent TB disease prevalence of 210 cases per 100,000 in the Marais *et al.* South African study could be explained in part by the younger study population (10–14 years) as compared to the other two South African studies (10–19 years), given that TB disease prevalence is three–fold higher in older (15–19 years) than younger adolescents (10–14 years).[41] The relatively lower adolescent TB disease notification rate of 274 cases per 100,000 in the Snow *et al.* South African study could be explained in part by use of routine health system TB notification data, which may underestimate TB prevalence by approximately one–third due to under–reporting and under–diagnosis.[50] Full text screening,[48, 51, 52] showed

that few studies disaggregate TB disease data in adolescents from young adults, which limits evaluation and future monitoring of adolescent TB disease burden. A mathematical modelling study estimated that 220,000 (30%) of the 727,000 adolescent TB disease cases occurring globally were in the WHO African region, resulting in an estimated regional adolescent TB prevalence of 100 per 100,000 (2012).[52]

We also report a relatively low infection–to–disease ratio in the Mahomed *et al.*[8] study (South Africa), although this estimate was consistent with 10 studies (1975–2008) for individuals of all ages conducted in the TB chemotherapy era that reported ratios ranging from 2.5–8.0:[53] Similarly, a literature review of 13 studies by Trunz *et al.*[54] for individuals of all ages reported infection–to–disease ratios of 5.7–7.2 in China, 3.8–7.4 in the Philippines, 4.8–7.9 in South Korea, and 7.8 in Cambodia.[54] The very high infection–to–disease ratio using all treated TB disease cases reported by Nduba *et al.*[3] in Kenya is biased by the large number of clinically diagnosed cases of unconfirmed TB disease, which likely over–estimates true TB disease prevalence in this study population. Using culture–confirmed TB only, infection–to–disease ratio was 5.7 and 2.5 for the Kenyan and South African studies, respectively. We also acknowledge that, as reviewed by Leth *et al.*[53], Styblo's[1] proposed ratio of 8–12 may no longer be accurate, since it was derived for populations without access to TB chemotherapy. In summary, although our infection–to–disease ratios were below Styblo's ratio of 8 to 12, they are comparable to more recent similar studies among adults (Van Leth *et al.).* Recent literature indicates Styblo's Rule does not apply under conditions of adequate (albeit imperfect) TB control (Van Leth *et al.).*

Methodological strengths of this study include the published, peer–reviewed systematic search strategy[55] and data reporting that adhered to the Preferred Reporting Items for Systematic Reviews and Meta–Analyses (PRISMA–R). Potential limitations to generalisability include the few studies meeting eligibility criteria for inclusion, which precluded a meta–analysis; the fact that included studies were from single communities, which might not be representative of country–level prevalence; restriction of analysis to articles published in English; and differences in TST cut–off for positivity across studies which could impact LTBI prevalence and thus ARTI estimates. We acknowledge limitations of Styblo Rule including

that the ratio is highly dependent on prevalence of disease in the general community, duration of exposure to source cases and on the probability of exposure.

In conclusion, we report high prevalence of both LTBI and TB disease among adolescents in high TB burden African countries for the period 1990–2018. Our review also highlights the variability of prevalence estimates across countries, the overall paucity of data on adolescent LTBI and TB disease prevalence in other high TB burden countries in Africa, and the need to benchmark cross–country comparisons against microbiologically–confirmed disease. Improved age disaggregation of adolescent data in research studies and WHO reporting would facilitate evaluation and future monitoring of trends in adolescent TB transmission in response to TB control interventions.

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Chapter 3

3. Temporal trends in prevalence of *Mycobacterium tuberculosis* infection in South African adolescents

3.1. Overview

Summary

This chapter compares prevalence of latent tuberculosis (TB) infection (LTBI) among adolescents living in a single endemic South African community, across two time periods spanning the decade 2005–2015.

Contribution to the thesis and novelty

Comparison of prevalence of LTBI among adolescents across two time periods in the same high TB burden community is important to evaluate medium–term impact of local and regional TB control measures.

Contributions of candidate

The candidate conceived and designed the study, managed the data, performed all analyses and wrote the manuscript with editorial input and guidance from supervisors. Co–authors contributed to research group discussions and commented on the final draft manuscript. The present analysis of change in LTBI prevalence over the decade 2005–2015 used existing baseline and comparative datasets published in part by Mahomed *et al.* (2005) and Nemes *et al.* (2015).

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Publication

Temporal trends in prevalence of *Mycobacterium tuberculosis* infection in South African adolescents

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3.2. Abstract

Setting

South Africa

Objective

To measure changes in adolescent prevalence of latent tuberculosis (TB) infection (LTBI) between 2005–2015, to evaluate medium–term impact of TB control measures on LTBI.

Design

We compared baseline data from a cohort study (2005–2007) and a vaccine trial (2014–2015), which enrolled adolescents from the same 8 South African high schools. LTBI was defined by positive QuantiFERON®–TB Gold In–Tube test.

Results

We analysed data from 4,880 adolescents between 2005–2007 and 1,968 adolescents between 2014–2015, when average LTBI prevalence was 43.8% (95% confidence interval (CI) 28.4–59.1) vs 48.5% (CI: 41.1–55.8), respectively. Age–specific LTBI prevalence increased between the ages of 12–18 years by 13% only in lower socio–economic quintile schools, in which average LTBI prevalence was unchanged between the two periods (54% vs 53%). In highest socio–economic quintile schools, LTBI prevalence did not increase with age, but average LTBI prevalence increased from 20% to 38% between the two periods.

Conclusion

Adolescent LTBI prevalence remained high and constant over a decade, suggesting *Mycobacterium tuberculosis* transmission to children was not impacted in the medium term by effective TB control efforts. Trends in adolescent LTBI prevalence should be interpreted in the context of socio–demographic factors that affect risk of transmission before and during adolescence.

3.3. Introduction

Childhood tuberculosis (TB) is considered a sentinel event that identifies an infectious adult contact, since children with pauci–bacillary disease rarely transmit to others. Similarly, prevalence of latent TB infection (LTBI) in children is an epidemiological indicator of recent *Mycobacterium tuberculosis* transmission from infectious adults.[1] Improved adult TB control measures, including early diagnosis and effective treatment to decrease infectivity, would be expected to reduce childhood LTBI prevalence over time; and short–term impact of improved TB control is likely to be noticed first in very young children. Adolescents have the highest force of *Mycobacterium tuberculosis* infection and adolescent LTBI rates are a barometer of medium–term TB transmission over a 5–10 year period.[1] Therefore, temporal changes in adolescent prevalence of LTBI are proxy measures for medium–term impact of local, regional and national TB programs on childhood TB control.[2]

Other measures of TB transmission include annual risk of tuberculosis infection (ARTI)[3] which is an average measure of risk of infection over the lifetime of study participants that measures recent transmission only if calculated in young children.[4, 5, 6, 7] In South Africa, 2005 was a key time–point in HIV and TB control due to scale–up of highly active antiretroviral therapy (HAART) in public health facilities.[5] Using estimated number of HIV+ individuals as the denominator for standardization, HAART coverage in the study community was <1% in 2004, 12% in 2007, 40% in 2013 and 55% in 2015[6]. Sputum smear microscopy was replaced by Xpert MTB/RIF as the primary test for TB diagnosis in 2013[7]. TB treatment supported by direct observation (directly observed treatment, short–course; DOTS) and passive TB case–finding were in place and remained unchanged between 2005–2015.We compared prevalence of LTBI and ARTI among school–going South African adolescents living in a single endemic community near Cape Town, across two time periods spanning the decade 2005–2015, during which regional TB disease notification rate in the general population fell by 34% from 1,038 (2005) to 682 (2015) per 100,000 per annum.[9]

3.4. Methods

The study population includes adolescents attending state–funded high schools near Cape Town, South Africa. We analysed baseline data from an adolescent cohort study for the period 2005–2007;[10–14] and screening data from an adolescent vaccine trial for the period 2014–2015 (ClinicalTrials.gov reference NCT02075203). LTBI status was defined by positive or negative QuantiFERON®–TB Gold In–Tube (QFT, Cellestis, Carnegie, VIC, Australia) test result[15], excluding individuals diagnosed and treated for TB disease. QFT testing was performed as previously reported,[10–15] and interpreted according to manufacturer's threshold.[16] QFT testing was performed by the same research laboratory for both studies, which were conducted in the same eight high schools.

State schools are classified by socio–economic status, in order to access government school subsidies, into five quintiles from lowest (quintile 1) to highest (quintile 5) socio–economic status.[17] We studied eight schools (A to H), including one school in quintile 1 (D), one in quintile 2 (B), two in quintile 4 (A and C) and four in quintile 5 (E, F, G and H) (see **Table 3.1)**. The quintile category of each school did not change and the annual student population remained similar across the two study periods. We analysed schools in two categories (quintiles 1, 2 and 4 vs. quintile 5), since half of the schools were quintile 5 schools for which parents paid higher fees. Choice of school is largely determined by affordability; we and others have previously demonstrated the relationship between socio–economic status and tuberculosis risk.[1,2]

Prevalence of LTBI was calculated by dividing the number of individuals with a positive QFT test by the total number of individuals. Statistics were adjusted for complex survey design that included simple random sampling with adjustment for clustering at school–level using the *svy* suite of complex survey analysis commands in STATA. ARTI was calculated from LTBI prevalence using the formula $\text{ARTI} = 1 - (1 - \text{Prevalence})^{1/(\text{mean age}+0.5)}$.[4] We used the Wilcoxon rank–sum test to compare medians, the two–sample test of proportions to compare prevalence and socio–demographic characteristics, and logistic regression for regression modelling. With a sample size of 4880 and 1968 per cohort, assuming LTBI prevalence of 55.2% in this community[11], the study had 80% power to detect a difference in LTBI rate of 3.8% at $\alpha=0.05$

Ethical approval was obtained from the Human Research Ethics Committee, University of Cape Town for the original studies, in which written informed consent was obtained from parents or guardians and assent from minor adolescents (HREC REF 045/2005 and HREC REF 471/2013), and for this analysis (HREC REF 163/2016).

3.5. Results

3.5.1. Socio–demographic characteristics

We analysed data for 6,848 participants with either a positive or a negative QFT result: 4,880 (71%) from the period 2005–2007 and 1,968 (29%) from the period 2014–2015 (See Appendix 1). The median age of study participants was 15.5 years (interquartile range (IQR) 14.4–16.7 years) overall and 15.3 (IQR: 14.3–16.4) years in 2014–2015 vs. 15.6 (IQR: 14.5–16.8) years in 2005–2007; p–value<0.001). The number of students enrolled per school varied from a maximum of 1,865 (27%) in school A to a minimum of 116 (2%) in school H (see **Table 3.1**), due to differences in size of the total available student population; 87% of all study participants were from four schools (schools A, B, C and D); and almost half of all students, 3159 (46%), were from two socio–economic quintile 4 schools (schools A and C); there were no students from a quintile 3 school.

Table 3.1: Socio–demographic characteristics of study population

Variable	2005–2007	2014–2015	P–value	Combined
Number of participants	4880 (71.3%)	1968 (28.7%)	NA	6848 (100%)
Age in years (Median (IQR))	15.6 (14.5–16.8)	15.3 (14.3–16.4)	<0.001	15.5 (14.4–16.7)
Gender n/N (%)				
Male	2207/4880 (45.2%)	840/1968 (42.7%)	0.055	3047/6848 (44.5%)
Female	2673/4880 (54.8%)	1128/1968 (57.3%)	0.055	3801/6848 (55.5%)
Ethnicity n/N (%)				
Black African	787/4880 (16.1%)	560/1968 (28.4%)	<0.001	1347/6848 (19.6%)
Mixed race	3697/4880 (75.8%)	1397/1968 (71.0%)	<0.001	5094/6848 (74.4%)
White	389/4880 (8.0%)	7/1968 (0.4%)	<0.001	396/6848 (5.8%)
Asian	7/4880 (0.1%)	4/1968 (0.2%)	0.576	11/6848 (0.2%)
Age				
12	50 (1.0%)	36 (1.8%)	0.007	86 (1.3%)

13	658 (13.5%)	345 (17.5%)	<0.001	1003 (14.6%)
14	1032 (21.2%)	468 (23.8%)	0.017	1500 (21.9%)
15	1084 (22.2%)	475 (24.1%)	0.086	1559 (22.8%)
16	975 (20.1%)	391 (20.0%)	0.917	1370 (20.0%)
17	720 (14.7%)	253 (12.8%)	0.042	973 (14.2%)
18	356 (7.3%)	0 (0.0%)	<0.001	356 (5.2%)
19	1 (0.0%)	0 (0.0%)	0.525	1 (0.0%)
School				
A	1413 (29.0%)	452 (23.0%)	<0.001	1865 (27.2%)
B	1180 (24.2%)	399 (20.3%)	<0.001	1580 (23.1%)
C	867 (17.8%)	427 (21.7%)	<0.001	1294 (18.9%)
D	678 (13.9%)	512 (26.0%)	<0.001	1190 (17.4%)
E	261 (5.4%)	94 (4.8%)	0.334	355 (5.2%)
F	215	20	<0.001	235

	(4.4%)	(1.0%)		(3.4%)
G	192 (3.9%)	21 (1.1%)	<0.001	213 (3.1%)
H	73 (1.5%)	43 (2.2%)	0.046	116 (1.7%)
Quintile				
1	678/4880 (13.9%)	512/1968 (26.0%)	<0.001	1190/6848 (17.4%)
2	1181/4880 (24.2%)	399/1968 (20.3%)	<0.001	1580/6848 (23.1%)
3	…	…	…	…
4	2280/4880 (46.7%)	879/1968 (44.7%)	0.122	3159/6848 (46.1%)
5	741/4880 (15.2%)	178/1968 (9.0%)	<0.001	919/6848 (13.4%)

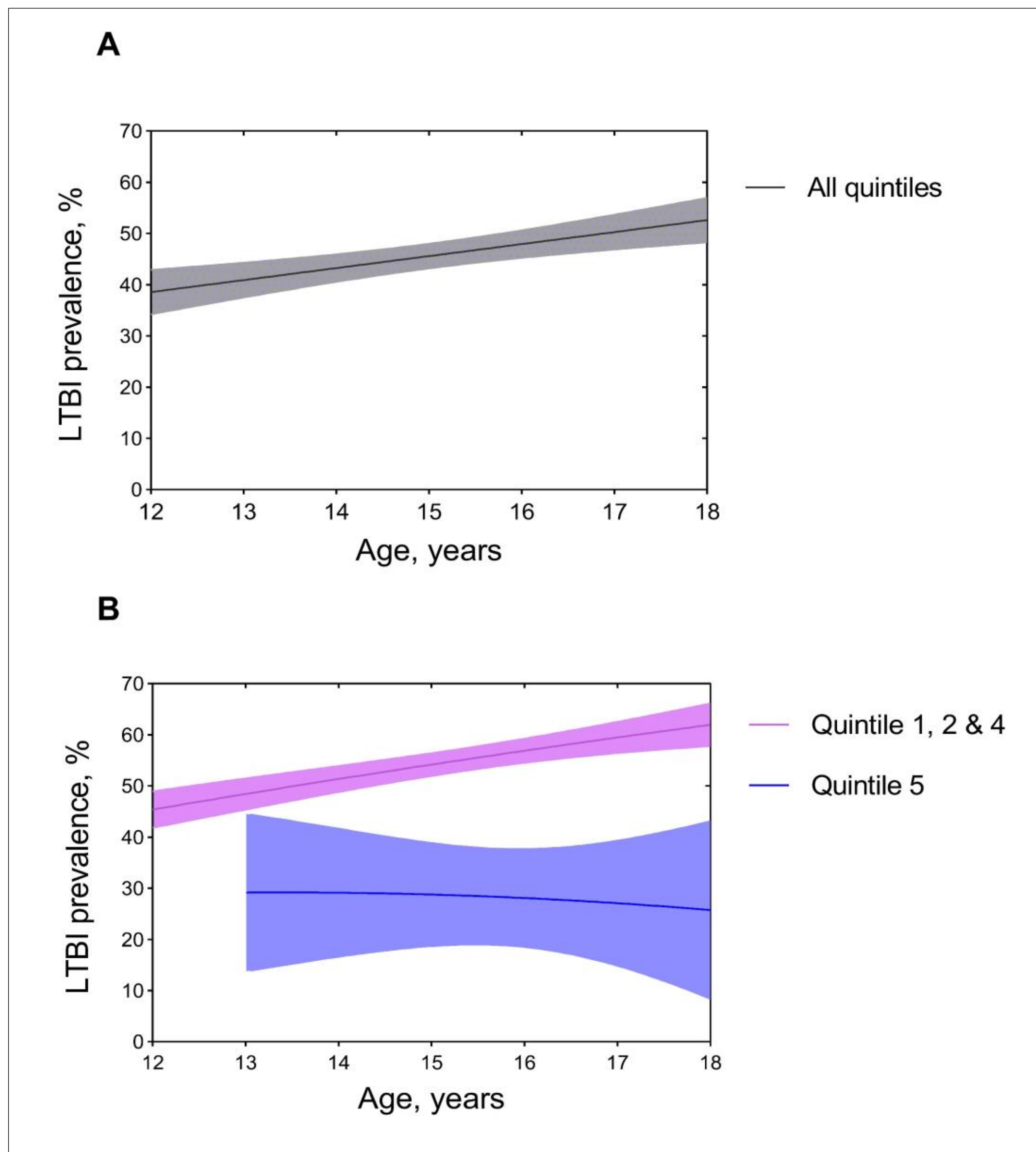
Legend: **n**=Number of study participants with specified attribute. **N**=Total number of participants forming the denominator; **%**=Percentage; **IQR**=Interquartile range; …=no data; NA=Not Applicable.

3.5.2. Prevalence and risk factors for LTBI

Overall, across both time periods, 3,459 (46.1%) of 6,848 study participants had LTBI. In multivariate analysis there was no significant association between risk of LTBI and sex, or time period, but a significant association was observed between LTBI and age, race and socio–economic quintile (**Figure 3.1A** and **Table 3.2**). There was an inverse association between LTBI prevalence and socio–economic quintile classification of schools. On average, students in quintile 5 schools had 51% lower odds of LTBI at any age; were less likely to be infected at school entry; and less likely to become infected between the ages of 12–18 years, than those in lower socio–economic quintile schools (**Figure 3.1B** and **Table 3.2**). LTBI

prevalence was significantly lower at school entry in socio–economic quintile 5 schools (34.0% (CI: 24.2–45.5)) than in lower socio–economic quintile schools (60.3% (CI: 51.5–68.6)), p–value=0.003.

Figure 3.1: Prevalence of LTBI by age and socioeconomic quintiles



Legend: LTBI=Latent Tuberculosis Infection. **Panel A** shows prevalence of LTBI by age among all students (grey). LTBI prevalence increased from 38.7% at 12 years to 50.3% at 18 years. **Panel B** shows prevalence of LTBI by age among students in quintile 5 schools (blue) and among students in quintiles 1, 2 and 4 schools combined (purple). LTBI prevalence increased from 48.2% at 12 years to 61.3% at 18 years among quintile 1, 2 and 4 schools. In quintile 5 schools, none of the 5 students aged 12 years had LTBI; LTBI prevalence was 26.6% at 13 years and 20.7% at 18 years. Shaded areas represent 95% confidence interval of prevalence estimates.

Table 3.2: Factors associated with risk of latent tuberculosis infection

Variable	n/N (Prevalence of LTBI; CI)	Crude OR (CI)	P–value	Adjusted OR* (CI)	P–value*
All	3459/6848 (46.1%; 39.2 – 58.4)	1.21 (0.84–1.73) †	0.255	NA	NA
Age (in years)	NA	1.14 (1.08–1.19)	<0.001	1.16 (1.10–1.22)	<0.001
Sex					
Female	1911/3801 (47.1%; 37.0 – 57.2)	1.00 (Ref)	NA	1.00 (Ref)	NA
Male	1548/3047 (45.0%; 30.4 – 59.6)	0.92 (0.58–1.47)	0.682	1.06 (0.91– 1.25)	0.391
Race					
Black African	669/1347 (49.4%; 45.8 – 53.1)	1.00 (Ref)	NA	1.00 (Ref)	NA
Mixed race	2741/5094 (49.7%; 40.6 – 58.8)	1.01 (0.73–1.40)	0.944	1.11 (0.84–1.48)	0.410
Asian	2/11 (12.2%; 2.8 – 21.5)	0.24 (0.06–1.02)	0.053	0.30 (0.05–1.82)	0.157
White	47/396 (18.9%; 0–41.6)	0.14 (0.06–0.32)	0.001	0.29 (0.11–0.56)	0.005
Socioeconomic category of school					
Quintile 1, 2 and 4	3236/5929 (53.8%; 49.3, 58.4)	1.00 (Ref)	NA	1.00 (Ref)	NA
Quintile 5	223/919	0.35	0.002	0.49	0.002

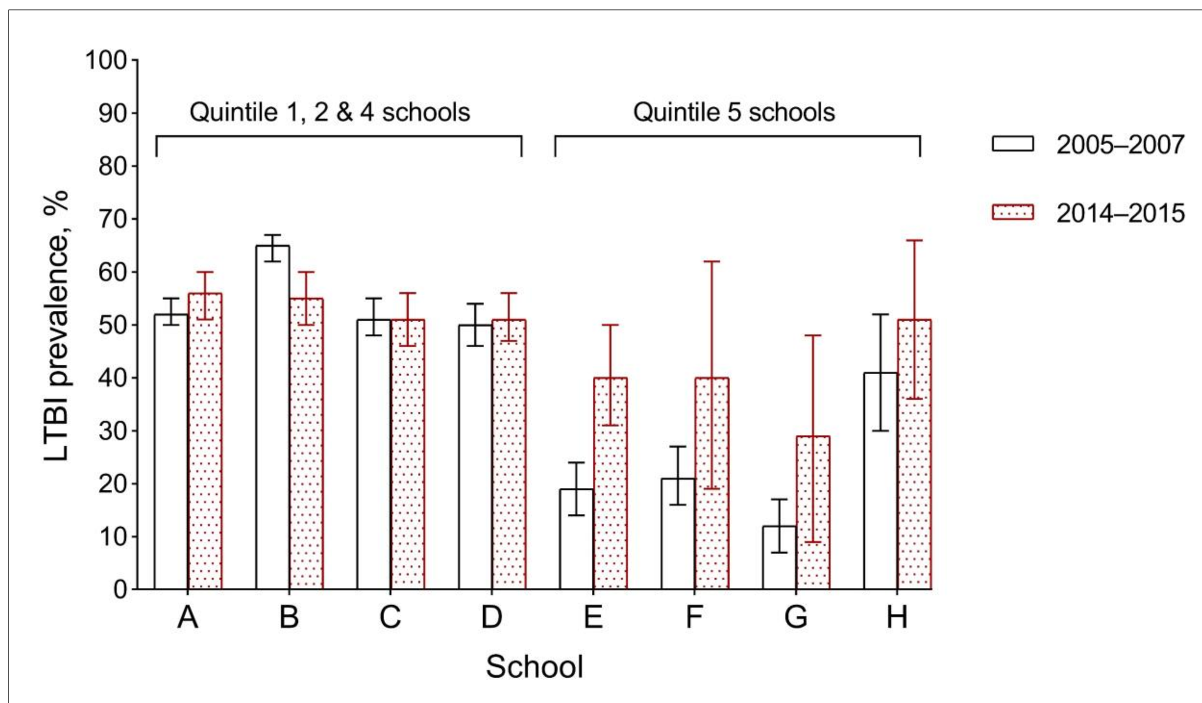
	(29.2%; 19.4, 39.0)	(0.21–0.59)		(0.34–0.69)	

Legend: * =Results from multivariate logistic regression adjusted for survey design with the following variables; age, race, school. †=the period 2014–2015 was the reference. There were no students from quintile 3 schools**. Ref**=Reference variable in analysis. **CI**=95% Confidence interval. **LTBI**=Latent tuberculosis infection. **n/N**=Number of participants with latent tuberculosis infection/total number of participants. Age of adolescents is between 12 and 19 years.

3.5.3. Prevalence of LTBI across time periods

Analysed by school and time period, prevalence of LTBI was highest in school B (quintile 2) in 2005–2007 (64.6%) and lowest in school G (quintile 5) in 2005–2007 (12.0%) (see **Figure 3.2** and **Appendix 2**). Prevalence of LTBI changed significantly over time only in three schools, increasing from 19.2% (CI: 14.4–23.9) to 40.4% (CI: 30.5–50.3) and from 12.0% (CI: 7.4–16.6) to 28.6% (CI: 9.2–47.9) in quintile 5 schools E and G respectively, but decreasing from 64.6% (CI: 61.9–67.3) to 55.1% (CI: 50.3–60.0) in quintile 2 school B (**Figure 3.2**).

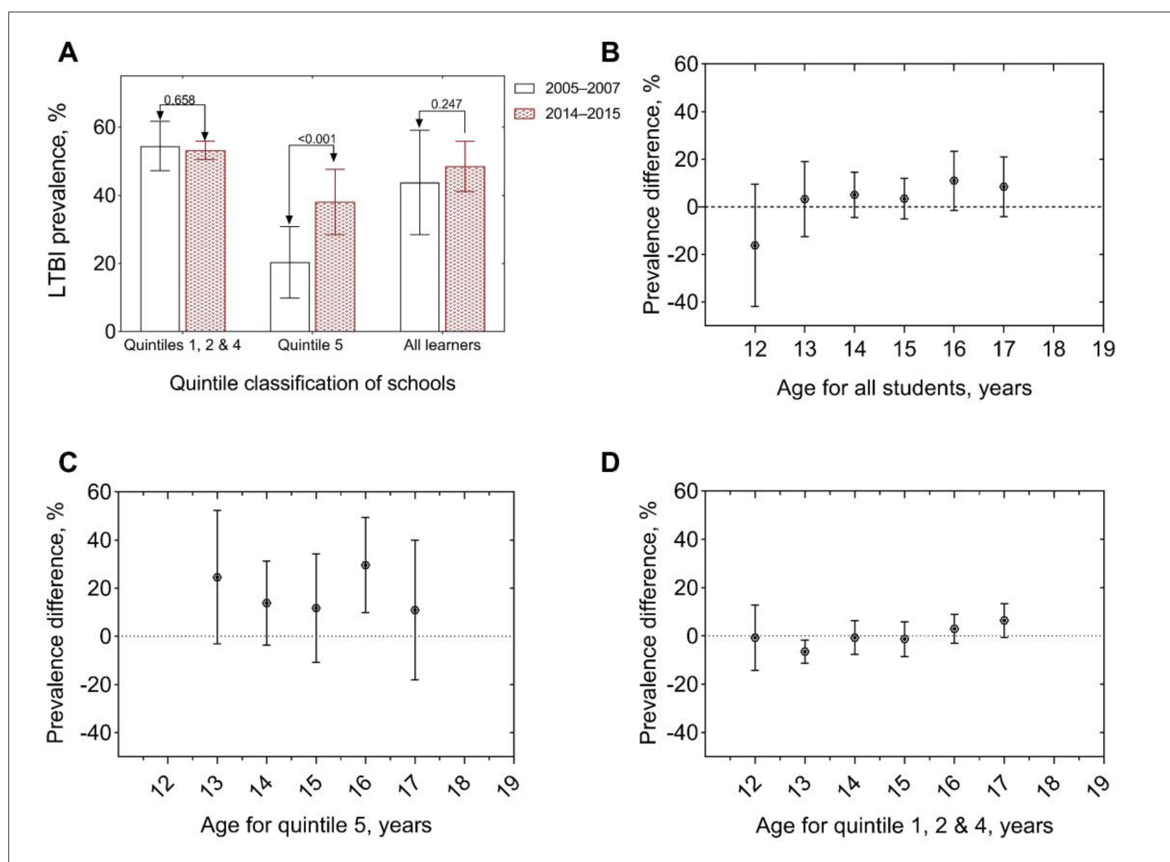
Figure 3.2: Prevalence of LTBI by school and time period



Legend: Letters A to H represent the 8 schools in the study area. Whiskers on bar charts represent 95% confidence intervals of the prevalence of LTBI at each school. Prevalence of LTBI significantly changed over time in 3 schools, increasing in quintile 5 schools E (p=<0.001) and G (p=<0.035), but falling in quintile 2 school B (p=0.001).

There was no significant difference in overall prevalence of LTBI by sex, age or race between the two time periods (**Table 3.3** and **Figure 3.3**). Students in socio–economic quintile 5 schools had the largest increase in prevalence of LTBI across the two time periods (from 20.4% to 38.0%), compared to all other quintile categories (from 54.4% to 53.2%) (**Figure 3.3** and **Appendix 3**), increasing at all ages, although a statistically significant difference was only observed among students aged 16 years (**Figure 3.3**). Each quintile 5 school recorded >10% increase in prevalence of LTBI, with a collective 17.6% increase in prevalence ($p < 0.001$) between the periods 2005–2007 and 2014–2015.

Figure 3.3: Prevalence of LTBI across two time periods, 2005–2007 and 2014–2015



Legend: **Panel A** shows prevalence of LTBI by socioeconomic quintile category of school and time period, 2005–2007 vs 2014–2015. Panel A shows prevalence of LTBI significantly increased among students in quintile 5 schools ($p<0.001$), but did not significantly change among students in quintile 1, 2 and 4 schools (p=0.658) across the two

time periods. **Panels B–D** show prevalence difference of LTBI by age across the two time periods, 2005–2007 vs 2014–2015: for all students (**Panel B)**; for students in quintile 5 schools (**Panel C);** and for students in quintile 1, 2 and 4 schools (**Panel D). LTBI**=Latent Tuberculosis Infection. Stars represent the difference in prevalence of LTBI between 2005–2007 and 2014–2015 (i.e. prevalence difference). Error bars represent 95% confidence interval of the prevalence difference. Stars above the X–axis denote an increase in prevalence of LTBI whereas those below the X–axis represent a reduction in prevalence of LTBI in 2014–2015 as compared to 2005–2007. Error bars completely above or below the X–axis represent a statistically significant increase or decrease in prevalence.

Table 3.3: Prevalence of latent tuberculosis infection by socio–demographic variables

Variable	**Prevalence 2005 – 2007** {n/N (%; CI)}	**Prevalence 2014 – 2015** {n/N (%; CI)}	**Prevalence difference** (%; CI)	**P–value**
All	2434/4880 (43.8%; 28.4 – 59.1)	1025/1968 (48.5%; 41.1 – 55.8)	4.7% (–4.1; 13.5)	0.247
Sex				
Male	1111/2207 (42.5%; 22.5 – 62.4)	437/840 (47.6%; 37.9 –57.3)	5.1% (–6.7; 16.9)	0.340
Female	1323/2673 (45.0%; 31.1 – 58.8)	588/1128 (49.3%; 42.5 –56.0)	4.3% (–3.9; 12.6)	0.257
Race				
Black African	386/787 (48.0.%; 41.4 – 54.7)	283/560 (50.7%; 48.0 – 53.5)	2.7% (–3.7; 9.1)	0.347
Mixed Race	2002/3697 (51.1%; 39.8 – 62.5)	739/1397 (48.5%; 40.0 – 57.0)	–2.6% (–11.1; 5.9)	0.488
White	45/389 (10.5%; 6.4 – 14.6)	2/7 (25.2%; 0.0 – 69.0)	14.7% (–25.9; 55.3)	0.421
Asian	1/7 (15.8%; 0 – 41.9)	1/4 (25.3%; 0 – 70.0)	9.5% (–42.3; 61.2)	0.678
Age				

12	24/50 (48.5%; 43.9–53.2)	15/36 (32.3%; 5.3–59.4)	–16.2 (–41.8; 9.4)	0.178
13	283/658 (36.8%; 20.7 – 52.9)	145/345 (40.0%; 30.4 – 49.6)	3.2 (–12.6; 19.0)	0.647
14	479/1032 (40.8%; 25.8 – 55.8)	230/468 (45.8%; 38.3 – 53.4)	5.0 (4.5; 14.5)	0.254
15	557/1084 (44.4%; 27.4 – 61.3)	253/475 (47.8%; 34.9 – 60.7)	3.4 (–5.1; 12.0)	0.374
16	507/979 (46.8%; 33.4 – 60.1)	226/391 (57.8%; 51.6 – 63.9)	11.0 (–1.5; 23.4)	0.075
17	379/720 (45.7%; 29.0 – 62.3)	156/253 (54.1%; 39.5 – 68.6)	8.4 (–4.1; 21.0)	0.156
18	204/356 (50.3%; 31.1 – 69.5)	…*	…*	…*
19	1/1 (…) *	…*	…*	…*
Socioeconomic quintile of school				
Quintiles 1, 2 and 4	2285/4139 (54.4%; 47.1–61.5)	951/1790 (53.2%; 50.5–55.9)	–1.2% (–7.2; 4.9)	0.658
Quintile 5	149/741 (20.4%; 11.8–32.8)	74/178 (38.0%; 29.0–47.9)	17.6% (13.2; 22.0)	< 0.001

Legend: …*=insufficient data for generation of estimates. **n/N**=Number of participants with latent tuberculosis infection/total number of participants. **CI**=95% confidence interval.

The demographic composition of study participants in quintile 5 schools changed significantly between the periods 2005–2007 and 2014–2015. In 2005–2007, 387/741 (52%) and 301/741 (41%) of participants were of White and Cape Mixed Race ancestry, respectively, whereas in 2014–2015, 136/178 (76%) and 36/178 (20%) of participants were of Cape Mixed Race and Black African ancestry. Among quintile 5

schools, prevalence of LTBI significantly increased only among students of Black African ancestry, in whom it increased by 24.0% (CI: 9.0, 39.0). Participants were significantly older in 2005–2007 as compared to 2014–2015 in quintile 5 schools, although the magnitude of the difference was small: 15.5 (CI: 14.5–16.8) vs 15.2 (CI: 14.1–16.4) respectively. Prevalence of LTBI in quintile 5 schools significantly increased in both sexes, by 14.3% (CI: 4.7, 23.9) and 20.0% (CI: 14.4–25.6) among female and male students, respectively.

Participants in quintile 1, 2 and 4 schools were also significantly older in 2005–2007 as compared to 2014–2015: 15.7 vs 15.3 respectively, although the magnitude of the difference was small. Prevalence of LTBI in these schools did not significantly increase in any racial group, or by sex, across the two time periods.

3.5.4. Annual risk of tuberculosis infection (ARTI)

Median age of study participants in each year of the two periods was between 14.7 and 16.0 years. Overall ARTI was 3.9% (CI: 2.7–5.3) per annum for the two time periods combined; and 3.6% (CI: 2.1–5.5) per annum in 2005–2007, compared to 4.2% (CI: 3.4–5.2) per annum in 2014–2015. ARTI varied by calendar year (see **Table 3.4** and **Appendices 4 & 5)**. ARTI in lower socio–economic quintile schools was 4.9% for school A, 5.6% for school B, 4.5% for school C, and 4.4% for school D. In highest socio–economic quintile schools, ARTI was 2.3% for school E, 2.3% for school F, 1.4% for school G and 3.8% for school H.

Table 3.4: Annual risk of tuberculosis infection

Year of study	Notification rate of TB disease in year	Number (%)	Median age (IQR)	Prevalence of LTBI (CI)	ARTI (CI)
2005	1038*	312 (3.9%)	16.0 (14.7–17.2)	37.9% (29.2–46.6)	2.94% (2.14–3.85)
2006	1031*	4871 (60.8%)	15.6 (14.5–16.8)	55.5% (48.8–62.2)	5.03% (4.18–6.02)
2007	1006*	854 (10.7%)	15.5 (14.5–16.8)	21.1% (9.8–32.4)	1.50% (0.65–2.47)
2014	710†	1325 (16.6%)	15.5 (14.5–16.5)	50.0% (44.1–56.0)	4.37% (3.67–5.15)
2015	682†	643 (8.0%)	14.7 (13.8–15.9)	45.4% (33.0–57.7)	3.98% (2.66–5.62)

Legend: IQR=Interquartile range; **CI** =95% confidence interval; **TB**=Tuberculosis; **LTBI**=Latent Tuberculosis Infection; **ARTI**=Annual risk of TB infection. Rates of TB disease are presented per 100,000 population. * / † Estimates were derived from the Health Systems Trust.

3.6. Discussion

We showed no overall change in LTBI prevalence or ARTI among South African adolescents over the decade 2005–2015. Notably, school–level prevalence of LTBI varied between 12% and 65%; and school–level ARTI varied between 1.4% and 5.6%. Students in socio–economic quintile 5 schools were less likely to be infected at school entry and less likely to become infected between the ages of 12–18 years, than those in lower socio–economic quintile schools, yet this was the only category in which overall prevalence of LTBI increased over the decade 2005–2015. Our finding of differential risk of LTBI by school and socioeconomic category, coupled with our previous finding that schools are a significant source of adolescent TB transmission,[19] emphasises the need to understand school–level factors that put some

adolescents at higher risk for LTBI. We observed no difference in overall prevalence of LTBI by sex, age or race between the two time periods.

Reduced TB transmission depends upon early TB case–finding and treatment. Thus LTBI prevalence and ARTI estimates reflect effectiveness of TB control and declining trends would reflect increasing impact of control programmes.[20] Short–term risk of LTBI, which is best measured by force of *Mycobacterium tuberculosis* infection in prospective cohort studies, changes quickly in response to improved diagnosis and treatment of active TB disease, whereas prevalence and ARTI rates change more slowly.[21] Nonetheless, prevalence and ARTI are useful to evaluate medium–term geographical and temporal trends in TB transmission and effectiveness of TB control.[22] Periodic LTBI surveys also have the advantage of being cheaper and logistically easier to conduct than cohort studies in resource–limited, high TB burden settings.[21] Our observation that adolescent prevalence of LTBI remained high and relatively unchanged, while notification rates of TB disease fell in the same calendar years, may be explained by the fact that the adolescent ARTI and LTBI prevalence represent cumulative lifetime measures[3] and the majority of latently infected individuals do not exhibit reversion of their positive IGRA result to negative.[4] Few studies compare prevalence of LTBI or ARTI across two time periods among African or Asian children or adolescents in the same source population. In Kenya, there was an increase in prevalence of LTBI from 5.7% to 8.7% in school children aged 6–14 years between 1986–1996.[24] In South Africa, among 6–9 year–old children, there was no significant change in prevalence of LTBI between 1998 and 2005 (26.2% vs 28.9%).[25] These prevalence are also representative of a period when TB notification rates were on an upward trend in the Western Cape Province [9] and South Africa.[26] By contrast, an Egyptian study reported a decline in prevalence of LTBI among children aged 6–7 years over a much longer period between 1949–1952 and 1995–1997;[27] and in India there was a significant decline in prevalence of LTBI, defined by positive TST, among children younger than 10 years of age (from 7.8% to 6.9% to 6.0% for the periods 1999–2001, 2001–2003 and 2004–2005, respectively), following implementation of DOTS.[28]

The key strengths of our study include the large sample size, the same community, and laboratory testing under rigorous conditions in the same research laboratory.[31] Interpretation of our data is subject to certain limitations. We acknowledge that the demographic composition of study participants may not be exactly representative of the local community, especially in respect of racial ancestry, which is critical when evaluating temporal changes in prevalence of LTBI. Changing racial ancestry in quintile 5 schools over time may partly explain differential risks for LTBI, given the long–standing racial disparities in socioeconomic status of South Africans, which is in turn associated with differential risk of TB. There is also a high drop–out rate (30%–55%) among South Africa high school students, primarily affecting poor underperforming students.[29] Differential drop–out by socioeconomic status might therefore lead to underestimation of true LTBI prevalence among older adolescents. HIV infection may also result in underestimation of LTBI prevalence defined by IGRA.[24] However, HIV prevalence is historically low among adolescents in this region, for example, only 1.6% of participants diagnosed with TB disease in 2005–2007 were confirmed HIV positive;[11] and all participants in the period 2014–2015 were confirmed HIV ELISA negative at the time of IGRA testing. Finally, our finding that adolescent prevalence of LTBI did not change over the decade 2005 – 2015 may not be generalizable to settings with different social determinants of health, regardless of the success of regional TB disease control efforts.

LTBI surveys for estimation of ARTI are often carried out among children younger than 10 years of age.[20] The cross–sectional adolescent LTBI prevalence and ARTI estimates reported here depict medium–term, not short–term changes in TB transmission. Compared to longitudinal cohorts, cross–sectional IGRA studies may underestimate the true rate of infection, owing to IGRA reversion after conversion.[23] For example in the same source population, force of *Mycobacterium tuberculosis* infection among adolescents almost doubled when QFT reversions were included.[23] Thus, changes in LTBI prevalence and ARTI reported in our cross–sectional analyses likely underestimate the true scale of TB transmission in this community. Mathematical modeling studies are planned to investigate the inter–relationships between these different measures of TB transmission in this adolescent population.

Conclusion

In conclusion, adolescent prevalence of LTBI and ARTI remained high and constant over the decade 2005–2015 in this South African community, suggesting that TB transmission to school–age children was not yet impacted significantly by successful programmatic TB control efforts. We also show that differential risk of LTBI before and during adolescence is closely associated with socio–economic factors that must be taken into account when conducting LTBI surveys in this population. The World Health Organisation recommends repeated LTBI surveys at 5–7 year intervals to monitor short–term impact of TB control programmes.[20] We additionally recommend that periodic IGRA surveys should be carried out among adolescents from a wide range of socio–economic backgrounds, to demonstrate if and when falling TB disease notification rates have a lasting impact on TB transmission to children.

Contributorship statement

EWB conceptualised, designed the study and wrote the study protocol, performed analysis and prepared the first draft of the manuscript with supervision from HG, RW and MH. EWB, HG, HMu, JS, AKKL, MT, EN, HMa, VR, RW, TS, JRA and MH critically reviewed, revised and approved the final version of the manuscript.

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Competing interest and funding

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and conclusions arrived at, are those of the authors and are not necessarily attributed to the NRF. EWB initiated the research. This research is not done on behalf of or commissioned by the NRF. The first author (EWB) would also like to appreciate supplementary departmental scholarship from the South African Tuberculosis Vaccine Initiative. Authors have no potential conflicts of interest to declare.

Data sharing

Dataset will be available on the Dryad repository.

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Chapter 4

4. Regional changes in tuberculosis disease burden among South African adolescents (2005–2015)

4.1. Overview

Summary

This chapter describes temporal changes in adolescent TB notification rates in a South African community over the decade 2005–2015.

Contribution to the thesis and novelty

Demonstration of temporal changes in the TB epidemic is important for re–appraisal of local and regional TB control efforts in line with global "End TB Strategy" targets.

Contributions of candidate

The candidate conceived and designed the study, managed the data, performed all analysis and wrote the manuscript with editorial input and guidance from supervisors. Co–authors contributed to research group discussions and commented on the final draft manuscript.

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Publication

Regional changes in tuberculosis disease burden among South African adolescents (2005–2015)

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4.2. Abstract

Background

Adolescents in the Western Cape Province of South Africa had high force of *Mycobacterium tuberculosis* infection (14% per annum) and high TB incidence (710 per 100,000 person–years) in 2005. We describe temporal changes in adolescent TB notification rates for the decade 2005–2015.

Method

We conducted an analysis of regional patient–level adolescent TB disease data, obtained from an electronic TB register, for 2005–2015. Numerators were annual TB notifications (HIV–related and HIV–unrelated); denominators were mid–year population estimates. Period averages of TB rates were obtained using time series modeling. The Agresti Coull method was used to derive confidence intervals for TB rates. Temporal trends in TB rates were explored using the Mann–Kendall test.

Findings

The average adolescent TB notification rate was 477 (95% Confidence Interval (CI): 313–641) per 100,000 for all cases (all–TB) and 361 (CI: 280–441) per 100,000 for microbiologically–confirmed cases. The

adolescent all–TB rate fell by 45% from 662 to 361 per 100,000 and the microbiologically–confirmed TB rate by 38% from 492 to 305 per 100,000 between 2005–2015, driven mainly by rapid decreases for the period 2005–2009. There was a significant negative temporal trend in adolescent TB rates between 2005–2015 (p=0·005), but that trend differed markedly pre– and post–2009. There was a statistically significant negative temporal trend in both all–TB (p=0·028) and microbiologically confirmed TB (p=0·027) for 2005–2009, which was not observed for the period 2009–2015 (p=0·764 and p=1·000, respectively).

Interpretation

We observed an encouraging fall in adolescent TB rates between 2005–2009 with a subsequent plateau during 2010–2015, suggesting that additional interventions are needed to sustain advances in TB control.

4.3. Background

Historical tuberculosis (TB) case notification rates in the greater Cape Town region of South Africa have changed significantly over the last century. The annual TB disease rate was approximately 450 per 100,000 general population between 1910–1950, but decreased in parallel with introduction of TB chemotherapy to the 20th century nadir of 250 per 100,000 in 1970,[1] followed by a sustained increase to 850 per 100,000 in 2005, driven primarily by the HIV epidemic.[1] The year 2005 was key for national HIV and TB control, due to scale–up of antiretroviral therapy (ART) in public health facilities, with increasing ART coverage over subsequent years.[2] It was hoped that introduction of the sputum Xpert MTB/RIF assay as the primary test for TB diagnosis in 2013[3] would improve diagnostic yield, laboratory efficiency, and ability to detect rifampicin–resistant *Mycobacterium tuberculosis* faster than liquid culture.[4] Introduction of these measures against the background of sustained programmatic TB control, including direct observation of treatment (directly observed treatment, short–course; DOTS),[5] raises the question of whether these interventions had measurable impact on TB rates in South African communities. Demonstration of temporal changes in the epidemic is important for re–appraisal of regional TB control in line with global "End TB Strategy" targets.[6]

Whereas early childhood TB disease by definition reflects only recent *Mycobacterium tuberculosis* transmission and adult TB disease is a function of cumulative lifetime risk of infection and progression to disease, adolescent TB rates represents an ideal proxy for medium–term *Mycobacterium tuberculosis* transmission over a 10–15 year period by which impact of TB control programs may be extrapolated.[7]

We aimed to describe temporal changes in adolescent TB notification rates over the decade 2005–2015, based on analysis of electronic health service data for the Breede Valley subdistrict, a high TB incidence setting near Cape Town, South Africa. Background TB rates among adults and children are provided for context.

4.4. Methods

4.4.1. Study design, population and setting

This is an analysis of electronic TB disease patient notification data from health authorities in the Breede Valley subdistrict, Western Cape Province, South Africa, between 1st January 2005 – 31st December 2015. Estimated population in 2005 was 154,565, of whom 28,643 (18·5%) were adolescents. Average annual population growth between 2005–2015 was 1·31%.[8] The general population composition in this subdistrict in 2010 was 63·3% Mixed Race, 24·3% Black African, 10·7% Caucasian, and 1·7% other classifications[8]; estimated general population HIV prevalence was 3·7% in 2005 and 4·6% in 2010.[9] In the local general population, the proportion of persons living with HIV and receiving antiretroviral therapy (ART) increased from <1% in 2005 to 55% in 2015. TB diagnostic, prevention and treatment guidelines did not change significantly between 2005–2015 with the exception of introduction of sputum Xpert MTB/RIF in place of sputum smear microscopy as the primary TB diagnostic test from 2013 onwards.[10]

4.4.2. Definitions

A TB disease case was defined as an episode of TB disease that was electronically notified by public health authorities. All treatment for TB disease is undertaken in public health facilities and is free of charge. The

regional TB control program maintains both a paper record and from 2001 onwards an electronic register of TB disease cases treated in health facilities. TB disease cases include all TB disease patients, regardless of clinical site or diagnostic approach (microbiologically confirmed or clinically diagnosed), that were treated and notified by public health authorities, with the exclusion of patients started on treatment outside the study area and referred to facilities within the study area; and (ii) patients re–treated after default or treatment failure and re–registered, in line with WHO recommendations[11, 12]. Due to high rates of re–infection after cure, relapse or re–infection TB disease cases were treated in statistical analysis as new TB disease events. Persons who received only isoniazid chemoprophylaxis were excluded.

Adolescents were defined as persons aged 10–19 years[13], adults as persons aged ≥ 20 years and children as persons aged <10 years. TB disease case notification rate was determined by dividing the annual number of notified TB disease cases by the estimated mid–year population, multiplied by 100,000. Annual percentage change in TB rate was obtained by dividing the difference between TB rate for a given year and TB rate for the previous year by the TB rate for the previous year multiplied by 100%. Microbiologically–confirmed TB was defined as sputum smear microscopy positive for acid–fast bacilli and/or positive liquid *Mycobacterium Tuberculosis* culture and/or positive Xpert MTB/RIF assay on one or more occasions. TB disease cases were defined as living with HIV if a positive HIV serology result was recorded, or if the patient was recorded to be receiving ART or co–trimoxazole prophylaxis. HIV–negative status was defined by a recorded HIV–negative serology result. All other cases were considered to be of unknown HIV status.

4.4.3. Statistical analysis

The annual mid–year population estimate was derived from the 2001 and 2011 national census[14] and adjusted for annual population growth assuming linear growth between census estimates.[8] Trend analysis for demographic and clinical characteristics was assessed using the Chi–squared test for trend (X^2_{trend}), except for age and TB rates for which the Mann–Kendall[15] test was used, allowing for quantification of the magnitude of trend using the Theil–Sen median slope estimator.[16] The Durbin–Watson statistic was

used to test and quantify autocorrelation in TB rates. The average TB rates for each period was estimated using a first order Box–Jenkins autoregressive integrated moving–average (ARIMA). The Agresti Coull method was used to obtain confidence intervals for TB rates. All statistical tests were 2–sided at alpha 0·05. This being a population level study, sample size considerations do not apply.

4.4.4. Ethical approval

Ethical approval was obtained from the University of Cape Town Human Research Ethics Committee (HREC REF 163/2016).

4.4.5. Role of the funding source

Funding sources 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.

4.5. Results

4.5.1. Demographic and clinical characteristics

A total of 21,250 TB disease cases were notified in Breede Valley subdistrict between 2005–2015, of whom 1,461 (6·9%) were among adolescents. The annual number of adolescent TB disease cases ranged between 102 (6·3%) – 187 (7·9%), with a statistically significant negative trend in proportion of all cases, falling from 8% to 6% between 2005–2015. The median age of adolescent TB disease cases also increased by an average of 1·0 (0·6–1·5) month annually over that period (p<0·001) (Table 4.1). 50% of adolescent TB disease cases occurred between the age of 17–19 years. 77% of adolescent TB disease cases were microbiologically–confirmed and this proportion did not change significantly after Xpert MTB/RIF scale–up in 2013. HIV testing of adolescent TB disease patients significantly increased from 1% to 88% between 2005–2015, when 45% of adolescent TB disease patients were known to be HIV–infected (2015).

Table 4.1: Demographic and clinical characteristics of adolescent TB disease cases

Variable	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**P–value**	**2005 – 2015**
Cases (Adolescents/All; %)	187/2372 (7·9%)	161/2155 (7·5%)	146/1963 (7·4%)	135/1788 (7·6%)	102/1631 (6·3%)	129/1821 (7·1%)	121/1927 (6·3%)	118/1761 (6·7%)	132/1798 (7·3%)	114/2047 (5·6%)	116/1987 (5·8%)	<0·001	1461/21250 (6·9%)
Age; Median (IQR)	16·0 (14·0–18·0)	16·0 (14·0–18·0)	17·0 (15·0–18·0)	17·0 (15·9–18·6)	17·9 (15·9–18·7)	17·6 (15·3–19·1)	16·7 (13·8–18·6)	17·1 (15·0–18·9)	17·2 (14·9–18·7)	17·4 (15·1–18·9)	17·2 (15·4–18·5)	<0·001	17·0 (15·0–18·6)
Sex (Male) n/N; %)	88/187 (47·1%)	69/161 (42·9%)	66/146 (45·2%)	55/135 (40·7%)	45/102 (44·1%)	70/129 (54·3%)	65/118 (53·7%)	46/118 (39·0%)	73/132 (55·3%)	57/114 (50·0%)	59/116 (50·9%)	0·350	693/1461 (47·4%)
Proportion of adolescent TB microbiologically confirmed (%)	139/187 (74·3%)	125/161 (77·6%)	117/146 (80·1%)	102/135 (75·6%)	75/102 (73·5%)	104/129 (80·6%)	92/121 (76·0%)	99/118 (83·9%)	97/132 (73·5%)	82/114 (71·9%)	98/116 (84·5%)	0·465	1130/1461 (77·3%)
Clinical type (PTB/(PTB+EPTB) (%)	168/187 (89·8%)	147/161 (91·3%)	129/146 (88·4%)	117/135 (86·7%)	80/102 (78·4%)	112/129 (86·8%)	106/121 (87·6%)	109/118 (92·4%)	115/132 (87·1%)	98/114 (86·0%)	110/116 (94·8%)	0·029	1291/1461 (88·4%)

Treatment episode (New/(New + Relapse)) (%)	172/187 (92·0%)	146/161 (90·7%)	135/146 (92·5%)	123/135 (91·1%)	94/102 (92·2%)	120/129 (93·0%)	116/121 (95·9%)	106/118 (89·8%)	125/132 (94·7%)	109/114 (95·6%)	111/116 (95·7%)	0·029	1357/1461 (92·9%)
Proportion with known HIV status of notified TB disease cases n/N (%)	1/187 (1·1%)	9/161 (5·6%)	35/146 (24·0%)	49/135 (36·3%)	62/102 (60·8%)	70/129 (54·3%)	31/121 (25·6%)	88/118 (74·6%)	108/132 (81·8%)	97/114 (85·1%)	102/116 (87·9%)	0·001	652/1461 (44·6%)
Proportion HIV+ of notified TB disease cases with known HIV status n/N (%)	0/1 (0·0%)	1/9 (11·1%)	3/35 (8·6%)	9/49 (18·4%)	6/62 (9·7%)	9/70 (12·9%)	6/31 (19·4%)	9/88 (10·2%)	10/108 (9·3%)	12/97 (12·4%)	46/102 (45·1%)	<0·001	111/652 (17·0%)
Proportion of adolescent TB disease patients that died (%)	1/179 (0·6%)	1/158 (0·6%)	0/139 (0%)	1/133 (0·8%)	1/93 (1·1%)	0/124 (0%)	0/117 (0%)	0/110 (0%)	1/126 (0·8%)	1/100 (1·0%)	0/99 (0%)	0·801	6/1378 (0·4%)
Proportion cured or completed treatment of	149/179 (83·2%)	141/158 (89·2%)	121/139 (87·1%)	119/133 (89·5%)	82/93 (88·2%)	109/124 (87·9%)	100/117 (85·5%)	99/110 (90·0%)	116/126 (92·1%)	87/100 (87·0%)	80/99 (80·8%)	0·861	1203/1378 (87·3%)

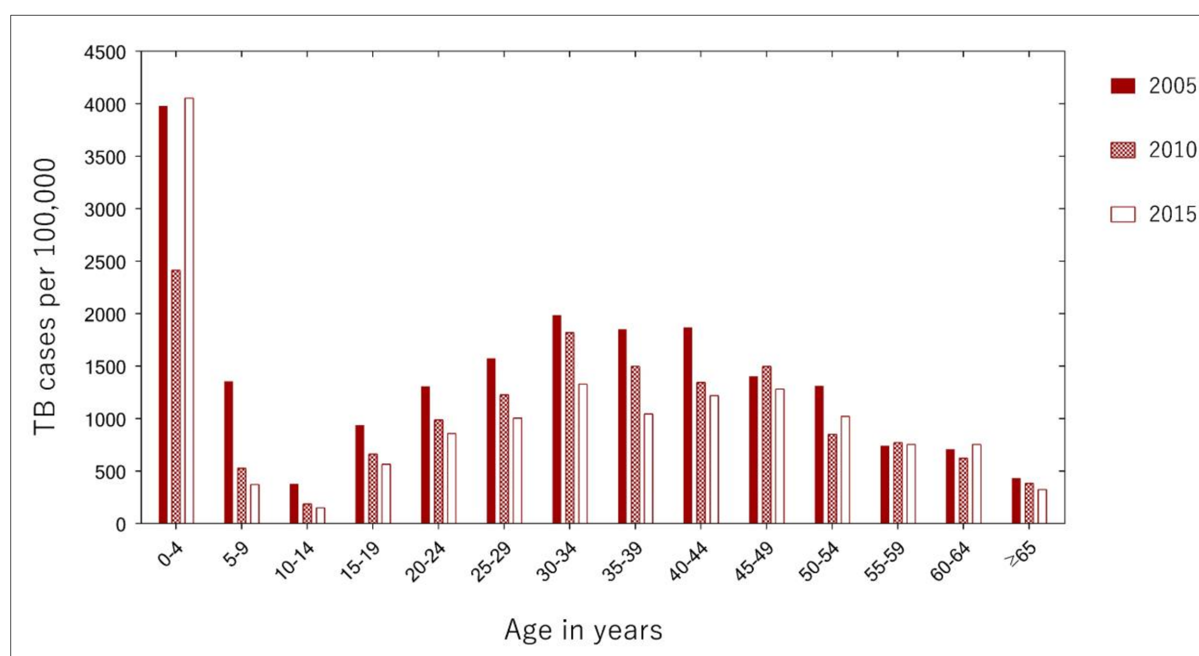
adolescent TB disease patients (%)													

Legend: TB=Tuberculosis; %=percent; PTB=Pulmonary TB; EPTB=Extra–Pulmonary TB; new=new cases of TB disease; relapse=cases of TB disease that relapsed. P–value is for temporal trend between 2005 and 2015 obtained using the Mann–Kendall test statistic for age but chi–squared test for trend for other variables. Proportion of all TB disease cases occurring among children < 10 years of age significantly increased (from 34% to 39%; p<0·001), those among adolescents significantly reduced (from 8% to 6%; p=0·008) whereas those among adults (≥20 years) significantly reduced between 2005–2015 (from 58% to 55%; p=0·043). However, this pattern is heavily influenced by the sudden upward increase in TB disease cases in children aged under five years in 2014 and 2015.

4.5.2. General population TB rates by age and year

TB rates followed a bimodal age pattern with the highest risk in children aged below five years old, with a second peak among individuals aged 30–45 years. Adolescents aged 10–14 years had the lowest risk of TB disease, with TB rates increasing in adolescence from 15–19 years of age. TB rates fell consistently from age 45–49 years onwards (Figure 4.1).

Figure 4.1: General population TB rates by age in 2005, 2010 and 2015.



Legend: TB=Tuberculosis disease (all cases). TB rates reduced from 379 to 188 to 151 cases per 100,000 among adolescents aged 10–14 years old and from 938 to 663 to 566 cases per 100,000 among adolescents aged 15–19 years old in 2005, 2010 and 2015 respectively.

4.5.3. Trends in childhood, adolescent and adult TB disease

Average adolescent TB rates for the decade 2005–2015 were 477 (CI: 313–641) cases per 100,000 and 361 (CI: 280–441) cases per 100,000 for all–TB and microbiologically–confirmed TB, respectively. There was a significant negative temporal trend in adolescent TB rates between 2005–2015 (see Figure 4.2, Table 4.2 and Appendix 1 and Appendix 2), but that trend differed markedly pre– and post–2009. Among adolescents there was a statistically significant negative temporal trend in both all–TB (p=0·028) and

microbiologically confirmed TB (p=0·027) for 2005–2009, which was not observed for the period 2009–2015 (p=0·764 and p=1·000, respectively). Between 2005–2015, adult all–TB rates fell by 30% from 1,423 to 994 per 100,000. Childhood all–TB rates dropped 15% from 2750 to 2331 per 100,000 during the same period, but rebounded thereafter and consistently increased between 2009–2015, consistent with a period of sustained *Mycobacterium tuberculosis* transmission (see Figure 4.2 and Appendices 3–5).

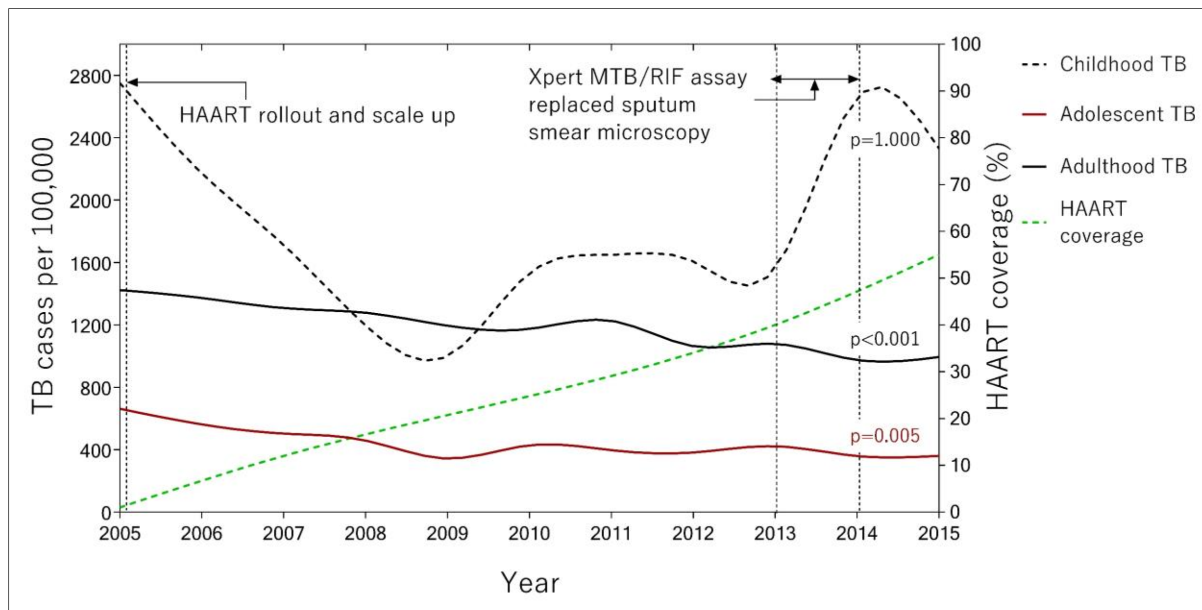
Table 4.2: TB rate by year, age and diagnostic approach

Year	Adolescent all–TB rates (CI)	Adolescent Annual change in TB rates (%)	Adolescent microbiologically confirmed TB rates (CI)	Overall TB rates (CI)	Overall Annual change in TB rates (%)	Overall microbiologically confirmed TB rates (CI)
2005	662 (570–763)	NA	492 (413–581)	1535 (1473–1598)	NA	890 (844–939)
2006	562 (479–656)	–15·1	436 (363–520)	1376 (1319–1435)	–10·4	817 (773–863)
2007	503 (425–592)	–10·5	403 (333–483)	1237 (1183–1293)	–10·1	785 (742–830)
2008	459 (385–544)	–8·7	347 (283–421)	1113 (1062–1165)	–10·0	714 (673–756)
2009	343 (279–416)	–25·3	252 (198–316)	1002 (954–1052)	–10·0	659 (620–699)
2010	428 (357–509)	24·8	345 (282–418)	1105 (1055–1157)	10·3	657 (619–698)
2011	397 (329–474)	–7·2	302 (243–370)	1155 (1104–1208)	4·5	694 (655–735)
2012	382	–3·8	320	1042	–9·8	577

	(316–457)		(260–390)	(994–1092)		(542–615)
2013	422 (353–500)	10·5	310 (251–378)	1050 (1002–1100)	0·8	588 (552–625)
2014	359 (296–432)	–14·9	258 (206–321)	1180 (1129–1232)	12·4	551 (517–587)
2015	361 (298–433)	0·6	305 (248–372)	1131 (1081–1181)	–4·2%	595 (559–632)
Period average	477 (313–641)	NA	361 (280–441)	1246 (1018–1474)	NA	720 (504–936)

Legend: CI=95% confidence interval; **TB**=Tuberculosis; **TB rates**=TB disease case notification rate per 100,000; **NA**=Not applicable. Period refers to 2005 to 2015. Overall refers to TB rates in the general population. Percentage annual change in TB rates varied between –25.3% and 24.8% and –10.4% and 12.4% among adolescents, and overall.

Figure 4.2: Comparison of adult and adolescent TB trends 2005–2015

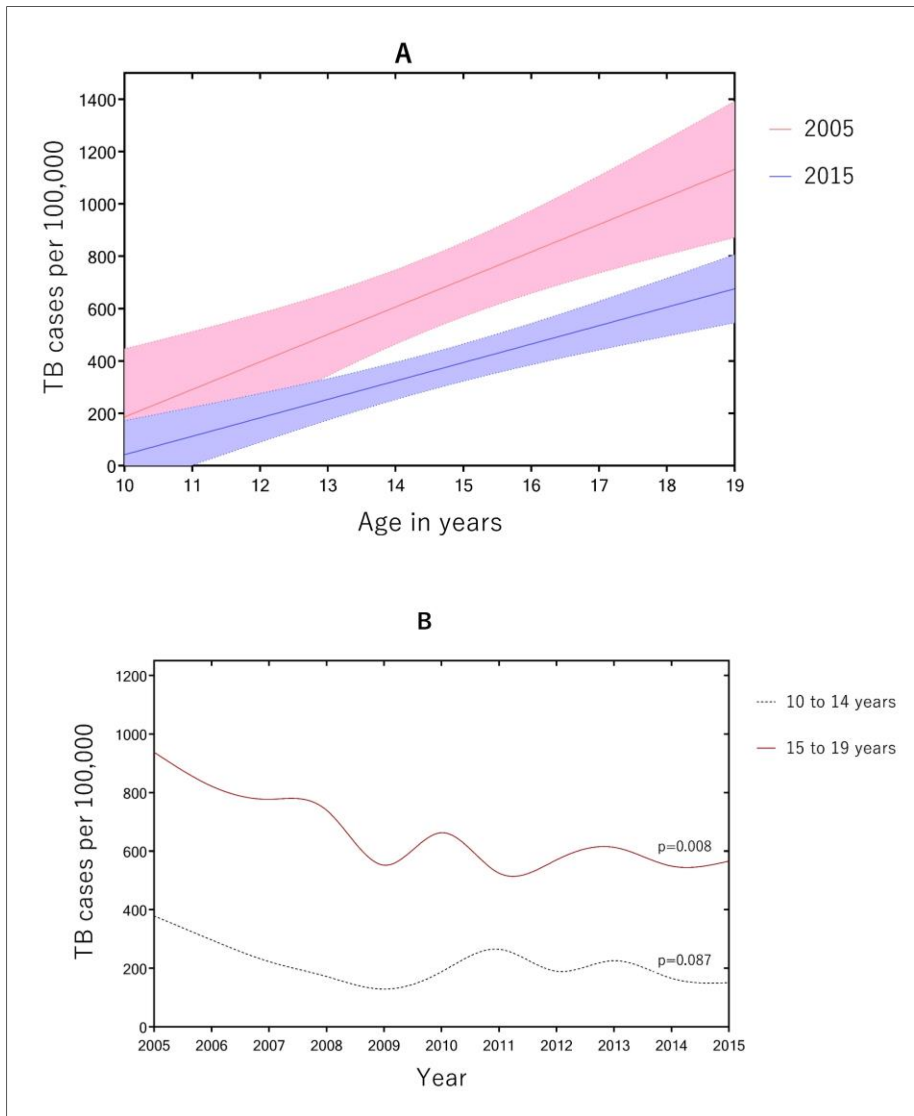


Legend: ART coverage= The proportion of persons living with HIV and receiving antiretroviral therapy; TB=Tuberculosis disease (all TB). Between 2005–2015 all–TB rates fell by 15% from 2750 to 2331 per 100,000 among children, by 45% from 662 to 361 per 100,000 among adolescents and by 30% from 1,423 to 994 per 100,000 among adults. The proportion of all persons living with HIV on ART in the general population was <1% in 2005, 12% in 2007, 40% in 2013 and 55% in 2015.[9] P–value is for temporal trend between 2005–2015.

4.5.4. Adolescent TB trends by age

Older adolescents contributed primarily to the overall reduction in adolescent TB rates (Figure 4.3A and Figure 4.3B). However, TB rates fell significantly in the period 2005–2009 among adolescents aged 10–14 years (p–value 0·028) and those aged 15–19 years (p–value 0·027).

Figure 4.3: Adolescent TB temporal trends by age and year

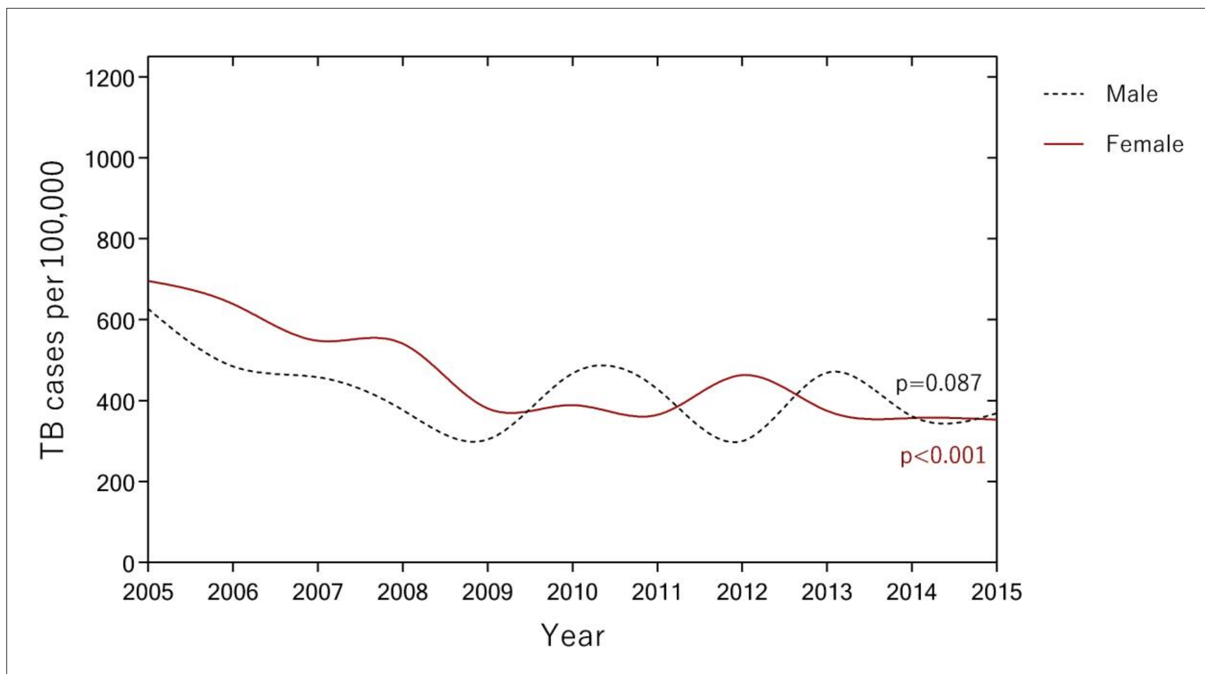


Legend: TB=Tuberculosis disease (all–TB). Panel 3A. Adolescent TB rates by age and year. Between 2005–2015, TB rates fell by 63% (from 429 to 157 per 100,000; p–value for temporal trend (p–value) =0·350) among adolescents aged 10 years old; by 29% (from 560 to 400 per 100,000; p–value =0·640 among adolescents aged 15 years old and by 40% (from 1120 to 677 per 100,000; p–value =0·020) among adolescents aged 19 years old. Panel 3B. Adolescent TB rates by five–year age groups. TB rates fell by 60% (from 379 to 151 per 100,000) among adolescents aged 10–14 years old and by 40% (from 938 to 566 per 100,000) among adolescents aged 15–19 years old. P–value is for temporal trend between 2005–2015.

4.5.5. Adolescent TB trends by sex

Average TB rates for the decade 2005–2015 were 425 cases per 100,000 among male adolescents and 496 cases per 100,000 among female adolescents. The temporal pattern of TB rates was similar by sex. Overall, a statistically significant temporal trend was only observed among female adolescents (see Figure 4.4 and Appendix 6) who had a higher baseline (2005) but a lower TB rate in 2015.

Figure 4.4: Adolescent TB temporal trends by sex



Legend: TB=Tuberculosis disease. Respectively among female and male adolescents, TB rates fell by 49% (from 696 to 353 cases per 100,000) and by 41% (from 627 to 369 cases per 100,000) from 2005 to 2015. P–value is for temporal trend between 2005 and 2015.

4.6. Discussion

A significant drop in adolescent TB rates was observed over the decade 2005–2015, although disease rates remained high compared to some high TB burden countries. The percentage fall in adolescent TB rates was greater than the fall in adult TB disease rates; was more marked among older than younger adolescents; and occurred primarily during 2005–2009, but appeared to plateau thereafter in parallel with increased TB rates in younger children compatible with a period of sustained *Mycobacterium tuberculosis* transmission.

The proportion of all TB disease cases occurring among adolescents also significantly reduced over this period, as it did that among adults, whereas childhood TB proportionally increased. The median age of TB in the general population dropped slightly from 25 to 24 years of age. We postulate that the demographic shift in the age of incidence of tuberculosis is due in part to a drop in HIV–associated TB that mainly affected young adults as supported by Hermans *et al.,*[1], but further studies would be required to model the relationship between HIV and TB for actual attributable fraction in this setting. This shift in median age of TB disease is therefore unlike the shift towards older age of incident tuberculosis reported in Brazil[17], which was due in part to population growth and aging, or that in Hong Kong due to immigration from higher TB burden regions of China.[18] While an epidemiologic shift towards the elderly due to endogenous reactivation of remote TB infection is generally regarded as a sign of successful TB control in developed countries with low HIV burden,[19] the significant fall in TB disease rates among South African adolescents who experience the highest force of *Mycobacterium tuberculosis* infection in this setting, is regarded as an initial sign of progress in TB control efforts.

Low HIV testing rates between 2005–2010 likely underestimated the scale of HIV burden in adolescent TB disease patients, which was 45% in 2015. HIV co–infection impacts TB rates among older adolescents as HIV incidence increases with age,[20] for example between 30–50% of TB was attributable to HIV in older adolescents and adults in Kenya. Since HIV increases TB risk up to 12–fold compared to that of HIV negative persons[21], the rapidly increasing population ART coverage from less than 1% in 2005 to 55% in 2015[9], and the increasing threshold for ART initiation from a cut–off CD4 cell count of 200 cells/ml in 2004[22] to 350 cells/ml in 2012; and to 500 cells/ml in 2015[23] might be expected to contribute to a decline in both adult and adolescent HIV–associated TB rates, as observed in these regional data[24], elsewhere in South Africa[1], and in mathematical modelling studies.[25] However the impact of improved ART access could not be determined for the adolescent population due to lack of HIV incidence or prevalence data for this age group during the period under study, and will require modelling or further epidemiologic studies. We note that TB rates substantially fell between 2005–2009 during nascent ART roll–out when HIV testing was less frequent: 21% (156/732) of adolescent TB notifications were HIV–

tested, 12% (19/156) of whom were living with HIV. We further observe that adolescent all–TB rates were 582 cases per 100,000 and 682 cases per 100,000 in 2003 and 2004 respectively (unpublished data from regional health authorities), suggesting the onset of the fall in adolescent TB rates either occurred at the same time or preceded ART roll–out. We acknowledge the temporal disconnect between ART roll–out and decrease in TB rates may not be sufficient to exclude an ART–related effect definitively. Our data show that the biggest increase in population ART coverage occurred between 2010–2015, when adolescent and adult TB disease rates appeared to plateau and childhood TB rates increased, a pattern compatible with a period of sustained *Mycobacterium tuberculosis* transmission that we are unable to explain. Whether this observation was due to improved diagnostic yield or clinical case detection in young children; or increased TB transmission to young children that did not affect adults or adolescents, requires further study. Similarly, additional research is required to estimate regional adolescent incidence or prevalence of HIV to study the impact of HIV control on TB transmission in this setting.

Socioeconomic factors affecting regional TB rates may also be at play. Unemployment decreased slightly between 2005–2009 (from 10.1% to 9.6%), but then increased slightly (from 9.6% to 11.4%) between 2009–2015. The proportion of informal dwellings also increased from approximately 13% in 2005 to >22% in 2015.[14] It is conceivable that the worsening housing situation could have contributed to stagnation in adolescent TB rates between 2009–2015, but we have no evidence for substantial impact. There were also no notable changes in year–on–year migration patterns for seasonal agricultural work that would likely have impacted adolescent TB trends in this region.[26]

We have no evidence to believe that other changes in TB program activity or reporting materially impacted these observations. Adolescent TB treatment success rates remained stable and relatively high throughout the study period at an average of 84%; and the TB health management information system was strengthened between 2000–2005, when electronic notification of TB disease cases was introduced. Passive TB disease case–finding and treatment supported by DOTS was in place and remained virtually unchanged between 2005–2015.

We previously analysed temporal trends in prevalence of latent TB infection (LTBI) and annual risk of tuberculosis infection (ARTI) for the same adolescent source population, in order to determine whether trends in LTBI and ARTI are in line with observed adolescent TB disease trends. By contrast, we showed that ARTI and LTBI remained high and relatively unchanged over the decade 2005–2015.[27] However, adolescent ARTI and LTBI prevalence represent cumulative lifetime measures[7] and the majority of latently infected individuals do not exhibit reversion of their positive interferon gamma release assay result to negative.[28] Force of *Mycobacterium tuberculosis* infection is a more accurate measure of short–term change or instantaneous adolescent TB risk. Therefore, prevalence of LTBI may not accurately correlate with declining TB rates in the short term.

Studies describing temporal trends in adolescent TB between 1990–2018 in high TB burden countries in Africa are scarce. Increasing temporal TB trends in the general population have previously been noted in Botswana, Zimbabwe, Lesotho, South Africa and Swaziland[29] due to the worsening HIV epidemic.

A key strength of this analysis is the large dataset, high quality public laboratory support and robust time series analysis. TB notification underestimates TB prevalence by at least 34% due to under–reporting and under–diagnosis[30]. Therefore, our estimates likely underestimate the true burden of adolescent TB. HIV testing among adolescents was limited between 2005–2008 and no reliable data on ART uptake among HIV–infected TB disease patients during TB treatment was available. Regional ART uptake data are maintained in a different health management information system that is unlinked to the TB information system. Lack of adolescent HIV incidence or prevalence data and reliable ART usage data limits our ability to accurately determine whether the decrease in TB rates was driven by a decrease in HIV–associated TB, HIV–negative TB, or both. Lastly, lack of racial ancestry data precludes analyses stratified by race which has been shown to be a useful proxy for socioeconomic status in this context due to historical factors. In high TB and HIV co–burdened countries, trends in TB incidence have been shaped substantially by management of the HIV epidemic and thus we appreciate that the finding of declining adolescent TB rates may not be generalisable to other settings.

In conclusion, the overall rate of reduction in adolescent TB over the decade 2005–2015 was almost two–fold that among adults and three–fold that among younger children. The substantial fall in adolescent TB disease case notification rates between 2005–2009 plateaued thereafter, coupled with an increase in the TB rate among younger children that is consistent with a period of sustained *Mycobacterium tuberculosis* transmission. It follows that additional TB and/or HIV control interventions are needed to sustain the encouraging early decline in adolescent TB rates. Given the apparent stagnation in adolescent TB and the marked increase in childhood TB rates between 2010–2015, close monitoring will be needed to guard against a rebound in adolescent TB and to ensure that these early gains are consolidated.

Authors' contributions

EWB conceptualised, designed the study and wrote the study protocol, performed analysis and prepared the first draft of the manuscript with oversight from MH. EWB, HM, AKKL, JS, SM, MT, RW, EN, TJS and MH critically reviewed, revised and approved the final version of the manuscript. All authors agreed on the final submitted version of the manuscript. EWB is the guarantor of the study. No medical editor is associated with this work.

Competing interests (Conflict of interest statements)

All other authors have no other potential conflicts of interest to declare.

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Ethics committee approval

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Data sharing

Dataset will be available on the Dryad repository.

About the Author

Dr. Bunyasi is a physician and PhD candidate at the University of Cape Town (Cape Town). His primary research interest is infectious disease epidemiology.

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Chapter 5

5. Indoor Air Quality and tuberculosis risk in South African schools

5.1. Overview

Summary

In this chapter we describe school classrooms as high–risk spaces for *Mycobacterium tuberculosis* transmission due to poor ventilation; pilot a strategy to test for RD9 *Mycobacterium tuberculosis* DNA by classroom air sampling; and examine the yield and feasibility of a pragmatic school–based tuberculosis screening strategy. The chapter is organised as follows: (1) Chapter Abstract; (2) Background and rationale; (3) Objectives; (4) Methods; (5) Results; (6) Discussion.

Contribution to the thesis and novelty

Investigation of classroom ventilation risk status is important to inform education authorities on whether classrooms contribute to *Mycobacterium tuberculosis* transmission among adolescent students; and to inform guidelines on engineering and environmental control measures for prevention of *Mycobacterium tuberculosis* transmission in the school setting. We further describe a proof of concept pilot study of classroom air sampling of RD9 *Mycobacterium tuberculosis* DNA to test whether this approach can distinguish classrooms at high risk for *Mycobacterium tuberculosis* transmission, based on ventilation status and/or presence of a known tuberculosis disease case.

Contributions of candidate

The candidate conceived and designed the study, conducted the fieldwork, collected the samples, managed the data, performed all data analysis and wrote the chapter with editorial input and guidance from supervisors. Trained field workers assisted in home language consenting and questionnaire administration,

and sample collection. The *Mycobacterium tuberculosis* ddPCR assay was performed by Desmond Tutu HIV Centre laboratory staff with support from the Warner laboratory.

Publication status

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5.2. Chapter abstract

Introduction

A South African study previously showed that high school students spend 60% of their time in school in inadequately ventilated spaces, defined as above the recommended threshold median ambient carbon dioxide concentration of 1000 parts per million (ppm), which is often used as a proxy for adequate ventilation. However, there is a lack of data on classroom risk of *Mycobacterium tuberculosis* transmission, and to the best of our knowledge there is no published study of air sampling for RD9 *Mycobacterium tuberculosis* DNA in schools. We aimed to: (1) determine the high school classroom ventilation risk for *Mycobacterium tuberculosis* transmission; (2) conduct a pilot study of classroom air sampling for RD9 *Mycobacterium tuberculosis* DNA; and (3) investigate the operational feasibility and yield of tuberculosis disease surveillance in high schools.

Methods

Between 1st September 2017 and 30th September 2018, we conducted a cross–sectional study of 72 high school classrooms (occupied by 1,836 students); and 7 clinic spaces that were selected based on high risk of *Mycobacterium tuberculosis* transmission, in Worcester, South Africa. We measured ambient carbon dioxide concentration for the first classroom session of the day, using portable carbon dioxide monitors to define high risk ventilation spaces for *Mycobacterium tuberculosis* transmission (threshold median carbon dioxide ≥1,000 ppm). We simultaneously sampled filtered air, using a Dry Filtration Unit, through a removable polyester felt filter of 47mm diameter and 1.0 µm pore size (American Felt and Filter Company, New Windsor, New York; Lockheed Martin, Alexandria, VA, USA), extracted DNA from each filter, and performed a droplet digital PCR (ddPCR) assay to detect and quantify RD9 *Mycobacterium tuberculosis* DNA copies in air samples. RD9 *Mycobacterium tuberculosis* DNA concentration was calculated for 180,000 litres of air (=180 cubic metres), based on the average estimated volume of classrooms. We performed active symptom screening and collected voluntary spontaneously expectorated sputum samples from participants

reporting at least one symptom (cough of ≥2 weeks, haemoptysis, weight loss for ≥2 months, fever for ≥2 weeks or nights sweats for ≥2 weeks) for Xpert MTB/RIF Ultra testing. The Wilcoxon rank–sum test was used to compare median carbon dioxide values by type of space. The two–sample test for equality of proportions was used to assess statistically significant differences between groups for categorical variables.

Results

Median duration of sampling for 44 clinic air sampling episodes was 82 minutes (interquartile range (IQR): 40–120) and median number of occupants was 6 (IQR: 3–22). Median duration of sampling for 72 classrooms was 40 minutes (35–54) and median number of occupants was 34 (29–39). No clinics, but 40% of classrooms were high risk ventilation spaces for *Mycobacterium tuberculosis* transmission, based on threshold median ambient carbon dioxide concentration ≥1,000 ppm. A positive *Mycobacterium tuberculosis* ddPCR assay was obtained in 4/39 (10.3%) clinics and 13/72 (18.1%) classrooms (p=0.276). Overall, the estimated average concentration of RD9 *Mycobacterium tuberculosis* DNA was 1.74 copies per 180,000 litres of air for all clinic spaces; and 17 copies (range 2–27) per 180,000 litres of air when limited to those clinics with a positive ddPCR assay. Similarly, the estimated average concentration of RD9 *Mycobacterium tuberculosis* DNA in classrooms was 3.61 copies per 180,000 litres overall; and 20 copies (range 8–82 copies) per 180,000 litres in those classrooms with a positive ddPCR assay. Across all classrooms, the average risk of an occupant inhaling 1 RD9 *Mycobacterium tuberculosis* DNA copy during one lesson of 35 minutes was 0.71%. The estimated risk for one academic day was 2.7%, for one academic week was 12.6%; for one academic month was 41.7%; and for one academic year was 100%. Therefore, we estimate each student will have inhaled on average 11 RD9 *Mycobacterium tuberculosis* DNA copies over the 1,155 hours South African high school students spend congregated in classrooms during approximately 199 school days each calendar year. We enrolled 1,836/2,262 (81.2%) of students in classrooms where air sampling was conducted. Median age was 16.2 years and 779 (45.8%) were male. The number of students reporting cough ≥2 weeks was 90/1820 (4.9%); 58/90 students with cough (64.4%) offered to provide a spontaneously expectorated sputum sample, but only 21 (36.2%) submitted a sputum sample for analysis.

One student (4.8%) was Xpert MTB/RIF Ultra positive (prevalence 55 (CI: 0–341) per 100,000). Air sampling from the classroom occupied by the student with confirmed tuberculosis disease yielded a positive ddPCR assay; was categorised as high ventilation risk based on ambient carbon dioxide concentration; and was occupied by three students with self–reported cough, only one of whom was sputum productive and Xpert MTB/RIF Ultra positive.

Conclusion

We showed that more than one–third of high school classrooms were a high ventilation risk for *Mycobacterium tuberculosis* transmission if they were occupied by a student with undiagnosed tuberculosis disease, whereas clinic spaces, where known tuberculosis patients were expected, were adequately ventilated. However, air sampling filters from classrooms were as likely to be *Mycobacterium tuberculosis* ddPCR assay positive as those from clinics, which reflects the combined contribution of bacillary droplet inoculation and ventilation to risk of *Mycobacterium tuberculosis* transmission. Estimated risk of inhalation of potentially infectious RD9 *Mycobacterium tuberculosis* DNA copies in classrooms was similar to that of clinics. However, one RD9 *Mycobacterium tuberculosis* DNA copy does not necessarily translate to one infectious quantum of *Mycobacterium tuberculosis* and more research is required to estimate the number of inhaled RD9 *Mycobacterium tuberculosis* DNA copies leading to established *Mycobacterium tuberculosis* infection in humans. Nonetheless, these findings highlight the importance of ventilation management for *Mycobacterium tuberculosis* infection control in congregate school settings. Education authorities should sensitize students and staff to open windows and allow adequate classroom ventilation, which is a low–cost structural intervention for tuberculosis control. Our tuberculosis screening strategy was pragmatic and designed to mimic real–life field conditions for a classroom–based tuberculosis symptom screening programme. Yield of screening for tuberculosis disease cases was low, but the *Mycobacterium tuberculosis* ddPCR data from all classrooms suggest additional cases of undiagnosed tuberculosis disease may have been missed by symptom screening and voluntary spontaneous expectorated sputum sampling of symptomatic students. Addition of chest radiography and/or IGRA testing might increase yield, but also increases cost and

logistic complexity of tuberculosis screening. Therefore, although we have shown that classroom air sampling for RD9 *Mycobacterium tuberculosis* DNA is feasible and potentially useful as a mass screening tool, further research is needed to determine the significance of *Mycobacterium tuberculosis* ddPCR air sampling for risk of *Mycobacterium tuberculosis* transmission, and the association between a positive *Mycobacterium tuberculosis* ddPCR assay and detection of an undiagnosed tuberculosis disease case in congregate spaces such as school classrooms.

5.3. Background and Rationale

The South African Integrated School Health Policy of 2012[1, 2] plans for tuberculosis disease screening of students in high schools, in combination with environmental assessment to prevent airborne infections as part of the package of healthcare to schools, but little is known about implementation of this policy.[3] There are 12 million students in the South African basic education sector, which comprises the first 12 years of formal schooling.[4] These 12 million students include a large pool of susceptible children, as yet uninfected with *Mycobacterium tuberculosis*, in addition to previously–infected children at risk of progression to tuberculosis disease.[5] Since adolescence is characterised by a rapid increase in tuberculosis disease incidence,[6] school–based infection control and tuberculosis disease surveillance measures may represent an opportunity to reduce the risk of *Mycobacterium tuberculosis* transmission and to detect previously undiagnosed tuberculosis disease cases for early therapeutic intervention.

There are limited published data that describe the yield of tuberculosis disease surveillance in schools in Africa. Three tuberculosis disease prevalence studies in Africa among school–age adolescents, which conducted tuberculosis disease case–finding by both symptom screening and Tuberculin Skin Test or Interferon Gamma Release Assay (TST/IGRA), detected one case of tuberculosis disease for every 303 (South Africa), 625 (Uganda) and 313 (Kenya) adolescents screened.[7–9] However, none of these studies reported on feasibility considerations or cost–effectiveness of school–based tuberculosis disease screening. A 2014–2015 study in Swaziland, where tuberculosis disease prevalence was 733 cases per 100,000 in 2015,[10] investigated utility of school–based symptom screening of tuberculosis disease contacts and

identified no secondary tuberculosis disease cases among 2,015 school contacts of 7 index GeneXpert–positive tuberculosis disease patients attending 6 different schools.[11] The cost/benefit ratio of school–based infection control and tuberculosis disease surveillance measures is yet to be determined in Africa. A South African community–based study that conducted sputum induction on all individuals with tuberculosis disease symptoms and symptomatic HIV–positive individuals reported a cost of US$1,117 per tuberculosis disease case detected and US$2,458 per tuberculosis disease case cured.[12] However, approaches to tuberculosis disease case–finding among adolescents in high schools may be more efficient if targeted at school classrooms with *a priori* evidence of increased risk for *Mycobacterium tuberculosis* transmission.

We conducted a study of classroom ventilation, in conjunction with a proof of concept study of classroom air sampling for RD9 *Mycobacterium tuberculosis* DNA, and evaluated the yield of symptom–based tuberculosis disease surveillance, to inform stakeholders in the education and health sectors of the feasibility and potential impact of these approaches to improve tuberculosis control among school–going adolescents.

5.3.1. Burden of adolescent tuberculosis

The estimated incidence of tuberculosis disease in the general South African population for 2018 was 520 cases per 100,000 people.[13] Annualized force of *Mycobacterium tuberculosis* infection in the study region during adolescence is approximately 14%,[14] with a latent tuberculosis infection prevalence of 49% (2014–2015)[5] that increases to approximately 90% in adulthood.[15] Snow *et al.* showed that regional (Western Cape Province, South Africa) tuberculosis disease notification rate is three–fold higher in older adolescents aged 15–19 years than in younger adolescents aged 10–14 years (418 tuberculosis disease cases/100 000 person–years vs. 141/100 000 person–years).[16]

Although South Africa has 12 million students in the basic education sector,[4] there is a paucity of data evaluating the fraction of *Mycobacterium tuberculosis* transmission that might be attributable to school

attendance.[17] A previous study in Cape Town, South Africa, showed that adolescents aged 10–19 years had an average of 12–14 close social contacts indoors with a mean duration of 86–96 minutes.[19] Although *Mycobacterium tuberculosis* infection among children aged 5–14 years was associated with residential exposure to an adult case of tuberculosis disease, there was no association among older adolescents aged 15–22 years.[17] This finding suggests increased significance of other congregate settings, such as school classrooms, as determinants of *Mycobacterium tuberculosis* infection and disease among adolescents.[20, 21] A statistical modeling study estimated that 25% and 50% of new *Mycobacterium tuberculosis* infections among South African individuals aged 0–14 and 15–19 years, respectively, occurs in the school setting.[18] Therefore, since schools may be important locations for *Mycobacterium tuberculosis* transmission from adolescents with tuberculosis disease to susceptible, uninfected adolescents, the classroom setting should be targeted for surveillance and prevention efforts.

5.3.2. Measurement of ventilation in classrooms

Students attending a South African high school will spend approximately 1,155 hours congregated in classrooms during the 199 school days of the academic year.[22] The indoor school environment and classroom ventilation are likely key determinants of transmission for airborne infections such as tuberculosis, as demonstrated by Wells and Riley.[23, 24] Given that *Mycobacterium tuberculosis* transmission occurs via aerosol droplet spread, classroom air quality may affect the risk of *Mycobacterium tuberculosis* transmission in the congregate school setting. Indoor air quality refers to the air quality within and around buildings, as it relates to the health and comfort of occupants.[25] Source control (e.g. isolation of infectious disease cases), particulate air filtration, and ventilation to dilute and remove airborne contaminants, are the primary methods for improving classroom ventilation.[25]

Ventilation in this study is defined as the provision of fresh air to a room to reduce the risk of transmission of tuberculosis disease,[25] and is measured using: (1) volume of the space (i.e. air changes per hour (ACH)); and (2) an occupancy–based approach that accounts for the number of people in a space (i.e. the amount of available fresh air per person per second).[26] The World Health Organization (WHO) defines adequacy

of ventilation using air changes per hour.[26] One air change has occurred when the volume of air entering or exiting a room is equal to the volume of the room.[26] Under ideal conditions, in which expired droplet nuclei are evenly distributed and room air is uniformly mixed, the proportion of infectious particles eliminated with one air change is 63%. At a ventilation rate of 10 air changes per hour, it would take 14 minutes to remove 90% of airborne contaminants in a room; and 28 minutes to remove 99%.[27]

The recommended ventilation for high risk clinic areas, such as sputum collection rooms that are known to be occupied by tuberculosis patients, is 12 ACH or 80–160 litres per person per second (l/p/s); and that for low risk clinic areas, such as outpatient consultation rooms, is 6 ACH or 40 l/p/s.[28] Ambient indoor carbon dioxide concentration is often used as a proxy for indoor ventilation due to ease of measurement. The WHO recommendation for classroom ventilation (12 air changes per hour or 8.6 litres per person per second), is equivalent to maintaining the median ambient carbon dioxide concentration at <1000 parts per million (ppm) for a standard classroom of approximately 180 cubic metres (180,000 litres), which is occupied by the average class size of 31 students, in South African high schools.[28] This 1,000 ppm carbon dioxide concentration threshold is also in line with classroom air quality regulations in Sweden, the United States and Portugal.[28]

The concentration of carbon dioxide in exhaled breath (~38,000 ppm) is roughly 100 times higher than that of open environmental air (~400 ppm);[29] and carbon dioxide decay curves have also been used for estimation of ventilation.[30] An unoccupied room has an ambient carbon dioxide concentration of about 400 ppm. Since exhaled breath is the primary source of excess carbon dioxide in indoor settings, ambient room carbon dioxide measurement allows estimation of the relative proportion of exhaled air from other occupants, and thus risk for *Mycobacterium tuberculosis* transmission from an infectious person to uninfected, susceptible occupants.[29] Indoor ambient carbon dioxide measurement can be used to estimate probability of *Mycobacterium tuberculosis* transmission using the equation developed by Issarow *et al.*[31] that builds on the historic work of Rudnick and Milton[29], and Wells and Riley.[28] The equation derived by Rudnick and Milton expanded upon the work of Wells and Riley and used rebreathed fraction to substitute for the more

difficult analysis of room ventilation and size.[29] The Rudnick–Milton equation does not require the assumption of steady–state conditions and assumes the loss of infectious particles to settling, filtration, and loss of viability is negligible compared to that removed by ventilation.[29]

Measurement of ambient room carbon dioxide using portable carbon dioxide monitors[32] (see Appendix 5.1) might therefore be used to identify poorly ventilated school classrooms that might pose increased risk for *Mycobacterium tuberculosis* transmission, but there is limited research into classroom ambient carbon dioxide measurement that would inform *Mycobacterium tuberculosis* transmission research. A previous South African study showed that high school students spend 60% of their time in school occupying spaces with median ambient carbon dioxide concentration above the recommended threshold of 1000 ppm.[28]

It is therefore important to study not only adequacy of classroom air quality in South African high schools, but also to determine whether inadequate ventilation in the classroom setting puts susceptible students at risk of *Mycobacterium tuberculosis* exposure and tuberculosis disease. These data may inform South African School Health Policy and help plan engineering and environmental controls for prevention of *Mycobacterium tuberculosis* transmission in the classroom setting.

5.3.3. Classroom air sampling for RD9 *Mycobacterium tuberculosis* DNA

The risk of *Mycobacterium tuberculosis* transmission in classrooms is determined not only by inadequate ventilation, but by the presence of exhaled *Mycobacterium tuberculosis* bacilli in suspended droplet nuclei. We have shown in previous chapters that half or more of adolescents in this study community are *Mycobacterium tuberculosis*–uninfected and would be susceptible to classroom *Mycobacterium tuberculosis* transmission from undiagnosed infectious tuberculosis cases. We have also shown that the incidence of tuberculosis disease in the adolescent age group is sufficiently high to constitute material risk of *Mycobacterium tuberculosis* transmission to susceptible students and staff in the school environment. Therefore, given the variable implementation of tuberculosis disease screening in South African schools,[3] and the attendant risk of *Mycobacterium tuberculosis* transmission to susceptible students due to delays in tuberculosis disease

diagnosis,[32, 33] it is important to investigate alternative tuberculosis surveillance and control strategies in the school setting.

Air sampling was informed by the landmark studies of Riley and colleagues in the 1960s as a tool to investigate aerosol *Mycobacterium tuberculosis* transmission;[34, 35] and subsequent studies in healthcare settings that used molecular detection methods (PCR) to test filtrate samples obtained from room air.[36–39] Air sampling has potential as a mass tuberculosis disease surveillance tool for students and occupants of high risk spaces. Targeted tuberculosis disease symptom screening in classrooms with high risk for tuberculosis transmission has potential for more efficient and faster identification of undiagnosed tuberculosis disease cases than indiscriminate screening of all 12 million South African students, as currently prescribed in the South African School Health Policy.[4]

Indoor air sampling can be used to obtain RD9 *Mycobacterium tuberculosis* DNA using air filtration devices such as the Dry Filtration Unit (see Appendix 5.2). The Dry Filtration Unit[40] is a portable biological sample collection device that samples room air via fine filters (polyester felt filter of 47mm diameter and 1.0µm pore size (American Felt and Filter Company, New Windsor, New York; Lockheed Martin, Alexandria, VA, USA) that allow collection and quantification of the DNA of bacteria and viruses [37, 40, 41] by Polymerase Chain Reaction (PCR), but there is limited data on use of the Dry Filtration Unit in tuberculosis research.[37, 40, 41] The Dry Filtration Unit samples approximately 1,000 litres of room air per minute.[42] Air sampling in tandem with a multiplex real–time PCR assay that targets *Mycobacterium tuberculosis* genes can provide quantitative data on amount of inoculum[43], which has potential as a tool for targeted mass screening of undiagnosed tuberculosis disease in schools and other congregate settings.

Therefore, our second objective was to conduct a school–based pilot study to show proof of concept that air sampling for RD9 *Mycobacterium tuberculosis* DNA could identify classrooms with and without RD9 *Mycobacterium tuberculosis* DNA detected by ddPCR assay. We hypothesized that positive ddPCR assays would correlate with high ventilation risk for transmission, identified by simultaneous ambient room

carbon dioxide measurement, and/or occupation of the sampled classroom by a student with undiagnosed tuberculosis disease.

5.3.4. Droplet digital polymerase chain reaction (ddPCR)

Droplet digital polymerase chain reaction (ddPCR) offers greater accuracy compared to traditional PCR methods, which is crucial if DNA copy numbers are low, as in the case of air sampling for *Mycobacterium tuberculosis*.[44] ddPCR is a form of PCR in which a sample is diluted and partitioned into multiple separate reaction chambers, each containing ≥1 or no copies of the DNA sequence of interest. The number of copies of DNA from the original sample may be determined by counting the number of positive versus negative partitions and applying Poisson distribution statistics.[44] This approach allows increased accuracy over real time PCR and accurate quantification of DNA copies,[44] although positive assay thresholds are not yet standardised.[44] An important potential limitation to the use of DNA–based technologies for tuberculosis disease surveillance is that the PCR assay does not distinguish between living or dead inoculum, and thus between infectious and non–infectious quanta.[43]

5.3.5. Classroom–based Symptom Screening for tuberculosis disease

The WHO defines occupants of congregate settings as a tuberculosis risk group[45] and recommends that close contacts of individuals with tuberculosis disease are systematically screened for tuberculosis disease.[45] However, there are few studies that report the yield of active symptom–based tuberculosis disease surveillance among adolescents in the high school setting, despite the fact that an estimated 50% of new adolescent *Mycobacterium tuberculosis* infections occur in schools.[18] Systematic analysis of the number of adolescents needed to screen in order to detect one undiagnosed tuberculosis disease case has not been estimated for school–based screening programmes.[46] A systematic review of community–based tuberculosis screening reported a weighted number–needed–to–screen of 100 (range: 16 – 6,355)[46]; and sensitivity of 71% and specificity 99% for any tuberculosis disease symptom and Xpert MTB/RIF testing in high tuberculosis burden countries.[47] Ugandan and Kenyan tuberculosis disease prevalence studies

detected one case of tuberculosis disease for every 625 and 313 adolescents screened, respectively.[7, 8] A South African study among school–going adolescents diagnosed one tuberculosis disease case for every 303 students screened by both symptoms and TST/IGRA,[9] but symptom screening alone performed poorly, with a sensitivity of 12% and specificity of 97%. However, testing all school students for tuberculosis using IGRA or TST, and then testing for tuberculosis disease using sputum Xpert MTB/RIF, is logistically challenging and expensive for resource–limited countries. A modelling study in South Korea reported that screening for tuberculosis among 1000 high–school adolescents using an IGRA–based strategy diagnosed 2 tuberculosis disease cases at a total cost of US$ 108,435 i.e. US$ 54,218 per tuberculosis case detected.[48] Our demonstration in prior chapters of the changing regional tuberculosis epidemic, in which adolescent tuberculosis disease case notification rates fell 45% from 662 to 361 per 100,000 between 2005–2015, is a strong motivation for feasibility studies to demonstrate the yield of school–based tuberculosis disease surveillance before adopting untested strategies as policy.

Therefore, our third objective was to investigate the yield of a pragmatic tuberculosis disease case–finding strategy among adolescents in the high school setting. A school–based tuberculosis disease surveillance strategy for endemic countries should be logistically simple, efficient, and require few resources. We conducted symptom–based screening for undiagnosed tuberculosis disease among students in the same classrooms that had undergone ambient carbon dioxide monitoring and *Mycobacterium tuberculosis* ddPCR air sampling; and followed symptom questionnaires with further investigation of symptomatic students who were willing and able to spontaneously expectorate a sputum sample for laboratory Xpert MTB/RIF Ultra testing.

5.4. Objectives

Our objectives were to: (1) determine the ventilation risk for *Mycobacterium tuberculosis* transmission based on classroom air quality in high schools in Worcester, South Africa; (2) conduct a pilot study of classroom ddPCR air sampling for RD9 *Mycobacterium tuberculosis* DNA; and (3) investigate the operational feasibility and yield of tuberculosis disease surveillance among adolescents in the high school setting.

5.5. Methods

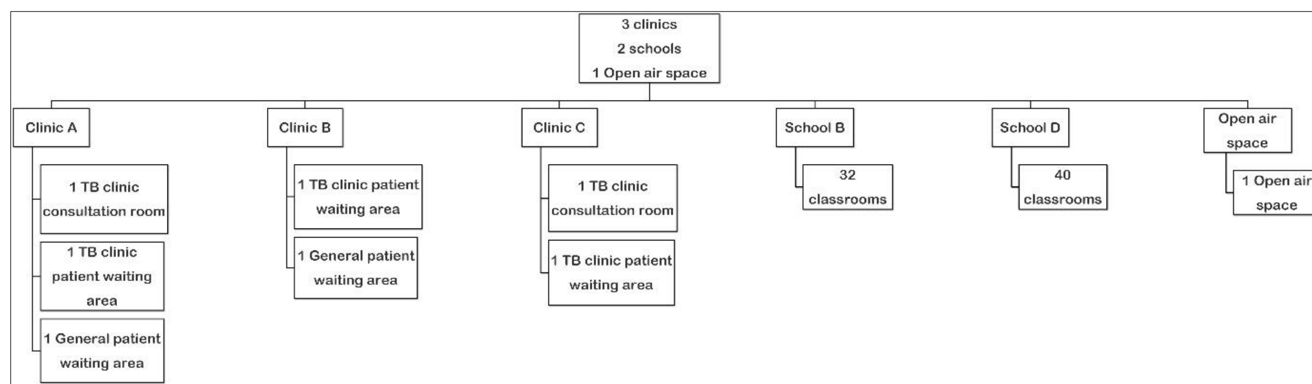
5.5.1. Study setting

This study was conducted in Breede Valley local municipality (Worcester), Western Cape Province, South Africa, between 1st September 2017 and 30th September 2018. Breede Valley local municipality had an estimated population of 180,375 in 2017, a figure obtained using the 2011 census estimate of 166,825 and applying the population growth rate of 1.31% per annum.[49] In 2017, the general population composition was 63·3% Mixed Race Ancestry, 24·3% African, 10·7% White, and 1·7% other classifications.[49] There were approximately 33,370 (18·5%) adolescents in Worcester in 2017.[49] In previous chapters, we reported a tuberculosis disease case notification rate among adolescents and adults in this region of 361 and 994 cases per 100,000 respectively[50], and adolescent latent tuberculosis infection prevalence of 48.5% (CI: 41.1–55.8), for 2015.[5] A summary of other key socioeconomic and health indicators for Worcester is provided in Appendix 5.3.

5.5.2. Study design

We conducted a cross–sectional study of 1,836 students in conjunction with classroom ambient carbon dioxide concentration measurements and ddPCR air sampling in 72 classrooms in two high schools; and from 7 different clinic spaces in primary public health care facilities (Figure 5.1). Ambient carbon dioxide concentration measurements and ddPCR air sampling were performed in both classrooms and clinic spaces, which were selected due to high *a priori* risk for presence of aerosolized RD9 *Mycobacterium tuberculosis* DNA. Classroom ambient carbon dioxide concentration measurements, ddPCR air sampling, and tuberculosis disease symptom screening were performed in classrooms once daily during the first classroom session of the day.

Figure 5.1: Sampling schema of spaces included in the study



Legend: ddPCR air sampling was conducted in all clinics (A, B and C) and both schools (B and D). Air quality was measured in clinics (A, B and C) and both schools (B and D) and one open air space. All classrooms in the two schools and all clinic spaces in the three primary public health care facilities were naturally ventilated.

Classrooms

In chapter 2,[5] we described prevalence of *Mycobacterium tuberculosis* infection among students in 8 high schools. We selected two of the 8 schools (school B and school D) for air sampling and tuberculosis disease surveillance, based on their high school–level prevalence of latent *Mycobacterium tuberculosis* infection among students. In 2015, school B students had latent tuberculosis infection prevalence of 60% (CI: 53%–67%) and school D students had latent tuberculosis infection prevalence of 51% (CI: 50%–52%).[5] Classrooms were approximately 7–10 metres in length, by 7–10 metres in width, for both schools.

An open–air space was included to provide a benchmark for outdoor vs classroom ambient carbon dioxide concentration and a negative control space for ddPCR air sampling. The open–air space was an unoccupied outdoor area with grass lawns, located 20 metres from the administrative office of the field research site.

Clinics

Three clinics (clinic A, clinic B and clinic C) were selected based on their high tuberculosis disease caseload. The absolute tuberculosis disease caseloads per annum differed between clinics due to differences in

population size of each clinic catchment area (Annual Notified tuberculosis disease cases in 2015 were 5,749 for clinic A; 6,098 for clinic B, and 12,240 for clinic C). However, the 2015 general population tuberculosis disease case notification rate was comparable for catchment areas of the three primary health care facilities (1,015 cases per 100,000 for clinic A catchment area, 1,029 cases per 100,000 for clinic B catchment area, and 1,055 cases per 100,000 for clinic C catchment area). All the three public primary health care facilities offer free outpatient care for tuberculosis disease patients, rely on passive tuberculosis disease case finding to identify notified tuberculosis disease cases, and did not offer in–patient services.

For all three clinics, the general patient waiting area was approximately 10–13 metres in length by 5–7 metres in width, with benches where clients waited for administrative processing and triage before being directed to the tuberculosis clinic consultation room. For all three clinics, the tuberculosis clinic consultation room where individuals suspected of having tuberculosis disease were clinically reviewed, and where individuals with tuberculosis disease were followed up and received medication, measured approximately 5 square metres. For all three clinics, the tuberculosis clinic patient waiting area was a corridor of variable length (approximately 8–12 metres for Clinic A and Clinic B and approximately 25–30 metres for clinic C) by approximately 2 metres in width.

5.5.3. Regulatory and administrative process and approval

Ethical approval of the project proposal was obtained from the University of Cape Town Human Research Ethics Committee (HREC REF 163/2016). Students were approached for written informed assent/consent for individual tuberculosis disease symptom screening. The HREC waived the need for parental consent for tuberculosis disease symptom screening of students due to the minimal risk and potential health benefit; and waived the need for individual assent/consent by all occupants of congregate spaces for ambient carbon dioxide concentration measurement and ddPCR air sampling due to the minimal risk of the procedure.

Approval was obtained from the regional Departments of Education and Health for conduct of this study. Clinic staff, teachers and students were sensitised about the study (i.e. education on *Mycobacterium tuberculosis* transmission, objectives of the study, how the study would be conducted, any potential benefits and risks/costs of the study for those involved, benefits of the study to society and when the study was estimated to start and conclude).

5.5.4. Training

Training of fieldworkers and laboratory staff covered *Mycobacterium tuberculosis* transmission, rationale and objectives of this study, study methods, anticipated impact of findings; and for field workers: the consent process, handling of samples, infection control, use of the carbon dioxide loggers and the ddPCR air sampling device; and for laboratory staff: the handling, processing and analysis of samples.

5.5.5. Study procedures

a) Ambient carbon dioxide concentration measurement

In both clinics and classrooms, we measured ambient carbon dioxide concentration as a proxy for ventilation and used the median ambient carbon dioxide concentration (threshold of 1,000 parts per million (ppm)) to define high (above 1,000 ppm) and low (below 1,000 ppm) ventilation risk for *Mycobacterium tuberculosis* transmission, in line with WHO recommendations and previous studies of congregate settings.[26–28] The median indoor carbon dioxide concentration of 1000 parts per million (ppm) in naturally ventilated classrooms is equivalent to the WHO recommendation of 12 air changes per hour, or a ventilation rate of 8.6 litres per person per second.[28] Carbon dioxide concentration was measured using a portable carbon dioxide monitor (Ethernet Multilogger thermo–hygro– carbon dioxide meter with 2 MiniDIN and 2 Terminals)[51] (see Appendix 5.1). The monitors were new, factory–calibrated and provided with calibration certificates. Simultaneous carbon dioxide measurement with multiple devices showed the measurements to be within the acceptable 10% range (as per factory manual). The carbon dioxide monitors recorded carbon dioxide concentration in parts per million (ppm) and were set to record

carbon dioxide concentration every 60 seconds for the entire duration of classroom ambient carbon dioxide concentration measurement. The maximum carbon dioxide concentration that the monitors could record was 2,100 ppm. The carbon dioxide monitors were placed 50 cm from the floor at the back of the clinic or classroom during ambient carbon dioxide concentration measurement.

For both clinics and classrooms, we recorded duration of ambient carbon dioxide concentration measurement and ddPCR air sampling, total number of windows and doors, and at the start and end of each ambient carbon dioxide concentration measurement and ddPCR air sampling episode, whether windows and doors were open or closed, and number of individuals in the space. The duration of ddPCR air sampling was the same as that of ambient carbon dioxide concentration measurement. We did not record the size of windows and doors, exact floor size or room volume, individuals entering or leaving these spaces, duration each individual spent in each space, or contact behavior during the sampling period. We did not measure temperature, humidity or wind speed.

Clinic ambient carbon dioxide concentration measurements and ddPCR air sampling was conducted in November and February of 2018. The start and end time for clinic ambient carbon dioxide concentration measurement and ddPCR air sampling was variable and between 8:00 am and 2:00 pm, except for 3 occasions when sampling was conducted for 5 hours and ended between 3.00 pm – 4:00 pm. Regional average temperature during the day for summer is 28 degrees Celsius, for autumn is 21 degrees Celsius, for Winter is 18 degrees Celsius and for spring is 23 degrees Celsius.[52]

Classroom ambient carbon dioxide concentration measurement was done during the first classroom session of the day to avoid overestimation due to carbon dioxide accumulation arising from previous occupants. Measurement was done once for each group of students who occupied that classroom for a single lesson, lasting approximately 35–45 minutes. Testing was stopped before a new group of students entered the classroom for the next lesson. Classroom ambient carbon dioxide concentration measurement and ddPCR air sampling for school B was conducted in August and September; and for school D in April and May, except for 7 instances (3 in March, 2 in July and 2 in August) 2018. The start and end time for

classroom ambient carbon dioxide concentration measurement and ddPCR air sampling was usually between 8:00 am and 10:00 am.

Calculation of rebreathed fraction

Rebreathed fraction (Appendix 5.15), as defined by Rudnick and Milton, is the fraction of inhaled air that has been exhaled previously by another person in the same space.[29] Rebreathed air volume is the absolute volume of rebreathed air expressed in litres.[29] Rebreathed fraction of air was calculated using Rudnick and Milton's equation that uses carbon dioxide concentration, i.e.:[29]

$$F=(C-C_o)/(C_a) \quad (1)$$

Where F is equivalent to the rebreathed fraction, C is the observed concentration of carbon dioxide in indoor air, C_o is the concentration of carbon dioxide in outdoor air and C_a is the concentration of carbon dioxide in exhaled air which was estimated as 38,000 ppm.[53] For low levels of physical activity, Ca was estimated to be 38,000 ppm based on a carbon dioxide production rate of 0.31 litres/minute and respiratory minute volume of 8.0 litres/minute.[53] Outdoor carbon dioxide concentration was defined as the minimum recorded value from outdoor carbon dioxide measurement.

b) ddPCR air sampling

We conducted ddPCR air sampling to detect and quantify RD9 *Mycobacterium tuberculosis* DNA in room air using the Dry Filtration Unit[40] (see Appendix 5.2). Briefly, the Dry Filtration Unit is a portable biological sample collection device that samples room air via filters. We used polyester felt filter of 47mm diameter and 1.0μm pore size (American Felt and Filter Company, New Windsor, New York; Lockheed Martin, Alexandria, VA, USA) filters that neither trap carbon dioxide nor impact simultaneous classroom ambient carbon dioxide concentration measurement. The Dry Filtration Unit samples approximately 1,000 litres of room air per minute[42] and has two separate holders for one filter in each holder. The filters are positioned side by side within the same air vent. We assumed the 1,000 litres was evenly distributed to each filter (i.e. 500 litres via each filter per minute). All ddPCR air sampling was accomplished using two

filters per Dry Filtration Unit for laboratory testing which were packaged separately, transported to the Desmond Tutu HIV Centre[54] laboratory at UCT, and stored at 1–4 degrees Celsius for 3–6 months prior to processing.

c) Droplet digital PCR (ddPCR) assay

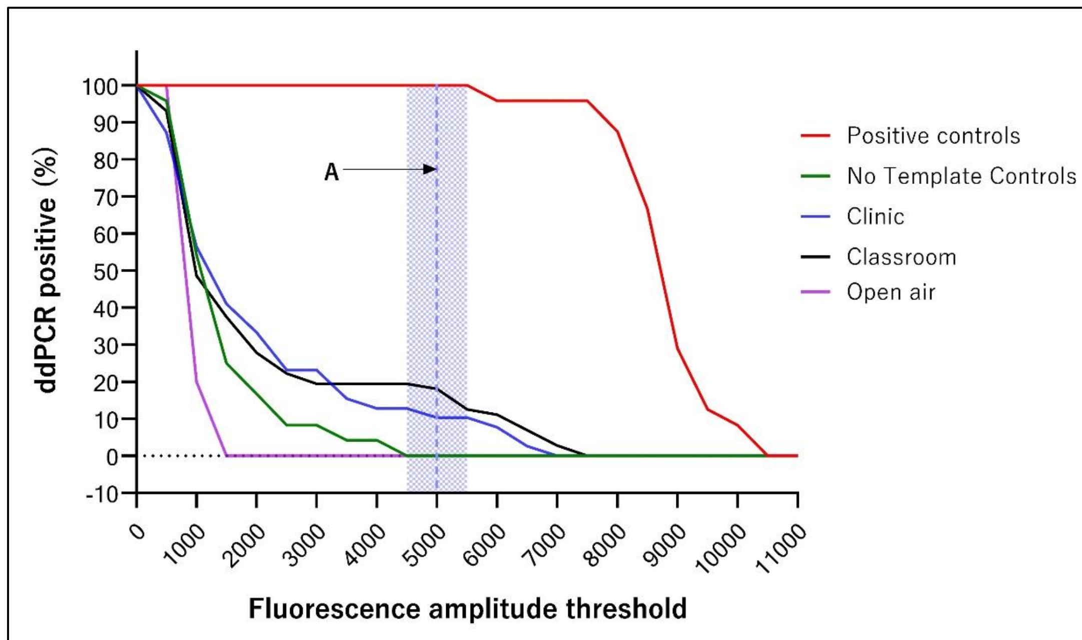
The ddPCR assay was performed by the Desmond Tutu HIV Centre[54] (University of Cape Town) in collaboration with the laboratory of Professor Digby Warner (University of Cape Town). The ddPCR assay was conducted using a previously published protocol,[55] described in Appendix 5.5. Briefly, filters were processed by vortexing in 10 ml sterile phosphate buffered saline (PBS) + 0.5% Tween 80 and centrifuged at 4,500 rpm for 15 minutes. The pellet obtained from centrifugation was lysed for DNA extraction and purification using a mastermix reagent from BioRad Laboratories, Inc (Denver, Colorado, USA).[56] Positive controls were *Mycobacterium tuberculosis* (H37RV) and No Template Controls (NTC) comprised of the mastermix, with no added template. Thermal cycler parameters used for DNA amplification were as follows: 50 °C for 2 min (incubation for Amperase); 95 °C for 10 minutes; 94 °C for 30 seconds; 60 °C for 1 minute; repeat 94 °C for 30 seconds (40 cycles); 98 °C for 10 minutes; holding at 4°C for between 5 hours to 24 hours before plate reading in the FAM and HEX channels using the QX100 BioRad Droplet Reader (BioRad Laboratories, Inc (Denver, Colorado, USA).[56] Genomic DNA extraction for PCR confirmation used primers RD9F (5'–gtgtaggtcagccccatcc–3'), RD9R (5'–gctaccctcgaccaagtgtt–3') and RD9Int (5'gctaccctcgaccaagtgtt–3') using a published protocol.[57] The QuantaSoft™ software[58] from BioRad Laboratories[56] which applies Poisson distribution statistics was used for RD9 *Mycobacterium tuberculosis* DNA copy quantification.[58]

d) Selection of fluorescence amplitude intensity positivity threshold for ddPCR assay

The range of fluorescence amplitude intensity that provided 100% discrimination between Negative (No Template Controls) and Positive Controls was 4,500–5,500 (Figure 5.2). The mid–point of the optimal

discriminatory range for fluorescence amplitude intensity, i.e. 5,000, was therefore selected as the positive threshold for all subsequent analyses. Using the 5,000 threshold, the proportion of ddPCR positive assays for control samples was Positive Controls 24/24 (100%), Negative (No Template) Controls 0/24 (0%), and open air spaces 0/5 (0%).

Figure 5.2: Proportion of positive ddPCR assay by fluorescence amplitude intensity threshold



Legend: The shaded region is the fluorescence amplitude intensity cut–off that provided 100% discrimination between No Template Controls and Positive Controls (i.e. 4,500–5500). Line A represents the optimal fluorescence amplitude intensity cut–off (5,000) selected for all subsequent ddPCR analyses.

e) Calculation of risk of inhaling RD9 *Mycobacterium tuberculosis* DNA

We adjusted concentration of RD9 *Mycobacterium tuberculosis* DNA to 180,000 litres of air (=180 cubic metres) for standardisation, based on the average volume of classrooms as per the education authorities. This was calculated as follows;

i.e. RD9 *Mycobacterium tuberculosis* DNA copies per 180,000= (2)

$$\frac{180{,}000}{Air\ via\ DFU(i.e.\,1{,}000\ l/min\ *\ duration\ in\ minutes)}\ x\ DNA\ copies\ from\ air\ sampling\ episode$$

Where: DFU=Dry Filtration Unit. The Dry Filtration Unit device samples approximately 1,000 litres of air per minute.

The rate of inhaling RD9 *Mycobacterium tuberculosis* DNA copies per sampling episode (i.e. "r") was estimated by: (3)

$$\frac{8\ l/min}{1000\ l/min}\ x\ DNA\ copies\ from\ ddPCR\ air\ sampling\ episode$$

Where: 8 l/min is the average adult human respiratory minute volume.[53] 1,000 l/min is the volume of air sampled via the Dry Filtration Unit per minute.

The risk of an individual occupant inhaling at least one RD9 *Mycobacterium tuberculosis* DNA copy was estimated by: (4)

$$1 - \exp(-r)$$

Where: exp= exponent; r= rate of inhaling RD9 *Mycobacterium tuberculosis* DNA copies per sampling episode obtained from equation (3) above.

The risk of an individual occupant inhaling at least one RD9 *Mycobacterium tuberculosis* DNA copy during a 35–minute lesson was obtained by; (5)

$$Class/room\ risk\ of\ inhaling\ 1\ DNA\ copy\ x\ \frac{35}{Duration\ in\ minutes}$$

Where: Duration=duration of ddPCR air sampling.

The estimated average risk of inhaling RD9 *Mycobacterium tuberculosis* DNA copies for classrooms or clinics was obtained by summing all classroom or clinic risk of inhaling one DNA copy, divided by the number of classrooms or clinics.

f) Symptom–screening for undiagnosed tuberculosis disease

All students in classrooms selected for ddPCR air sampling were given a health education talk that covered tuberculosis disease symptoms and tuberculosis transmission, and information about the study that included the rationale and objectives, eligibility for participation, how the study would be conducted, potential benefits and risks, confidentiality and autonomy (optional participation). Any queries were addressed. Students who expressed interest to participate in the study were individually requested to provide their written informed assent or consent (signature or thumbprint), and to complete a self–administered, tuberculosis disease symptom–based screening questionnaire written in their home language. Study staff were available to respond to any enquiries. Questionnaires included participant socio–demographic data and presence/absence of the following symptoms: cough for ≥2 weeks; haemoptysis; weight loss of ≥2 months; fever of unknown cause for ≥2 weeks; or night sweats for ≥2 weeks. Symptom screening was performed on the same day as classroom ambient carbon dioxide concentration measurement and ddPCR air sampling. Participants with cough or any other of the five tuberculosis–related screening symptoms who were willing to spontaneously expectorate sputum were given a sputum bottle and requested to provide one early morning sputum for Xpert MTB/RIF Ultra testing at the study laboratory, in order to evaluate the rate of laboratory testing using the screening algorithm and the yield of tuberculosis disease diagnosis among those screened and tested. It was acknowledged that participants without cough were unlikely to be able to produce a sputum sample; and that some participants with cough might be sputum unproductive. Students with any symptom/s were also given a referral letter asking them to report to the nearest public health facility where additional tuberculosis diagnostic and treatment, and HIV testing, is offered free of charge. Further testing for clinical purposes, including for tuberculosis and HIV, was managed by routine clinic staff.

5.5.6. Statistical analysis

Descriptive statistical analysis was done to describe socio–demographic variables and distribution of tuberculosis disease symptoms. Box and whisker plots were used to compare ambient carbon dioxide

concentration in clinics and classrooms. Categorical data were summarized using frequency counts and percentages. Carbon dioxide values by type of space were described using medians and inter–quartile ranges and the Wilcoxon rank–sum test was used to compare median carbon dioxide values by type of space. The *Chi–squared* test was used to assess statistically significant differences between groups for categorical ddPCR assay results. The Spearman rank correlation (correlation coefficient=rs) was used to determine correlation between ambient carbon dioxide concentration and mean temperature by month. The two–sample test of equality of proportions was used to compare proportions of high/low ventilation risk spaces for *Mycobacterium tuberculosis* transmission and spaces with positive ddPCR assay for clinics and classrooms. The Agresti–Coull method was used to obtain confidence intervals for tuberculosis disease prevalence. Analysis was conducted in STATA statistical software version 15.[59] For all hypothesis tests the alpha threshold was set at 0.05.

5.6. Results

5.6.1. Characteristics of clinic and classrooms

a) Duration of sampling and number of individual occupants

For classrooms, the median duration of ambient carbon dioxide concentration measurement and ddPCR air sampling was 40 minutes (IQR: 35,54; Appendix 5.6). The number of students in classrooms was usually unchanged at the end compared to the start of ddPCR air sampling (median 34 (IQR: 29, 39)).

For clinics, the median duration of ambient carbon dioxide concentration measurement and ddPCR air sampling was 82 minutes (IQR: 40,120; see Appendix 5.6). The number of individuals in clinics at the start of ambient carbon dioxide concentration measurement and ddPCR air sampling varied by type of space (see Appendix 5.7) and was highest in general patient waiting areas (42 (median) (IQR: 27, 45)) and lowest in tuberculosis clinic consultation rooms (3 (IQR: 3, 5)). The number of individuals in clinic spaces was usually unchanged at the end compared to the start of ddPCR air sampling, except for the general patient

waiting areas where the former was almost half the latter, 20 (IQR: 5, 24) vs. 42 (IQR: 27, 45) respectively. For clinics, the number of patients or clients got fewer as the day progressed from 10:00 am onwards.

b) Windows and doors

For classrooms, the proportion of all windows that were open was 27%, this varied by month (75% in March, 13% in May). The proportion of open classroom doors was approximately 50%.

All clinic spaces had existing carbon dioxide monitors with alarm functions that had previously been installed by hospital authorities to monitor ambient carbon dioxide concentration. The overall proportion of all clinic windows that were open was 70% (range 46% to 89% (Appendix 5.8 and Appendix 5.9)). The proportion of open clinic windows was similar for the two months that ambient carbon dioxide concentration measurement and ddPCR air sampling was conducted (71% vs 69% for February and November, respectively). The proportion of open clinic doors was 20% (Appendix 5.10).

c) Ambient carbon dioxide concentration and season

Classroom median ambient carbon dioxide concentration was highest in July (1297 ppm ((IQR: 1235, 2100); the coldest month of the year) and lowest in March (835 ppm ((IQR: 751, 893); the third warmest month of the year). There was a statistically significant negative correlation between ambient carbon dioxide concentration and average daytime temperature of the month of sampling for classrooms ($p<0.001$); and a statistically significant negative correlation between the proportion of open classroom windows and ambient carbon dioxide concentration ($P<0.001$).

Clinic median ambient carbon dioxide concentration was similar for the two months of clinic space sampling (see Appendix 5.10 and Appendix 5.11). There was no statistically significant correlation between ambient carbon dioxide concentration and average daytime temperature of the month of sampling for clinic spaces ($p=0.547$), but there was a statistically significant negative correlation between the proportion of open clinic windows and ambient carbon dioxide concentration ($p<0.001$).

d) Ambient carbon dioxide concentration and time of sampling

For classrooms, there was a statistically significant direct relationship between time of sampling and ambient carbon dioxide concentration ($rs=0.211$; $p<0.001$).

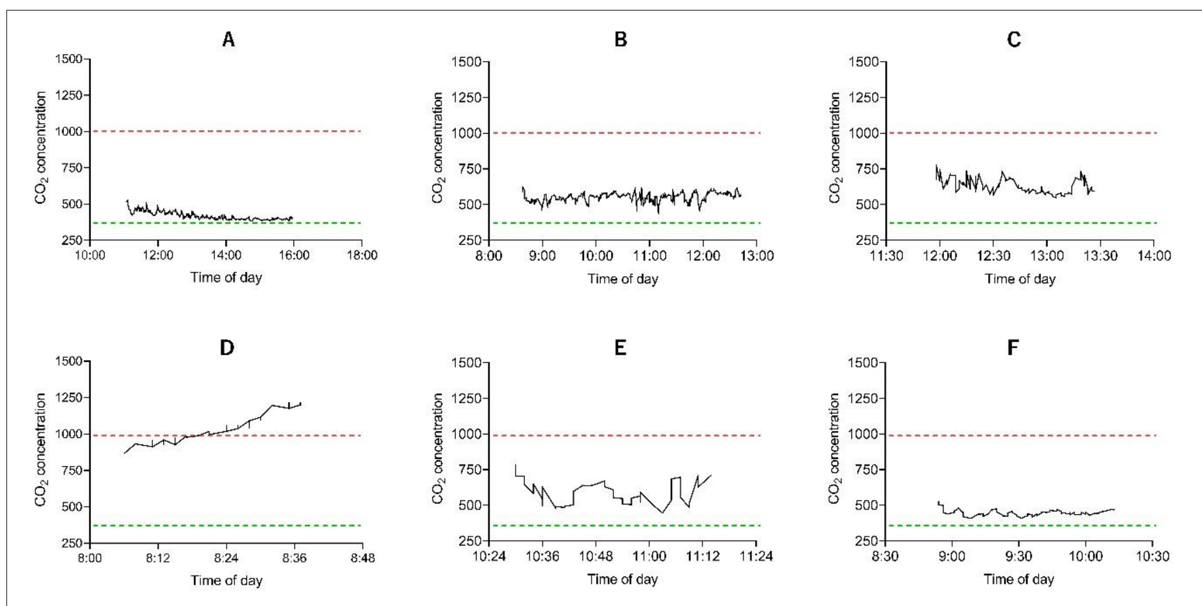
For clinics, there was a statistically significant inverse relationship between time of sampling and ambient carbon dioxide concentration ($rs= -0.273$; $p<0.001$).

e) Ambient carbon dioxide concentration

The distribution of classroom ambient carbon dioxide concentration measurements was variable; and was impacted by the number of people in the space, and the number and proportion of open windows and doors. Representative examples for clinics and classrooms are shown in Figure 5.3(a) and Figure 5.3(b), respectively.

All clinics had median ambient carbon dioxide concentration below 1,000 ppm (see Appendix 5.12 and Appendix 5.13); and the majority (>70%) of clinic ambient carbon dioxide concentration readings did not achieve steady state by the end of the sampling period.

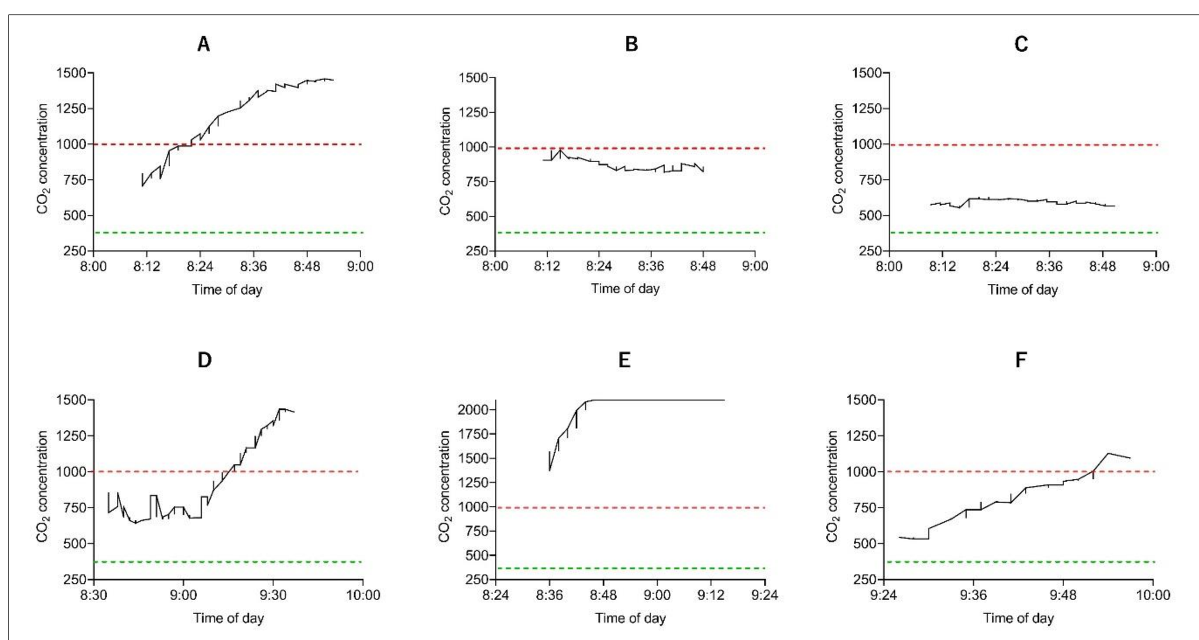
Figure 5.3(a): Representative examples of clinic ambient carbon dioxide concentration over time



Legend: CO_2 =Carbon dioxide. Carbon dioxide concentration was measured in parts per million and showed variable distribution over the duration of measurement. Panel A=general patient waiting area, Panel B=tuberculosis clinic consultation room, Panel C = general patient waiting area; Panel D=general patient waiting area, Panel E=tuberculosis patient waiting area, Panel F = tuberculosis patient waiting area. Green dotted line= The median carbon dioxide level of open–air environmental space (387 ppm). The dotted red line is the upper threshold for recommended median carbon dioxide concentration of 1,000 ppm. Portable carbon dioxide monitors recorded carbon dioxide concentration every 60 seconds for clinic spaces.

Steady state ambient carbon dioxide concentration was observed rarely in classrooms (e.g. Figure 5.3(b) panel E), despite peak concentration of >1,000 in several cases (Figure 5.3(b) Panel D).

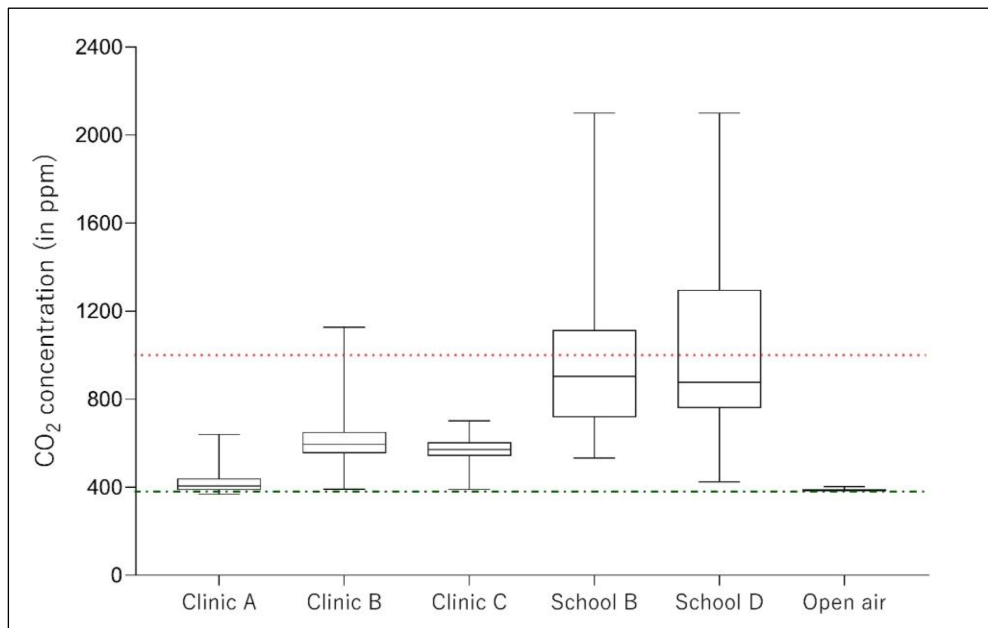
Figure 5.3(b): Representative examples of classroom ambient carbon dioxide concentration over time



Legend: CO_2 =Carbon dioxide. carbon dioxide concentration was measured in parts per million and showed variable distribution over the duration of IAQ sampling. Panel A–F = classrooms. Green dotted line= The median carbon dioxide level of open–air environmental space (387 (384–403 ppm). The dotted red line is the threshold for recommended median carbon dioxide concentration of 1,000 ppm.

In Figure 5.3, we show that Clinic A, B and C had ambient carbon dioxide concentration of 405 (median) (IQR: 387–440), 595 (555–651), and 571(544–605), respectively. School B and D had carbon dioxide concentration of 904 (median) (719–1114) and 877 (761–1297), respectively. The carbon dioxide concentration of the open–air space was 387 (384–403) (Figure 5.4).

Figure 5.4: Comparison of ambient carbon dioxide concentration in clinics and classrooms



Legend: CO_2=Carbon dioxide. Carbon dioxide concentration was measured in parts per million (ppm). Green dotted line= The median ambient carbon dioxide concentration of open–air space (387 ppm). The dotted maroon line is the threshold for recommended median carbon dioxide concentration of 1,000 ppm.

Overall, clinic spaces were better ventilated than classroom spaces; respectively for all clinics vs all classrooms, the median ambient carbon dioxide concentration was 490 ppm (IQR: 405–587) vs 886 ppm (IQR: 747–1223) (p–value <0.001). No clinic spaces and 40% of classrooms met the definition of high–risk spaces for *Mycobacterium tuberculosis* transmission if defined by median ambient carbon dioxide concentration of ≥1,000 ppm (Table 5.1). Median classroom ventilation rate as estimated by air changes per hour was 6.10 (4.38, 8.21) in school B and 6.46 (3.69, 8.37) in school D.

Classroom proportion of rebreathed air from fellow students was median 1.40% in school B and 1.26% in school D. Volume of rebreathed air per classroom learning session (35 minutes) was median 3.53 litres (2.70, 4.85) in school B and 3.21 litres (1.75, 5.50) in school D.

Table 5.1: Classification of ventilation risk status in clinics and classrooms

Setting	Total duration of sampling (hours)	Proportion of sampling duration with carbon dioxide readings ≥1000ppm (n/N (%))	Proportion of spaces with median carbon dioxide ≥1,000 ppm (n/N (%))	Proportion of spaces with peak carbon dioxide ≥1000 ppm (n/N (%))	Proportion of rebreathed air (%) Median (IQR)
Clinic A	22.0	0 (0%)	0/9 (0%)	0/9 (0%)	0.03% (0.02, 0.10)
Clinic B	14.3	0.15 (1.0%)	0/7 (0%)	3/7 (42.9%)	0.58% (0.47, 0.63)
Clinic C	8.7	0 (0%)	0/5 (0%)	0/5 (0%)	0.43% (0.33, 0.52)
School B	17.4	6.2 (35.6%)	11/32 (34.4%)	20/32 (62.5%)	1.40% (1.02, 1.81)
School D	24.4	9.5 (38.9)	14/31 (45.2%)	19/31 (61.3%)	1.26% (1.15, 2.42)
Open–air space	1.1	0 (0%)	0/3 (0%)	0/3 (0%)	NA

Legend: CO_2=Carbon dioxide. Indoor ambient carbon dioxide concentration is in parts per million. IQR=Interquartile range. NA=Not applicable.

5.6.2. ddPCR assay results

Using the selected fluorescence amplitude intensity positivity threshold (5,000), ddPCR air sampling yielded a positive *Mycobacterium tuberculosis* ddPCR assay result from four clinics (10.3%) and 13 classrooms (18.1%) (p=0.276).

For classrooms, there was no statistically significant association between a positive ddPCR assay result and high–risk ventilation status (χ^2 p–value=0.170). Overall, the estimated average concentration of RD9 *Mycobacterium tuberculosis* DNA for all classrooms was 3.61 copies per 180,000 litres but 20 copies (range 8–

82 copies) per 180,000 litres when analysis was limited to classrooms with ddPCR assay positive filters only. The average risk of a classroom occupant inhaling one RD9 *Mycobacterium tuberculosis* DNA copy over one lesson (35 minutes) was 0.71%; and the estimated risk over one academic day was 2.7%, over one academic week was 12.6%, over one academic month was 41.7% and over one academic year was 100%. Students in South African high schools spend approximately 1,155 hours congregated in classrooms during approximately 199 school days (academic year) each calendar year.[22] We estimate each student will have inhaled on average 11 RD9 *Mycobacterium tuberculosis* DNA copies per year. For only those classrooms with a positive ddPCR assay, the estimated average proportion of occupants who inhaled at least one copy of RD9 *Mycobacterium tuberculosis* DNA was 3.8% (range: 2.4%–20%). It is important to note that one RD9 *Mycobacterium tuberculosis* DNA copy does not necessarily translate to one infectious *Mycobacterium tuberculosis* quantum.

For clinic spaces, the overall estimated average concentration of RD9 *Mycobacterium tuberculosis* DNA was 1.74 copies per 180,000 litres of air but 17 copies (range 2–27) per 180,000 litres of air for clinics with ddPCR assay positive filters only, and was similar for the tuberculosis clinic consultation room (19 copies) and patient waiting area (15 copies). The average risk of a clinic occupant inhaling one RD9 *Mycobacterium tuberculosis* DNA copy over a 35–minute period was 0.29% for all clinics.

5.6.3. Sociodemographic characteristics of students screened for tuberculosis disease

We enrolled 1,836 out of 2,262 students (81.2%) in the classrooms selected for ddPCR air sampling (Table 5.2). The median age of study participants was 16.2 years. 779 (45.8%) of all study participants were male. School B predominantly had students of Mixed race (96.1%) whereas school D predominantly had students of Black African race (62.2%) (Table 5.2 and Appendix 5.14).

Table 5.2: Sociodemographic characteristics of enrolled students

Variable	School B	School D	Total
Enrolled participants (n (%))	643 (73.0%)	1,193 (86.4%)	1,836 (81.2%)
Age (median (IQR)	15.7 (14.8; 16.8)	16.6 (15.2; 17.9)	16.2 (15.1; 17.5)
Sex (male)	272 (46.3%)	507 (45.6%)	779 (45.8%)
Race			
Black African	10 (1.6%)	1073 (96.7%)	1083 (62.2%)
Cape Mixed Ancestry	608 (96.1%)	36 (3.2%)	644 (36.9%)
White	14 (2.2%)	0 (0.1%)	14 (0.8%)
Indian/Asian	1 (0.2%)	1 (0.1%)	2 (0.1%)

Legend: n (%) = number (percentage). IQR= Interquartile range.

5.6.4. Symptom screening for tuberculosis disease

90 students reported cough ≥2 weeks (4.9%), 14 reported haemoptysis (0.8%), 79 reported weight loss >2 months (4.3%), 68 reported fever of unknown cause >2 weeks (3.7%), and 48 reported nights sweats >2 weeks (2.6%). These proportions did not differ substantially by school (Table 5.3).

215 (11.7%) students reported one or more tuberculosis–related symptoms, of whom 90 (41.9%) reported a cough ≥2 weeks and 125 (58.1%) reported no cough ≥2 weeks. 58 (64.4%) of those reporting cough ≥2 weeks indicated they could produce sputum, but only 21 (36.2%) submitted sputum for Xpert MTB/RIF Ultra testing, one (4.8%) of which was positive, giving an estimated tuberculosis disease prevalence of 55 (CI: 0–341) per 100,000).

Table 5.3: Screening for self–reported symptoms

Symptom or attribute	School B n or n/N (%)	School D n or n/N (%)	All n or n/N (%)
Cough ≥ 2 weeks	36/640 (5.6%)	54/1180 (4.6%)	90/1820 (4.9%)
Haemoptysis	5/640 (0.8%)	9/1174 (0.8%)	14/1814 (0.8%)
Weight loss >2 months	30/636 (4.7%)	49/1185 (4.1%)	79/1821 (4.3%)
Fever of unknown cause >2 weeks	32/637 (5.0%)	36/1180 (3.1%)	68/1817 (3.7%)
Nights sweats >2 weeks	16/642 (2.5%)	32/1189 (2.7%)	48/1831 (2.6%)
Household tuberculosis disease contact	28/640 (4.4%)	62/1186 (5.2%)	90/1826 (4.9%)
≥1 tuberculosis disease screening symptom*	86/642 (13.4%)	129/1190 (10.8%)	215/1832 (11.7%)
Sputum positive Xpert MTB/RIF Ultra (N= tested individuals)	0/3 (0.0%)	1/18 (5.6%)	1/21 (4.8%)
Number screened to detect 1 case of tuberculosis disease	NA	1190	1832

Legend: Differences in denominator due to the fact that not all students responded to all questions on tuberculosis–related symptoms.

5.6.5. Relationship between classroom ambient carbon dioxide concentration, ddPCR air sampling, and tuberculosis disease symptoms

Relationship between classroom ambient carbon dioxide concentration and ddPCR air sampling

There was no statistically significant association between a positive ddPCR assay and high–risk ventilation status (χ^2 p–value=0.170) for classrooms. χ^2 p–value not applicable for clinics since all were low ventilation risk. Three (12%) of high-risk ventilation classrooms and 10 (26%) of low ventilation risk classrooms had a positive *Mycobacterium tuberculosis* ddPCR assay. However, the median ambient carbon dioxide concentration for ddPCR assay positive classrooms was significantly lower than ddPCR–negative classrooms (829 ppm (IQR: 700–1114) vs 914 ppm (IQR: 761–1243); p–value < 0.001), respectively. The

median ambient carbon dioxide concentration for ddPCR assay positive clinic spaces was higher than ddPCR–negative clinic spaces (581.5 ppm (IQR: 475.0–645.0) vs 468.5 ppm (IQR: 402–577); p–value <0.001). The median ambient carbon dioxide concentration for ddPCR assay positive classrooms was much higher than that for ddPCR assay positive clinics.

Relationship between tuberculosis–related symptoms and *Mycobacterium tuberculosis* ddPCR air sampling

There was a statistically significant direct association between self–reported weight loss of >2 months and classroom ddPCR assay positive result; but statistically significant inverse associations between fever >2 weeks, nights sweats >2 weeks and classroom ddPCR assay positive result (Table 5.4).

Table 5.4: Screening for self–reported tuberculosis disease symptoms among students by ddPCR assay result

Symptom	**ddPCR assay positive** **n or n/N (%)**	**ddPCR negative assay** **n or n/N (%)**	**P–value**
Cough > 2 weeks	14/272 (5.2%)	76/1548 (4.9%)	0.868
Haemoptysis	0/273 (0%)	14/1541 (0.9%)	0.114
Weight loss >2 months	20/272 (7.3%)	59/1548 (3.8%)	0.009
Fever >2 weeks	2/273 (0.7%)	66/1544 (4.3%)	0.004
Nights sweats >2 weeks	2/274 (0.7%)	46/1557 (3.0%)	0.034
≥1 tuberculosis disease screening symptom*	33/274 (12.0%)	182/1558 (11.7%)	0.864

Legend: tuberculosis=tuberculosis. Numbers are n (%) or n (IQR).

Relationship between tuberculosis–related symptoms and classroom ambient carbon dioxide concentration

There was no statistically significant association between the presence of self–reported tuberculosis–related symptoms and low/high classroom ventilation risk status based on median ambient carbon dioxide concentration threshold (1,000 ppm) (Table 5.5).

Table 5.5: Self–reported tuberculosis disease symptoms among students by ventilation risk status of classrooms

Symptom or attribute	Low ventilation risk classrooms n or n/N (%)	High risk ventilation classrooms n or n/N (%)	P–value
Cough > 2 weeks*	34/936 (3.6%)	31/708 (4.4%)	0.442
Haemoptysis	9/942 (1.0%)	5/711 (0.7%)	0.580
Weight loss >2 months	41/940 (4.4%)	29/709 (4.1%)	0.139
Fever>2 weeks	34/940 (3.6%)	20/708 (2.8%)	0.371
Nights sweats >2 weeks	22/944 (2.3%)	14/711 (2.0%)	0.618
≥1 tuberculosis disease screening symptom*	102/944 (10.8%)	67/711 (9.4%)	0.358

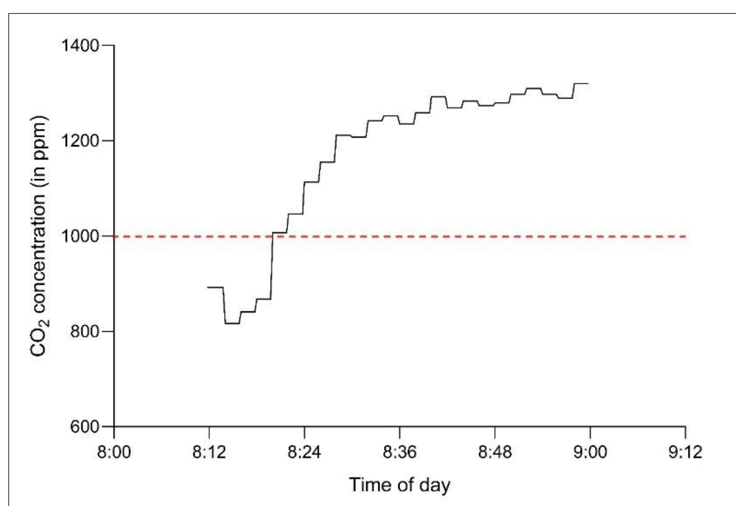
Legend: tuberculosis=tuberculosis; *=Any symptom refers to any one or more of cough, haemoptysis, weight loss, self–reported fever and/or night sweats. Numbers are n (%) or n (IQR). * Not all classrooms had ambient carbon dioxide concentration readings hence the different denominators.

5.6.6. Profile of classroom occupied by a student with undiagnosed tuberculosis disease

The classroom with a microbiologically confirmed case of tuberculosis disease was a grade 12 classroom in school D that was occupied by 41 students in total, 29 (71%) of whom agreed to take part in this study. The median age of all students in this classroom was 18.4 years (IQR: 17.8, 19.6). Students spent 40 minutes of the 58–minute sampling period (83%) with indoor ambient carbon dioxide concentration levels

above the recommended median ambient carbon dioxide concentration threshold. The median ambient carbon dioxide concentration was 1,242 ppm (IQR: 1,046–1,283 ppm; Figure 5.5). ddPCR air sampling of this classroom yielded a positive ddPCR assay.

Figure 5.5: Indoor ambient carbon dioxide concentration monitoring of classroom with undiagnosed tuberculosis disease case



Legend: CO_2=Carbon dioxide. This Figure shows that students spent 40 minutes exposed to indoor ambient carbon dioxide concentration levels above the recommended median carbon dioxide concentration of 1,000 ppm (dotted red line).

Three male students reported at least one tuberculosis–related symptom. The student with confirmed tuberculosis disease was aged 20 years old and reported a cough of ≥2 weeks, but was unsure of any household contact with tuberculosis disease. The second student with self–reported symptoms had a cough of ≥2 weeks with no known household tuberculosis disease contact, and the third student had weight loss and self–reported fever, but was unsure whether he had a household contact with tuberculosis disease. It is not known whether other students in this classroom who did not consent to take part in symptom–screening (29%) were symptomatic or had a household tuberculosis disease contact. Only one of these three students was able to provide sputum for Xpert MTB/RIF Ultra assay; the other two students with self–reported symptoms were sputum unproductive.

5.7. Discussion

We built on the seminal work of Riley,[23] Wells,[24] Rudnick[29] and Wood[32] to study classroom ambient carbon dioxide concentration and tuberculosis symptom screening, in conjunction with air sampling for *Mycobacterium tuberculosis* using a highly sensitive molecular test,[55] ddPCR, that to the best of our knowledge has not been previously used in school classrooms in a high tuberculosis burden setting. We showed that more than one–third of 72 classrooms in two schools in the study area were inadequately ventilated. One–fifth of classrooms had evidence of airborne *Mycobacterium tuberculosis* DNA detected by ddPCR air sampling. However, yield from school–based TB symptom screening was low, consistent with the presence of undiagnosed subclinical TB cases and risk of ongoing transmission in the school setting.

We also studied clinics as comparator spaces with high *a priori* risk of occupation by known tuberculosis patients. We showed that all clinics were adequately ventilated, based on a median ambient carbon dioxide concentration ≥1,000 ppm, although 14% of clinics had a high peak carbon dioxide ≥1,000 ppm indicating intermittent periods of poor ventilation during which *Mycobacterium tuberculosis* transmission risk might be elevated. All clinic readings were conducted during summer when ventilation due to open windows could be assumed maximal. However, we obtained positive ddPCR assay results on filters from 10% of clinic spaces, all of which were defined as low ventilation risk by median ambient carbon dioxide concentration, which emphasises the need for strict adherence to other infection control measures, given the high probability of individuals with tuberculosis disease in this setting.

In contrast to the findings in clinics, 40% of classrooms were inadequately ventilated with median carbon dioxide concentration ≥1,000 ppm. This finding is similar to the 60% observed in another South African study, which tracked spaces that 64 students visited over 91 school days using individual monitors for a total of 509 hours of school time.[28] Inadequate classroom ventilation is also not limited to schools in developing countries. A study of 62 classrooms in 27 naturally–ventilated schools in Greece found 52% to be high risk ventilation spaces with mean indoor ambient carbon dioxide concentration of more than 1,000 ppm.[60] A systematic review on classroom ambient carbon dioxide concentration in European

schools (287 classrooms from 182 naturally–ventilated schools and 900 classrooms from 220 mechanically–ventilated schools) also found 52% of classrooms to be high risk ventilation spaces.[60] However, the potential risk of *Mycobacterium tuberculosis* transmission would likely be higher in classrooms in high tuberculosis burden compared to low tuberculosis burden settings, given the same degree of sub–optimal ventilation to which students are exposed. The poor ventilation of classrooms in this study is likely a result of the low percentage (27%) of open windows, which in turn was likely influenced by prevailing weather conditions in winter months. However, in this study, the mean rebreathed air volume for one classroom session (35 minutes) of 5.0 litres is lower than the 6.7 litres reported for an equivalent period in another adolescent study in a South African school.[32]

A limitation of the study is time of year and season in which ddPCR air sampling was conducted, which limits our ability to compare adequacy of ventilation directly between clinics and classrooms. A study of both clinics and classrooms in winter only might not replicate our findings of an inverse association between average monthly temperature and median ambient carbon dioxide concentration in classrooms, but not clinics.

The study of classrooms during the winter months with sub–optimal ventilation can be assumed as the worst–case scenario for tuberculosis transmission risk. Indoor environment and ventilation, as demonstrated by Wells and Riley are key determinants of transmission for airborne infections.[23, 24] The following factors impacted ventilation in this study: month/season, proportion of open windows or doors, time of day and number of occupants in rooms. Other factors that impact indoor ventilation status and air quality include light intensity, wind direction, wind speed,[61] desiccation rate,[62] size of windows, relative humidity, temperature, use of mechanical ventilation and adaptive behaviours by room occupants to keep windows or doors closed or open, parameters which were not systematically measured in this study.[60]

Mycobacterium tuberculosis ddPCR assay positive results were obtained from 10 (26%) adequately ventilated and three (12%) inadequately ventilated classrooms, including 12 (17%) classrooms in which tuberculosis disease symptom–screening did not detect additional undiagnosed tuberculosis disease cases, suggesting

additional undiagnosed tuberculosis disease cases in these classrooms were missed. We demonstrated the relationship between high risk ventilation and increased risk of ddPCR assay positive signal in clinics. Although we did not demonstrate the same relationship in classrooms, this may be due to the fact that even in ddPCR negative classrooms ventilation was not optimal. Median ambient carbon dioxide concentration in ddPCR negative classrooms was higher than that in ddPCR positive clinics.

We report an average risk of a classroom occupant inhaling 1 DNA copy per classroom learning session of 35 minutes of 0.71%. Considering students in South African high schools spend approximately 1,155 hours congregated in classrooms during approximately 199 school days (academic year) each calendar year,[22] we estimate each student will have inhaled on average 11 RD9 *Mycobacterium tuberculosis* DNA copies per academic year. A South African study reported a concentration of approximately 40 colony forming units of *Mycobacterium tuberculosis* per 40 minutes of sampling, similar to that in our study.[55] However, the only way to measure whether presence of RD9 *Mycobacterium tuberculosis* DNA from ddPCR air sampling was resulting in *Mycobacterium tuberculosis* transmission would be to measure prospective serial interferon gamma release assays (IGRA) in a fixed population of susceptible individuals. This study was designed pragmatically, to reflect the likely design of a future school–based tuberculosis disease screening strategy, which would not use TST/IGRA, chest X–ray or sputum induction due to feasibility and cost considerations. We set out to identify undiagnosed tuberculosis disease cases in classrooms with a positive ddPCR signal, but did not plan to use IGRA to identify students who might have been infected by these tuberculosis disease cases, because after each lesson there is a change of classes with mixing of students. Thus, it would not be possible to link individual infections with individual tuberculosis disease cases.

The confidence interval of our adolescent tuberculosis disease prevalence estimate overlaps that of a 2005–2007 estimate (prevalence 300 per 100,000 CI: 100–400 per 100,000 population) in this study community[9] and two other South African studies (476 cases per 100,000; CI: 0–1,305[63] (2002) and 274 cases per 100,000; CI: 263–284[16] (2013)). Possible reasons for the relatively low prevalence estimate include underdiagnosis, since some students did not consent to participate in tuberculosis disease symptom

screening; some students who consented might have had asymptomatic, subclinical tuberculosis disease and were not detected by symptom screening; and some symptomatic students with tuberculosis disease might not have been able and/or willing to provide an expectorated sputum sample. All of these reasons for lower yield of our pragmatic tuberculosis disease surveillance approach would be equally applicable to the likely approach of a school–based tuberculosis disease surveillance system.

Studies have postulated that quantitative airborne sampling may serve as a clinically relevant measure of infectivity to target high–risk spaces for more intensive tuberculosis disease surveillance efforts.[55] A periodic classroom ddPCR air sampling strategy that identified ddPCR assay positive classrooms, followed by intensive symptom–screening accompanied by Xpert MTB/RIF Ultra testing and chest radiography of all students might be logistically feasible and cost–effective in resource–limited, high tuberculosis burden settings like South Africa.

Further research is required to understand whether the *Mycobacterium tuberculosis* ddPCR assay arises from DNA incorporated in viable or non–viable *Mycobacterium tuberculosis* cells or, possibly, cell–free DNA; and how that ddPCR assay correlates with *Mycobacterium tuberculosis* transmission risk.[55] The recommended dynamic range of the QX200 ddPCR system is 1–100,000 copies per 20 µl reaction.[64] Estimation error is negligible compared with other error sources such as pipetting, sample processing, and biological variation.[65]

Although ddPCR air sampling offers promise in *Mycobacterium tuberculosis* transmission research, further studies using a longitudinal study design that involves ddPCR air sampling, *Mycobacterium tuberculosis* culture and more intensive tuberculosis disease case detection, supported by sputum induction, may help determine the potential role of ddPCR air sampling for targeted classroom tuberculosis disease screening. Further research is also required to investigate the potential clinical role of ddPCR air sampling as a measure of infectivity in other high tuberculosis burden congregate settings.[55] It is crucial to note that one RD9 *Mycobacterium tuberculosis* DNA copy does not translate to one infectious *Mycobacterium tuberculosis* quantum. Therefore, more research is required to estimate the number of inhaled RD9 *Mycobacterium*

tuberculosis DNA copies leading to established latent *Mycobacterium tuberculosis* infection in one person. This research is crucial to inform the accuracy and impact of school–based tuberculosis disease symptom–screening program, which, based on our ddPCR air sampling data, might miss infectious undiagnosed tuberculosis disease cases in South African schools.

Conclusion

In conclusion, students in South African high school classrooms are exposed to inadequate ventilation during the winter months. Given the high tuberculosis disease prevalence rates in this population, inadequate ventilation exposes susceptible students to risk of *Mycobacterium tuberculosis* transmission. We have shown that ddPCR air sampling detects RD9 *Mycobacterium tuberculosis* DNA copies in South African classrooms, which suggest that the risks of inadequate ventilation are real. This highlights the importance of ventilation management (e.g. sensitization for window opening), tuberculosis infection control and surveillance for early TB disease case detection in the congregate school settings. However, yield from school–based tuberculosis symptom screening was low and thus school–based tuberculosis symptom screening alone as a surveillance strategy may be economically unfeasible due to the high likelihood of missing tuberculosis disease cases. Further, we have quantified the risk of the average student inhaling one RD9 *Mycobacterium tuberculosis* DNA copy per 35–minute lesson at 0.71% and more than 100% after two months. While acknowledging the limitations of seasonal sampling on generalizability of our results, we infer that the risk of tuberculosis transmission in South African classrooms in the winter months is at least as high, or greater than the risk of tuberculosis transmission in clinic settings where infection control measures have been deployed during the summer months.

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Chapter 6

6. Discussion

In this chapter we discuss overarching themes, conclusions, outstanding research questions and public health implications of the findings presented in the preceding chapters. Briefly, the main findings of this project are:

1) There is a paucity of literature reporting adolescent LTBI and TB disease prevalence in high TB burden African countries (1990–2018). Based on this limited available data, both LTBI and TB disease prevalence rates are high, but corresponding infection–to–disease ratios are inconsistently low compared to that expected from Styblo's Rule.[1]
2) In the study area, adolescent LTBI prevalence remained high and relatively unchanged over a decade.[2]
3) Adolescent TB disease notification rates in the study area fell 45% over the same period.[3]
4) More than one–third of 72 classrooms in two schools in the study area were inadequately ventilated. One–fifth of classrooms had evidence of airborne *Mycobacterium tuberculosis* DNA detected by ddPCR air sampling. However, yield from school–based TB symptom screening was low, consistent with the presence of undiagnosed subclinical TB cases and risk of ongoing transmission in the school setting.

To the best of our knowledge, we present the first estimates of adolescent TB infection–to–disease ratios from high TB burden African countries. We note the change in estimated ratio from six–fold to two–fold higher for Nduba *et al.*[4] (Kenya) vs. Mahomed *et al.*[5] (South Africa) study, respectively, if culture is used in place of sputum smear microscopy. We infer that comparison of the infection–to–disease ratio across different studies and settings may require benchmarking against microbiologically–confirmed TB disease. However, it should be noted that Leth *et al.*[6] argues the decrease in the infection–to–disease ratio compared to the pre–chemotherapy era (per Styblo's Rule) may be due to improvements in treatment of TB disease.[6]

We compared regional adolescent LTBI prevalence data for the period 2014–2015, obtained from a clinical trial,[7] with baseline LTBI prevalence data from an adolescent cohort study published in part by Mahomed *et al.*[5], which was included in the systematic review (Chapter 1). The systematic review did not obtain a published manuscript that evaluated temporal trends in prevalence of adolescent LTBI or TB disease from a high TB burden African country (1990–2018), the sub–population with the highest force of *Mycobacterium tuberculosis* infection in this setting.[8] These regional datasets permitted a comparison of changes in LTBI prevalence over the decade 2005–2015. Analysis of temporal trends in prevalence of both LTBI (Chapter 2) and TB disease (Chapter 3) provided novel data on the adolescent TB epidemic in the study region to inform monitoring and outcomes of adolescent TB control.

The 45% fall in adolescent TB disease notification rate in the face of constant LTBI prevalence in the same study area implies that the infection–to–disease ratio among adolescents in this community increased over the period 2005–2015, since ARTI is derived using LTBI prevalence. Using microbiologically–confirmed TB, the adolescent infection–to–disease ratio increased from 2.5 in 2005 to 6.9 in 2015 in the study area. However, the ratio remains lower than that expected from the Styblo Rule (8–12). Differences in estimates of infection–to–disease ratio within the same country or the same community over time were also observed by Trunz *et al.*[9] and Leth *et al.*[6] Leth *et al.*[6] argues that given these observations, the Styblo Rule should not be used to estimate incidence of smear–positive TB, a view endorsed by the WHO.[6] It follows that TB prevalence surveys in high TB burden countries are likely the best tools for monitoring progress in TB control in the absence of adequate routine TB surveillance systems.[6] We also appreciate that the observed trends in adolescent TB disease notification rates in the study area may not be extrapolated directly to all communities in South Africa, or to all high TB burden countries, because TB disease burden varies across communities based on contextual socioeconomic factors. Therefore, the first South African national TB disease prevalence survey, for which results are anticipated in 2020, would provide generalizable estimates of national TB disease prevalence, including that among adolescents.[10]

Over time, we would expect a decrease in adolescent *Mycobacterium tuberculosis* infection prevalence to precede any decrease in TB disease case notification rate in the same population, provided that duration of observation is sufficient to observe the expected changes. Our finding that adolescent prevalence of LTBI remained high and relatively unchanged, while adolescent notification rates of TB disease fell 45% over the same period,[3] may be explained by the fact that the adolescent ARTI and LTBI prevalence represent cumulative lifetime measures,[11] such that the majority of latently infected individuals have survived without reversion of their positive IGRA result to negative.[8] Compared to force of *Mycobacterium tuberculosis* infection, ARTI underestimates adolescent rates of *Mycobacterium tuberculosis* infection, due to IGRA (and TST) reversion after conversion.[8] For example, the point estimate of adolescent force of *Mycobacterium tuberculosis* infection (14%) in the study area is three–fold the adolescent ARTI estimate (3.6%, Chapter 2), which would impact the estimated adolescent infection–to–disease ratio.[2, 8] Instantaneous or short–term risk of *Mycobacterium tuberculosis* infection is best measured by force of *Mycobacterium tuberculosis* infection estimated from serial IGRA testing in prospective cohort studies, because force of *Mycobacterium tuberculosis* infection changes quickly in response to TB control interventions, whereas LTBI prevalence rates change more slowly, especially in older age groups.[12] It is not known whether force of *Mycobacterium tuberculosis* infection has changed in parallel with changing adolescent TB disease case notification rates.

It appears from our data that a pragmatic school–based TB disease screening program for adolescents that relied primarily on self–reported TB symptom screening, without additional TST/IGRA, chest radiography, or sputum induction for sputum unproductive and/or asymptomatic individuals, would likely not be effective. We showed relatively low rates of symptom–positive learners among those interviewed, low rates of sputum production and testing among symptomatic learners, and a low yield of microbiological confirmation among symptomatic learners who provided a sputum sample for testing. The low yield of confirmed TB diagnosis from school–based symptom screening appears not only operationally inefficient, but is likely associated with missed subclinical TB disease cases, which is supported by the relatively high rate of positive *Mycobacterium Tuberculosis* ddPCR assay results from classroom air sampling.

There is limited published data on the cost/benefit ratio of school–based infection control and TB disease surveillance measures in Africa.[13–15] This lack of data supports the need for research into more efficient strategies to target active TB disease case–finding. Our preliminary data suggest that *Mycobacterium tuberculosis* ddPCR air sampling might allow more intensive TB case–finding efforts, potentially including TST/IGRA, chest radiography and sputum induction, to be targeted at high risk learners who occupy ddPCR positive classrooms. This approach might be more efficient, cost–effective and operationally feasible in low– and middle–income high TB burden countries than mass symptom–based screening.

Additional research is needed to further evaluate and optimize ddPCR air sampling for TB case–finding in congregate settings such as schools, including re–designing primers to obtain a robust ddPCR assay, and varying the annealing temperature to optimise specificity of primer binding.[16] Some available PCR enhancers such as dithiotreitol, Beatine monohydrate, formamide are incompatible with the Biorad ddPCR chemistry platform used in the present study, and further research is needed to identify PCR enhancers compatible with the Biorad ddPCR chemistry platform to improve diagnostic accuracy.[16] We can't discount possibility of false positive ddPCR results, however we note that selection of ddPCR amplitude threshold was compatible with 100% positive controls and 100% negative controls samples. The ddPCR assay is sensitive and offers superior DNA copy quantitation capability compared to real time PCR and other available molecular diagnostic approaches, but limitations of ddPCR air sampling include the fact that the ddPCR assay is costly to set up and run, and requires specialised training and high safety laboratory support (Biosafety level II or better). Results for ddPCR assays performed in the research laboratory are not rapidly available, due to the need to process samples in batches for cost–saving and efficient use of reagents and laboratory personnel.

New studies are also needed to facilitate translation of ddPCR air sampling of congregate spaces from an early–stage research tool to an operational public health surveillance intervention. Intensive TB disease case–finding targeted at ddPCR positive classrooms would only be effective if all classroom occupants can be traced from classroom schedules and daily attendance rosters, in the event of a positive ddPCR assay

signal. It would also be crucial that the all learners occupying ddPCR positive classrooms participate in TB investigations and therefore sensitization to the importance of TB control measures and issues of potential TB stigma would have to be addressed.

Our finding, based on ddPCR air sampling of high school classrooms, that cumulative risk of exposure to *Mycobacterium tuberculosis* DNA approached 100% after 2.5 months of classroom attendance, is consistent with our published finding that adolescent LTBI prevalence in this study area remained high through the decade 2005–2015, which suggests ongoing *Mycobacterium tuberculosis* transmission in the medium–term. It appears that inadequate classroom ventilation would increase the risk for *Mycobacterium tuberculosis* transmission in the event of a learner or teacher with TB disease occupying the same space as susceptible IGRA negative students. We acknowledge that seasonal differences in air sampling between clinics and classrooms limits direct comparison of transmission risk. However, based on the proportion of ddPCR positive spaces, classroom risk of *Mycobacterium tuberculosis* transmission in the winter months appeared at least as high, or even greater than in clinics during the summer months, which highlights the impact of seasonal environmental measures on air quality and risk for TB transmission. Although clinics would have been deemed 'high risk' for transmission due to the likelihood of occupancy by infectious TB patients, based on our ddPCR air sampling findings, the actual risk of exposure in clinics appears significantly mitigated by optimal ventilation practices, which was not the case in classrooms. There is a need to ensure schools are safe places for learning as envisaged in the South African Integrated School Health Policy.[17, 18] We therefore recommend strategies be put in place to ensure adequate ventilation in all school classrooms by for example, health education and sensitization of staff and learners to the need for adequate ventilation e.g. open windows whenever seasonal weather conditions permit; and install carbon dioxide monitors in classrooms with an alarm function that is triggered when carbon dioxide levels exceed the recommended maximum safe threshold of 1,000 ppm, similar to measures that are already in place in 'high risk' clinics.

Development of effective TB intervention strategies requires identification of key locations for *Mycobacterium tuberculosis* transmission. Regional molecular epidemiological data indicate recent

Mycobacterium tuberculosis transmission is responsible for at least 55% of the regional TB disease burden,[19] and statistical modeling estimates that 25% and 50% of new *Mycobacterium tuberculosis* infections among South African individuals aged 0–14 and 15–19 years, respectively, occurs in the school setting.[20] We therefore recommend, as a matter of urgency, new studies of classroom ddPCR air sampling that incorporate *Mycobacterium tuberculosis* culture of parallel air filtrate samples and intensive TB case detection, supported by serial IGRA, chest radiography, and sputum induction for Xpert MTB/RIF Ultra and *Mycobacterium tuberculosis* molecular epidemiology testing (e.g. IS6110 Restriction Fragment Length Polymorphism (RFLP) or Mycobacterial Interspersed Repetitive Unit–Variable Number Tandem Repeat (MIRU–VNTR) to genotype *Mycobacterium tuberculosis*) to define relatedness of strains in incident TB disease cases.

In conclusion, despite the decline in regional TB disease notification rates observed between 2005–2015, the adolescent burden of *Mycobacterium tuberculosis* infection in the study area remains high compared to other high TB burden countries in Africa. This finding, coupled with our observation of inadequate classroom ventilation during the winter months, very high cumulative risk of exposure to *Mycobacterium tuberculosis* DNA within months of classroom attendance, and the likelihood of classrooms being occupied by learners and/or staff with undiagnosed, subclinical TB disease, suggests that schools are high risk settings for exposure of susceptible students to *Mycobacterium tuberculosis* transmission. Our data suggest that pragmatic school–based TB symptom screening is low yield and inefficient. New and more efficient targeted TB case–finding strategies are needed for congregate settings, including schools, in high TB burden countries. Based on our preliminary data, classroom ddPCR air sampling for *Mycobacterium tuberculosis* DNA appears feasible for this purpose, but requires further research to optimise diagnostic accuracy and demonstrate the cost–effectiveness and public health value of this approach in high TB burden countries.

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7. Appendices

7.1. Appendix 2.1

Checklist of items in design of a systematic review protocol

Domain	**Line item & PRISM–P code**	**Page**
Section 1: Administrative information		
Title:		
	Identification (1a)	√
	Update (1b)	NA
Registration	Registration (2)	√
Authors:		
	Contact (3a)	√
	Contributions (3b)	√
Amendments	Amendments (4)	√
Support:		
	Sources (5a)	√
	Sponsor (5b)	√
	Role of sponsor or funder (5c)	√
Section 2: Introduction		
Rationale	Rationale (6)	√
Objectives	Objectives (7)	√
Section 3: Methods		
Eligibility	Eligibility criteria (8)	√
Information	Information sources (9)	√
Search	Search strategy (10)	√

Study records:		
	Data management (11a)	√
	Selection process (11b)	√
	Data collection process (11c)	√
Data	Data items (12)	√
Outcomes	Outcomes and prioritization (13)	√
Bias	Risk of bias in individual studies (14)	√
Data synthesis		
	Quantitative synthesis criteria (15a)	√
	Appropriateness of data for synthesis (15b)	√
	Sensitivity and subgroup analyses (15c)	√
	Qualitative synthesis? (15d)	√
Meta–bias(es)	Meta–bias(es) (16)	√
Confidence in cumulative evidence	Assessment of strength of cumulative evidence (17)	√

7.2. Appendix 2.2

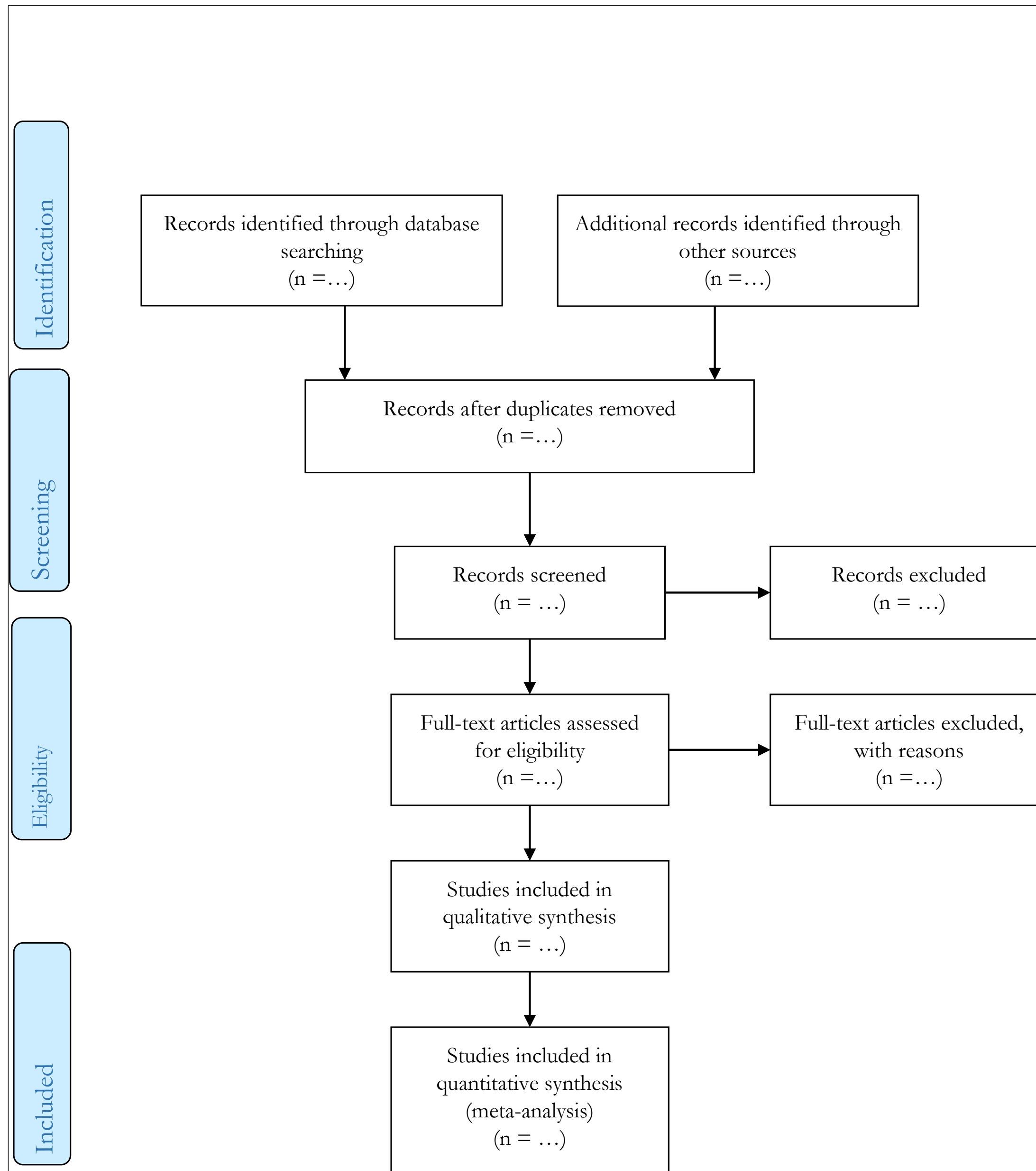
Search strategy

Item	**Search term**	**Boolean operator**
Adolescents	("adolescent" (All Fields) OR "adolescence" (All Fields) OR "adolescent" (MeSH Terms) OR "adolescence" (MeSH Terms) OR "teen*" (All Fields) OR "child" (All Fields) OR "person" (All Fields) OR "persons" (MeSH Terms) OR "people" (All Fields))	AND
Tuberculosis	("tuberculosis" (All Fields) OR "tuberculosis" (MeSH Terms) OR "TB" (All Fields) OR "TB" (MeSH Terms) OR "LTBI" (MeSH Terms) OR "LTBI" (All Fields) OR "latent" (MeSH Terms) OR "latent" (All Fields))	AND
Countries	("Africa" (All Fields) OR "Africa" (MeSH Terms) OR "east*" (All Fields) OR "south*" (All Fields) OR "Congo" (All Fields) OR "Zaire" (All fields) OR "Ethiopia" (All Fields) OR "Kenya" (All Fields) OR "Uganda" (All Fields) OR "Tanzania" (All Fields) OR "Zimbabwe" (All Fields) OR "South Africa" (All Fields) OR "Mozambique" (All Fields) OR "Nigeria" (All Fields) OR "Angola" (All Fields) OR " Sierra Leone " (All Fields) OR ("Central" AND "African" AND "Republic") (All Fields) OR "Lesotho " (All Fields) OR "Liberia " (All Fields) OR "Namibia " (All Fields) OR "Zambia " (All Fields) OR "Botswana " (All Fields) OR "Cameroon " (All Fields) OR "Chad" (All Fields) OR " Ghana " (All Fields) OR "Guinea–Bissau " (All Fields) OR "Malawi " (All Fields) OR "Swaziland " (All Fields) OR "Somalia" (All Fields))	AND
Prevalence	("epidemiology" (Subheading) OR "epidemiology" (MeSH Terms) OR "epidemiology" (All Fields) OR "prevalence" (All Fields) OR "prevalence" (MeSH Terms)	AND
Period	Between 1st January 1990 and 13th August 2018	

*Covers both Democratic Republic of Congo and Congo

7.3. Appendix 2.3

PRISMA 2009 flow diagram



7.4. Appendix 2.4

Data Extraction Form

Prevalence of latent TB infection **and TB disease among adolescents in high TB burden countries in Africa**

Part A: Article identity

Title of article ……………………………………………………………………....................

First author ……………………………… Publication year ……………………..……….

Part B: Study characteristics

Outcome: □ TB disease prevalence only □ LTBI prevalence only □ Both LTBI & TB disease prevalence

Country of study ……………………………

Study design

□ Cross–sectional study

□ Cohort study

□ Other. State if other ……………………………………………………………………….

Population

□ Non–students only

□ Students only

□ Both students and non–students

□ Undefined

Period of study conduct

□ State ……………………………

Age range of study participants

□ State ………………

□ Mean age ………….

Source population

□ Nationally representative study

□ Sub–national study

Response rate

□ State ……………………………

Diagnostic modality for TB disease or latent TB infection

Condition	**No. of events**	**Population size tested/evaluated**
TB disease		
Clinical		
Sputum smear		
Culture		
Xpert MTB/RIF		
X–ray		
LTBI		
IGRA		
TST		
Either IGRA or TST (Only if not disaggregated)		

Study participants by gender, HIV and outcome

Gender	No. with LTBI	Population size tested for LTBI	No. with TB disease	Population denominator for TB disease	No. with HIV	Population size tested for HIV
Male						
Female						
Total						

Include: □ Yes □ No

If no, reason;

- Age range ineligible
- Country ineligible
- Year ineligible
- Study design ineligible
- Language ineligible
- Sampling non representative
- Case definition or methodology unclear
- Adolescent data unobtainable
- Additional data unobtainable
- Duplicate study
- Other

Part C: Quality assessment

Item under review	Score (Yes=1 or No=0)
External Validity	…
Was the study's target population a close representation of the national population in relation to relevant variables?	…
Was the sampling frame a true or close representation of the target population?	…
Was some form of random selection used to select the sample, OR was a census undertaken?	…
Was the likelihood of non–response bias minimal?	…
Internal validity	…
Were data collected directly from the participants (as opposed to a proxy)?	…
Was an acceptable case definition used in the study?	…
Was the study instrument that measured the parameter of interest shown to have validity and reliability?	…
Was the same mode of data collection used for all participants? (1 point)	…
Was the length of the shortest prevalence period for the parameter of interest appropriate?	…
Were the numerator(s) and denominator(s) for the parameter of interest appropriate?	…
Total	…
Summary item on the overall risk of study bias: low, moderate or high risk of bias	

Legend: Response options for individual items are either low (1) or high risk of bias (0). If there is insufficient information in the article to permit judgment of the specific item, then the article is deemed to be at high risk of bias with respect to that item.

7.5. Appendix 2.5

Amendments to systematic review protocol

The published protocol was amended as follows:

1) BMS left the organisation and was replaced by Simon C. Mendelsohn.
2) The period of study conduct was changed from "studies reported between 1st January 1990 and 1st July 2016" to "studies conducted and reported between 1st January 1990 and 11th August 2018": (1) In order to avoid inclusion of historical studies reported over this period that would not meet our core objective of studies conducted in mature HIV epidemics between ≥1990; (2) Due to delay occasioned by research fieldwork; and (3) To leverage on the delay to increase probability of obtaining eligible articles.
3) To reduce chances of missing eligible articles we supplemented our search by searching reference lists of 'all full–text articles screened' and not just reference lists of 'selected articles' as published in the protocol.
4) We used 'Endnote' software instead of 'RefWorks' bibliographic management software. Endnote was found to be superior to 'RefWorks' in systematic review bibliographic management.
5) We used time–series modelling and linear regression to estimate time–adjusted adolescent TB disease prevalence rates for 2010 for the South African studies. Using these estimates, we compared with the published adolescent TB prevalence rates in the studies conducted in 2010 in Kenya and Uganda. This was done by assuming adolescent TB trends followed a pattern similar to the corresponding general population TB trends. We assumed the rate of change (i.e. gradient) for adolescent TB rates was the same as that of the line obtained by linear regression of WHO–reported general population TB rates for 1999–2010, a finding we observed for South African regional data (chapter 3).[24].
6) Styblo *et al.*[10] did seminal work on infection–to–disease ratios of tuberculosis by calculating the ratio between the number of tuberculous infections (based on ARTI estimates) and the number

of smear–positive TB disease cases per year and obtained a ratio of 8–12. We calculated the ratio between the number of *Mycobacterium tuberculosis* infections based on Annual Risk of TB Infection (ARTI) estimates and the number of smear– and culture–positive TB disease cases per year for the same study population; and compared the observed ratio to the expected ratio of 8–12 published by Styblo *et al.*[10] We used 8–12 as a benchmark for description purposes and not to indicate our agreement or disagreement with Styblo's rule. As in literature, this sub–analysis was only done for studies with infection and disease data for the same population. ARTI was calculated from LTBI prevalence using the formula $ARTI = 1 - (1 - Prevalence)^{1/(mean\ age+0.5)}$.[25] The number of TB infections per annum was obtained by multiplying the ARTI by the number of LTBI negative individuals in a given study. Reported sputum positive TB disease cases in the study were used as TB disease cases in our calculation, as in the literature.

7.6. Appendix 2.6

Results of quality assessment and evaluation of risk of bias of included studies

Author, year (Ref)	Outcome measure	External validity				Internal validity						Summary of risk of bias
		1	2	3	4	5	6	7	8	9	10	
Mahomed, 2011[2]	LTBI											Moderate
Middelkoop, 2014[1]	LTBI											Moderate
Mumpe–Mwanja, 2015[5]	LTBI											Moderate
Marais, 2005[3]	LTBI, TB											Low
Nduba, 2015[4]	LTBI, TB											Low
Mahomed, 2013[7]	TB											Moderate
Snow, 2017[8]	TB											High
Waako, 2013[5]	TB											Moderate

Legend: TB=Tuberculosis; LTBI=Latent Tuberculosis Infection. **Green box**=score of 1 on Hoy *et al.* quality assessment tool for prevalence studies (i.e. low risk of bias); **Red box**=score of 0 on Hoy *et al.*[26] quality assessment tool for prevalence studies (i.e. high risk of bias). Numbered items in 2nd row refer to items listed below obtained from Hoy *et al.*[26] quality assessment tool;

External Validity

1. Was the study's target population a close representation of the national population in relation to relevant variables?
2. Was the sampling frame a true or close representation of the target population?
3. Was some form of random selection used to select the sample, OR was a census undertaken?
4. Was the likelihood of non–response bias minimal?

Internal validity

5. Were data collected directly from the participants (as opposed to a proxy)?

6. Was an acceptable case definition used in the study?

7. Was the study instrument that measured the parameter of interest shown to have validity and reliability?

8. Was the same mode of data collection used for all subjects?

9. Was the length of the shortest prevalence period for the parameter of interest appropriate?

10. Were the numerator(s) and denominator(s) for the parameter of interest appropriate?

Summary item interpretation: Summary item on the overall risk of study bias was scored as low, moderate or high for risk of bias. Low=Low risk of bias i.e. Further research is very unlikely to change our confidence in the estimate; Moderate=Moderate risk of bias i.e. Further research is likely to have an important impact on our confidence in the estimate and may change the estimate; High=High risk of bias i.e. Further research is very likely to have an important impact on our confidence in the estimate and is likely to change the estimate.

Narrative summary of quality assessment

Both LTBI and TB prevalence studies scored poorly on external validity items numbered (1) and (2) because the Hoy *et al.* tool for assessment of external validity gives weight to national surveys with sampling frames that include all households in the country of study conduct or that perform a census. Such studies are often costly and resource intense and are rarely commissioned by national TB control programs. This quality assessment highlights limitation of community surveys in determining country–level adolescent TB burden and the need for more research on adolescent LTBI and TB prevalence or revised reporting by surveillance systems or surveys to profile adolescent TB burden for monitoring.

Marais, 2005[3] and **Nduba**, 2015[4] *et al.* aspects of the criteria save the fact that they were not national in scope, otherwise the studies were rigorously designed meeting all other key assessment items.

Snow, 2017[8] study was rated as high risk of bias because by nature passive surveillance likely grossly underestimates TB incidence and the fact that all items on the Hoy *et al.*'s external validity scoring had an unfavorable score.

Waako, 2013[5] and **Mumpe–Mwanja**, 2015[5]: these two are research outputs from the same study with two publications, for LTBI and for TB prevalence. The main reason for classification as 'moderate' on overall risk of bias is that sample selection was by a convenience sample, no random sample selection.

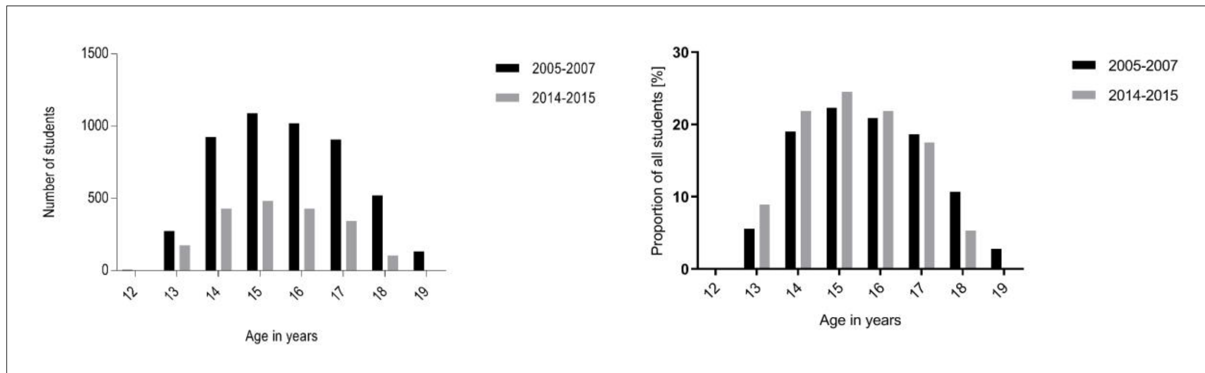
Mahomed, 2011[2] and **Mahomed**, 2013[7]: these two are research outputs from the same study with two publications, for LTBI and for TB prevalence. The main reason for classification as 'moderate' on overall risk of bias is that sample selection was by a convenience sample and only conducted in schools, thus no random sample selection and non–school–going adolescents are not represented. **Middelkoop**, 2014[1], similar to Mahomed *et al.*, used a convenience sample of nearly schools, thus no random sample selection and non–school–going adolescents are not represented.

References

See reference list for chapter 2

7.7. Appendix 3.1

Distribution of study participants by age



Legend: There were only 5 and 0 study participants aged 12 years in 2005–2007 and 2014–2015 respectively. There were 134 and 0 study participants aged 19 years in 2005–2007 and 2014–2015 respectively. Study participants were aged between 12.3 and 19.1 years.

7.8. Appendix 3.2

Prevalence of latent TB infection **by school and quintile category among high school students in Worcester**

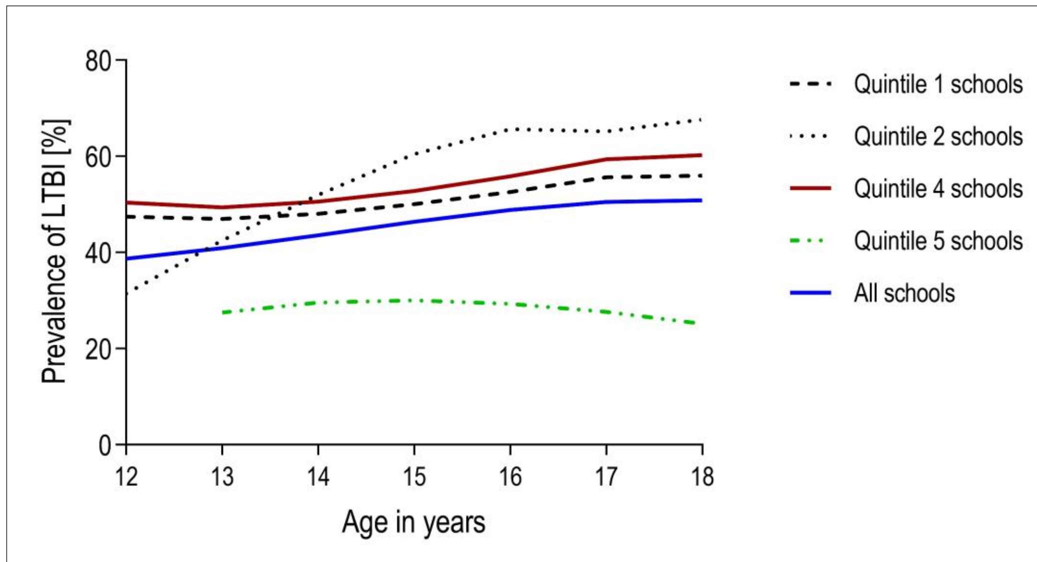
Variable	Prevalence for 2005 – 2007 {n/N (%; CI)}	Prevalence for 2014 – 2015 {n/N (%; CI)}	Prevalence difference (PD) (%; CI)	P–value for prevalence difference	Prevalence for both periods {n/N (%; CI)}
School					
A	740/1413 (52.4%; 49.8 – 55.0)	251/452 (55.5%; 50.9 – 60.1)	3.2% (–2.1; 8.4)	0.241	991/1865 (53.9%; 51.6 – 56.3)
B	763/1181 (64.6%; 61.9 – 67.3)	220/399 (55.1%; 50.3 – 60.0)	–9.5% (–15.1; –3.9)	0.001*	983/1580 (59.9%; 52.8 – 67.0)
C	444/867 (51.2%; 47.9 – 54.5)	218/427 (51.2%; 46.3 – 55.8)	0% (–5.9; 5.6)	0.958	662/1294 (51.1%; 51.0 – 51.2)
D	338/678 (49.9%; 46.1 – 53.6)	262/512 (51.2%; 46.8 – 55.5)	1.3% (–4.4; 7.1)	0.652	600/1190 (50.5%; 49.5 – 51.5)
E	50/261 (19.2%; 14.4 – 23.9)	38/94 (40.4%; 30.5 – 50.3)	21.3% (10.3; 32.3)	<0.001*	88/355 (29.8%; 13.9 – 45.7)
F	46/215 (21.4%; 15.9 – 26.9)	8/20 (40.0%; 18.5 – 61.5)	18.6% (–3.6; 40.8)	0.059	54/235 (30.7%; 16.8 – 44.6)
G	23/192 (12.0%; 7.4 – 16.6)	6/21 (28.6%; 9.2 – 47.9)	16.6% (–3.3; 36.5)	0.035*	29/213 (20.3%; 7.9 – 32.7)
H	30/73 (41.1%; 29.8; 52.4)	22/43 (51.2%; 36.2; 66.1)	10.1% (–8.7; 28.8)	0.292	52/116 (46.0%; 38.5 – 53.5)
Quintile of school					

1	338/678 (49.9%; …)	262/512 (51.2%; …, …)	1.3% (–4.4, 7.1)	0.652	600/1190 (50.5%; …)
2	763/1181 (64.6%; …)	220/399 (55.1%; …)	–9.5% (–15.1, –3.9)	<0.001	983/1580 (59.9%; …)
3	…	…	…	…	…
4	1184/2280 (51.8%; 50.8, 52.8)	469/879 (53.3%; 49.3, 57.3)	1.5% (–2.5, 5.3)	0.472	1653/3159 (52.6%; 50.0, 55.1)
5	149/741 (20.4%; 11.8, 32.8)	74/178 (38.0%; 29.0, 47.9)	17.6% (13.7, 29.3)	<0.001	223/919 (29.2%; 20.4, 39.8)

Legend: *=statistically significant; **=insufficient data for comparison of proportions; …***=insufficient data; NA=not applicable e.g. because the study was not conducted over this period; HS=high school; SES=Socioeconomic status. ****=Classification into low and high SES of the school was obtained from the National Department of Basic Education.

7.9. Appendix 3.3

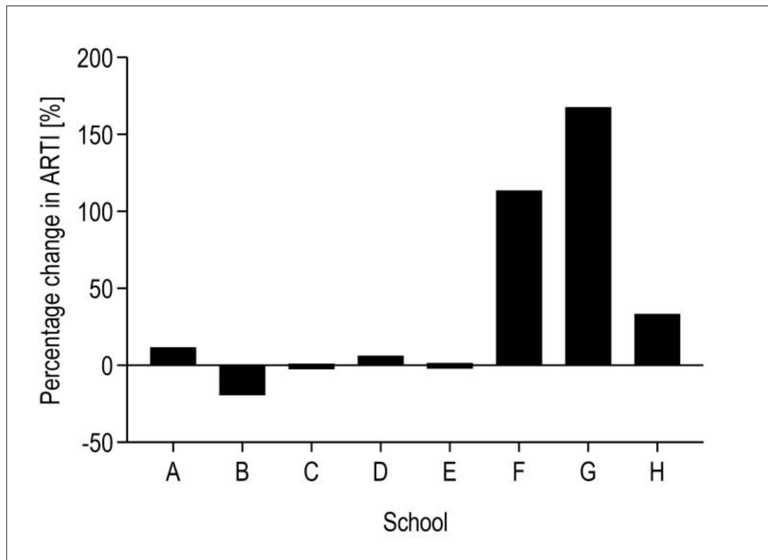
Changes in prevalence of LTBI by socioeconomic quintile category of the school



Legend: LTBI=Latent Tuberculosis Infection. There was no quintile 3 school in our study population. We observed a significant direct relationship between age and prevalence of LTBI for quintile 1 (r_s= 0.100; p–value <0.001), quintile 4 (r_s= 0.099; p–value <0.001) and quintile 5 (r_s= 0.077; p–value <0.019) schools but not for quintile 2 schools (r_s= 0.044; p–value=0.078).

7.10. Appendix 3.4

Percentage change in Annual risk of Tuberculosis Infection **across the two periods by school**



Legend: ARTI= Annual risk of Tuberculosis Infection. This figure shows the change in ARTI in 2014–2015 as compared to 2005–2007 and demonstrates that ARTI substantially increased in schools F, G and H and substantially reduced in school B.

7.11. Appendix 3.5

Annual Risk of Tuberculosis Infection **by sociodemographic characteristics**

Variable	**ARTI for 2005 – 2007 (%)**	**ARTI for 2014 – 2015 (%)**	**Overall ARTI (%)**
All participants	3.60%	4.23%	3.89%
Sex			
Male	4.10%	4.10%	4.08%
Female	4.24%	4.34%	4.26%
Race/Ethnic group			
Black	4.81%	4.50%	4.68%
Mixed Race	4.97%	4.24%	4.58%
White	0.67%	1.86%	0.79%
Asians/Indians	1.45%	1.74%	1.54%
Grade			
8	4.21%	6.80%	5.21%
9	4.44%	4.06%	4.26%
10	4.50%	4.23%	4.35%
11	3.98%	2.96%	3.45%
12	2.60%	0.13%	1.60%
Age			
12	5.05%	3.00%	3.75%
13	3.32%	3.69%	3.53%
14	3.55%	4.14%	3.86%
15	3.71%	4.11%	3.91%
16	3.75%	5.09%	4.39%
17	3.43%	4.37%	3.85%

18	3.73%	…	…
19	…	…	…
School			
A	4.66%	5.21%	4.90%
B	6.29%	5.07%	5.60%
C	4.51%	4.56%	4.52%
D	4.28%	4.55%	4.44%
E	1.34%	3.38%	2.25%
F	1.53%	3.27%	2.32%
G	0.81%	2.17%	1.44%
H	3.31%	4.42%	3.83%

Legend: ARTI= Annual risk of Tuberculosis Infection.

7.12. Appendix 4.1

TB disease case notifications by age and year in Breede Valley subdistrict

Age (yrs)	2005 N (%)	2006 N (%)	2007 N (%)	2008 N (%)	2009 N (%)	2010 N (%)	2011 N (%)	2012 N (%)	2013 N (%)	2014 N (%)	2015 N (%)	Total N (%)
0–4	618 (26.0%)	536 (24.9%)	453 (23.1%)	301 (16.8%)	275 (16.9%)	400 (22.0%)	436 (22.6%)	439 (24.9%)	440 (24.5%)	788 (38.5%)	716 (36.0%)	5,402 (25.4%)
5–9	185 (7.8%)	107 (5.0)	60 (3.1%)	62 (3.5%)	32 (2.0%)	77 (4.2%)	84 (4.4%)	74 (4.2%)	65 (3.6%)	83 (4.1%)	58 (2.9%)	887 (4.2%)
10–14	53 (2.2%)	42 (2.0%)	32 (1.6%)	25 (1.4%)	19 (1.2%)	28 (1.5%)	40 (2.1%)	29 (1.6%)	35 (2.0%)	26 (1.3%)	24 (1.2%)	353 (1.6%)
15–19	134 (5.7%)	119 (5.5%)	114 (5.8%)	110 (6.2%)	83 (5.1%)	101 (5.6%)	81 (4.2%)	89 (5.1%)	97 (5.4%)	88 (4.3%)	92 (4.6%)	1,108 (5.2%)
20–24	185 (7.8%)	168 (7.8%)	158 (8.1%)	162 (9.1%)	134 (8.2%)	149 (8.2%)	161 (8.4%)	153 (8.7%)	143 (7.9%)	128 (6.3%)	138 (6.9%)	1,679 (7.9%)
25–29	220 (9.3%)	196 (9.1%)	216 (11.0%)	196 (11.0%)	190 (11.6%)	183 (10.1%)	157 (8.1%)	174 (9.9%)	156 (8.7%)	142 (6.9%)	160 (8.1%)	1,990 (9.4%)

30–34	228 (9.6%)	234 (10.9%)	200 (10.2%)	179 (10.0%)	187 (11.5%)	223 (12.3%)	188 (9.8%)	189 (10.7%)	188 (10.5%)	165 (8.1%)	174 (8.8%)	2,155 (10.1%)
35–39	207 (8.7%)	196 (9.1%)	200 (10.2%)	230 (12.9%)	197 (12.1%)	179 (9.8%)	199 (10.3%)	170 (9.6%)	165 (9.2%)	152 (7.4%)	133 (6.7%)	2,028 (9.5%)
40–44	203 (8.6%)	187 (8.7%)	164 (8.4%)	182 (10.2%)	162 (9.9%)	156 (8.6%)	187 (9.7%)	142 (8.1%)	156 (8.7%)	164 (8.0%)	151 (7.6%)	1,854 (8.7%)
45–49	122 (5.1%)	158 (7.3%)	139 (7.1%)	133 (7.4%)	159 (9.8%)	139 (7.6%)	159 (8.3%)	115 (6.5%)	132 (7.3%)	109 (5.3%)	127 (6.4%)	1,492 (7.0%)
50–54	104 (4.4%)	98 (4.6%)	88 (4.5%)	87 (4.9%)	87 (5.3%)	72 (4.0%)	102 (5.3%)	73 (4.2%)	99 (5.5%)	95 (4.6%)	92 (4.6%)	997 (4.7%)
55–59	45 (1.9%)	57 (2.7%)	65 (3.3%)	55 (3.1%)	54 (3.3%)	50 (2.8%)	54 (2.8%)	57 (3.2%)	61 (3.4%)	47 (2.3%)	52 (2.6%)	597 (2.8%)
60–64	33 (1.4%)	33 (1.5%)	33 (1.7%)	27 (1.5%)	21 (1.3%)	31 (1.7%)	41 (2.1%)	32 (1.8%)	32 (1.8%)	33 (1.6%)	40 (2.0%)	356 (1.7%)
>65	35 (1.5%)	24 (1.1%)	41 (2.1%)	39 (2.2%)	31 (1.9%)	33 (1.8%)	38 (2.0%)	25 (1.4%)	29 (1.6%)	27 (1.3%)	30 (1.5%)	352 (1.7%)

Total	2372 (100%)	2155 (100%)	1963 (100%)	1788 (100%)	1631 (100%)	1821 (100%)	1927 (100%)	1761 (100%)	1798 (100%)	2047 (100%)	1987 (100%)	21,250 (100%)

Legend: % is number of notified TB disease cases/total number of notified TB disease cases in specified year.

7.13. Appendix 4.2

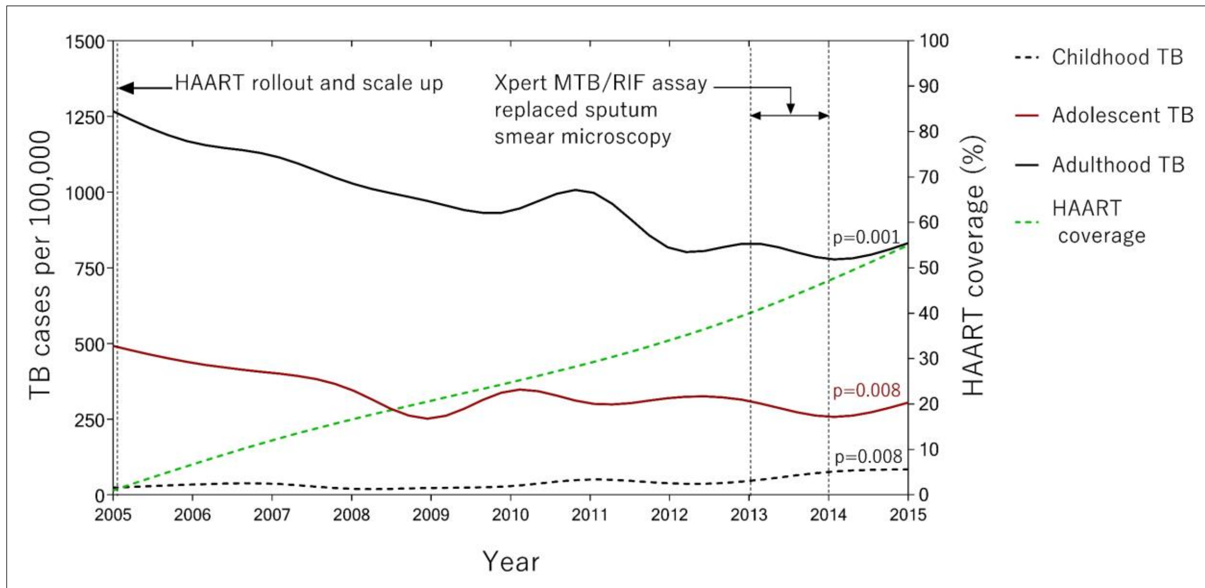
Temporal trends in TB disease case notification rate by age

Age	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	P–value
0–4	3979	3406	2842	1864	1682	2416	2601	2585	2557	4521	4055	0.876
5–9	1353	773	428	436	222	528	569	495	429	541	373	0.276
10–14	379	297	223	172	129	188	265	190	226	166	151	0.087
15–19	938	822	777	740	552	663	525	570	613	549	566	0.008
20–24	1309	1173	1089	1102	900	989	1055	990	913	807	859	0.003
25–29	1574	1384	1505	1349	1291	1228	1041	1138	1007	905	1007	<0.001
30–34	1984	2009	1695	1498	1545	1820	1515	1504	1476	1279	1331	0.003
35–39	1851	1729	1742	1978	1673	1501	1648	1390	1332	1211	1046	<0.001
40–44	1867	1697	1469	1610	1415	1346	1593	1194	1295	1344	1221	0.003
45–49	1403	1793	1557	1471	1736	1499	1694	1209	1370	1117	1284	0.062
50–54	1312	1220	1082	1056	1043	852	1192	842	1128	1068	1023	0.062
55–59	743	928	1045	873	846	774	826	860	909	691	755	0.275
60–64	708	699	690	557	428	624	815	628	620	631	755	1.000
≥65	433	293	494	464	364	383	435	283	324	298	326	0.213
All	1535	1376	1237	1113	1002	1105	1155	1042	1050	1180	1131	0.161

Legend: TB=Tuberculosis; TB disease case notification rate is per 100,000 for all notified cases of TB in the respective age group. The *Mann–Kendall test* was used to test for trend. "All" refer to all TB disease cases in the general population.

7.14. Appendix 4.3

Temporal trends in adolescent microbiologically confirmed TB disease case notification rate



Legend: ART coverage= The proportion of persons living with HIV receiving antiretroviral therapy; **TB**=Tuberculosis disease. The figure shows that between 2005–2015, there was a significant negative temporal trend in microbiologically–confirmed TB among adolescents (TB rates fell 38% (from 492 to 305 per 100,000)) and adults (TB rates fell 34% (from 1267 to 832 per 100,000)), but a marked increase in childhood microbiologically–confirmed TB rates (TB rates rose 250% (from 24 to 84 per 100,000)). The proportion of all persons living with HIV in the general population on ART was <1% in 2005, 12% in 2007, 40% in 2013 and 55% in 2015.[9] P–value is for temporal trend between 2005–2015.

7.15. Appendix 4.4

TB disease case notifications in the general population by age, HIV status and laboratory test result status in 2015

Age–group	**All TB disease cases**					**HIV negative TB**			**HIV positive TB**			**TB with unknown HIV status**		
	All cases1	New cases[2]	lab confirmed[3]	HIV–4	HIV+5	All cases	New cases	lab confirmed	All cases	New cases	lab confirmed	All cases	New cases	lab confirmed
0–4	716 (36%)	703 (98%)	17 (2%)	516 (86%)	87 (14%)	516 (50%)	513 (99%)	11 (2%)	87 (12%)	82 (94%)	3 (3%)	113 (52%)	108 (96%)	3 (3%)
5–9	58 (3%)	56 (97%)	11 (19%)	47 (87%)	7 (13%)	47 (5%)	47 (100%)	9 (19%)	7 (1%)	5 (71%)	0 (0%)	4 (2%)	4 (100%)	2 (50%)
10–14	24 (1%)	23 (96%)	15 (63%)	11 (52%)	10 (48%)	11 (1%)	10 (91%)	5 (45%)	10 (1%)	10 (100%)	7 (70%)	3 (2%)	3 (100%)	3 (100%)
15–19	92 (5%)	87 (96%)	83 (90%)	45 (56%)	36 (44%)	45 (4%)	42 (95%)	41 (91%)	36 (5%)	34 (94%)	33 (92%)	11 (5%)	11 (100%)	9 (82%)
20–24	138 (7%)	116 (84%)	117 (85%)	56 (47%)	64 (53%)	56 (5%)	49 (88%)	48 (84%)	64 (9%)	52 (81%)	57 (89%)	18 (8%)	15 (83%)	12 (67%)

25–29	160 (8%)	142 (89%)	136 (85%)	64 (43%)	85 (57%)	64 (6%)	56 (88%)	56 (88%)	85 (12%)	76 (89%)	69 (81%)	11 (5%)	10 (91%)	11 (100%)
30–34	174 (9%)	133 (76%)	137 (79%)	57 (34%)	109 (66%)	57 (6%)	46 (81%)	48 (84%)	109 (15%)	81 (74%)	84 (78%)	8 (4%)	6 (75%)	5 (63%)
35–39	133 (7%)	101 (77%)	108 (81%)	41 (33%)	82 (67%)	41 (4%)	32 (80%)	34 (83%)	82 (11%)	63 (78%)	65 (79%)	10 (5%)	6 (60%)	9 (90%)
40–44	151 (8%)	102 (68%)	129 (85%)	54 (38%)	89 (62%)	54 (5%)	39 (72%)	48 (89%)	89 (12%)	57 (64)	73 (82%)	8 (4%)	6 (75%)	8 (100%)
45–49	127 (6%)	86 (68%)	111 (87%)	52 (44%)	66 (56%)	52 (5%)	38 (73%)	47 (90%)	66 (9%)	44 (67%)	56 (85%)	9 (4%)	4 (44%)	8 (89%)
50–54	92 (5%)	66 (73%)	78 (85%)	32 (40%)	49 (60%)	32 (3%)	22 (69%)	28 (88%)	49 (7%)	35 (73%)	40 (82%)	11 (5%)	9 (82%)	10 (91%)
55–59	52 (2%)	38 (73%)	45 (87%)	26 (51%)	25 (49%)	26 (3%)	19 (73%)	23 (8%)	25 (3%)	19 (76%)	21 (84%)	1 (<1%)	0 (0%)	1 (100%)
60–64	40 (2%)	31 (78%)	34 (85%)	18 (51%)	17 (49%)	18 (2%)	12 (67%)	17 (94%)	17 (2%)	14 (82%)	14 (82%)	5 (2%)	5 (100%)	3 (60%)
≥65	30	21	24	17	7	17	14	15	7	6	4	6	1	5

	(1%)	(70%)	(80%)	(71%)	(29%)	(2%)	(82%)	(88%)	(1%)	(86%)	(57%)	(3%)	(17%)	(83%)
All	1987 (100%)	1,705 (86%)	1,045 (53%)	1,036 (59%)	733 (41%)	1036 (100%)	939 (91%)	430 (42%)	733 (100%)	578 (79%)	526 (72%)	218 (100%)	188 (86%)	89 (41%)

Legend: Denominator; for 1=all notified TB disease cases in 2015; 2=new + relapse cases; 3=lab + non–lab confirmed; 4=HIV– + HIV+

7.16. Appendix 4.5

Overall TB disease case notification rate in the general population in Breede Valley subdistrict by year

Year	Notified TB disease cases	Population size	TB CNR (CI)	Annual change in CNR (%)	CNR for microbiologically confirmed TB (CI)	Proportion with known HIV status of notified TB disease cases n/N (%)	Proportion HIV+ of notified TB disease cases with known HIV status n/N (%)
2005	2372	154565	1535 (1473–1598)	NA	890 (844–939)	6/2372 (0.3%)	4/6 (66.7%)
2006	2155	156611	1376 (1319–1435)	–10.4%	817 (773–863)	129/2155 (6.0%)	46/129 (35.7%)
2007	1963	158654	1237 (1183–1293)	–10.1%	785 (742–830)	456/1963 (23.2%)	124/456 (27.2%)
2008	1788	160701	1113 (1062–1165)	–10.0%	714 (673–756)	666/1788 (37.3%)	260/666 (39.0%)
2009	1631	162747	1002 (954–1052)	–10.0%	659 (620–699)	1025/1631 (62.8%)	389/1025 (38.0%)
2010	1821	164794	1105	10.3%	657	938/1821	366/938

<table>
<tr><td></td><td></td><td></td><td>(1055–1157)</td><td></td><td>(619–698)</td><td>(51.5%)</td><td>(39.0%)</td></tr>
<tr><td>2011</td><td>1927</td><td>166826</td><td>1155
(1104–1208)</td><td>4.5%</td><td>694
(655–735)</td><td>712/1927
(37.0%)</td><td>320/712
(44.9%)</td></tr>
<tr><td>2012</td><td>1761</td><td>169010</td><td>1042
(994–1092)</td><td>–9.8%</td><td>577
(542–615)</td><td>1308/1761
(74.3%)</td><td>385/1308
(29.4%)</td></tr>
<tr><td>2013</td><td>1798</td><td>171226</td><td>1050
(1002–1100)</td><td>0.8%</td><td>588
(552–625)</td><td>1550/1798
(86.2%)</td><td>431/1550
(27.8%)</td></tr>
<tr><td>2014</td><td>2047</td><td>173468</td><td>1180
(1129–1232)</td><td>12.4%</td><td>551
(517–587)</td><td>1774/2047
(86.7%)</td><td>436/1774
(24.6%)</td></tr>
<tr><td>2015</td><td>1987</td><td>175740</td><td>1131
(1081–1181)</td><td>–4.2%</td><td>595
(559–632)</td><td>1769/1987
(89.0%)</td><td>733/1769
(41.4%)</td></tr>
<tr><td>2005–2015
average</td><td>NA</td><td>NA</td><td>1246
(1018–1474)</td><td>NA</td><td>720
(504–936)</td><td>NA</td><td>NA</td></tr>
</table>

Legend: CI=95% confidence interval for rate per 100000; CNR=Case Notification Rate per 100000; Incidence rate is per 100000; NA=Not applicable; TB=Tuberculosis; n/N=. Note, patients 'transferred in' and 'moved in' are excluded in this calculation, but transfer out, moved out or relapses are included. TB disease notification rate in the general population fell by 26% from 1,535 (2005) to 1,131 (2015) per 100,000 per annum.

7.17. Appendix 4.6

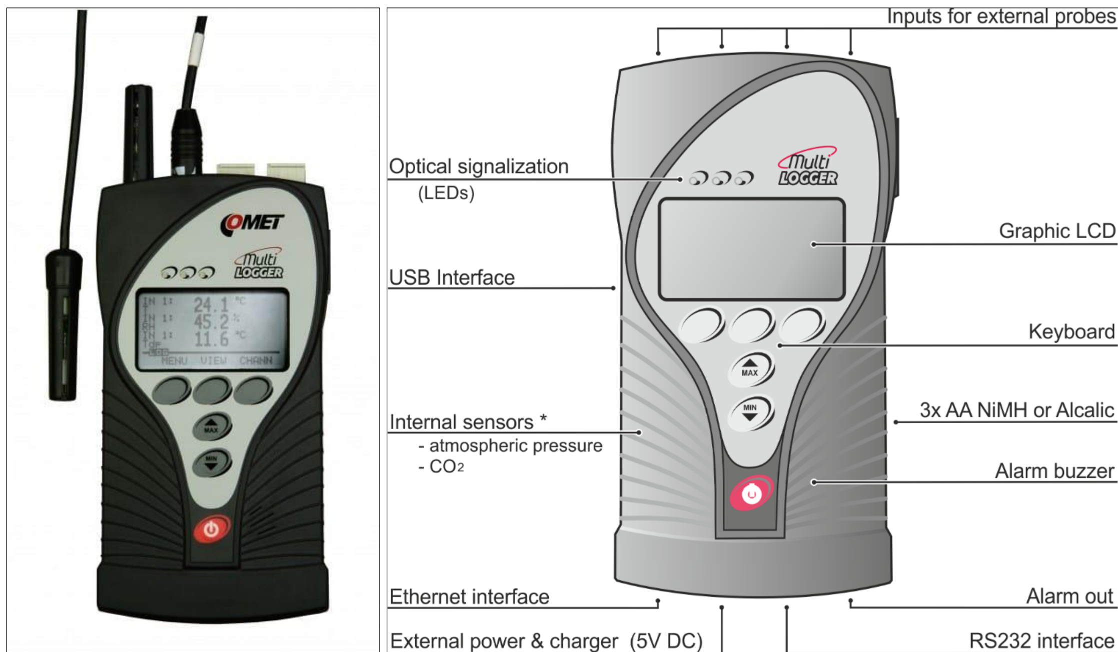
Adolescent TB disease case notification rate by sex

Year	All TB disease cases in males	All TB disease cases in females	TB rate in males	TB rate in females
2005	88	99	627	696
2006	69	92	485	639
2007	66	80	458	548
2008	55	80	377	541
2009	45	57	304	381
2010	70	59	467	389
2011	65	56	429	365
2012	46	72	300	463
2013	73	59	469	375
2014	57	57	362	357
2015	59	57	369	353
Average	63	73	425	496
p–value	NA	NA	0.087	<0.001

Legend: TB rates refer to adolescent TB disease case notification rate per 100,000 adolescents. All TB disease cases refer to the total number of notified TB disease cases in the regional electronic tuberculosis notification register.

7.18. Appendix 5.1

Carbon dioxide logger



Legend: Brief description of device and how it works

This device is a carbon dioxide logger (Ethernet Multilogger – thermo–hygro–carbon dioxide meter with 2 MiniDIN and 2 Terminals).[51] It measures 17.8 cm by 9.5 cm by 3.7 cm. It has an internal carbon dioxide sensor that uses non-dispersive infrared technology to sense carbon dioxide and measure carbon dioxide concentration in the air as a function of transmitted light. The device is designed for measuring and recording temperature, humidity, carbon dioxide concentration with adjustable recording intervals from 1 second to 24 hours. The internal carbon dioxide concentration sensor measures carbon dioxide concentration in the air with a range of 0 to 2,100 ppm with an accuracy of 50 ppm + 2% of the measured value.

7.19. Appendix 5.2

The Dry Filtration Unit

Brief description of The Dry Filtration Unit and how it works[42]

This device is a Dry Filtration Unit, a novel, portable, electricity–powered, ddPCR air sampling device that measures approximately 0.5 metres by 0.5 metres by 0.5 metres. The device works by filtering air through removable filters. Air is suctioned through an air inlet vent of approximately 10–centimetre diameter on the very top part of the chimney (the chimney is the black device on the top part of the Dry Filtration Unit with two white protruding "horns" (that serve as removable filter holders) on opposite ends/sides of the black protruding chimney). Air is suctioned via removable filters then out through an air outlet vent of approximately 5–centimetre diameter on the bottom–right side of the Dry Filtration Unit device. The Dry Filtration Unit device samples approximately 1,000 litres of air per minute. The removable filters within the "white horns" collect/trap particles from room air that may include any dust, bacteria or viruses onto the two filters in each of the two filter holders. After a period of ddPCR air sampling, the filters are extracted and transported to a laboratory for processing using sensitive analytical technologies such as Polymerase Chain Reaction for detection of bacteria and viruses and electrochemical luminescence for detection of toxins for routine samples.[42]

7.20. Appendix 5.3

Key socioeconomic and health indicators for Breede Valley local municipality[66]

Indicator	Estimate (2015, unless otherwise stated)
Demographic indicators	
General population size	174,198
Households	46,963
Annual growth rate	1.31%
Economic indicators	
Unemployment (2014)	25%
Per Capita Income (2013), US$	2,502
Households earning less than 38.6 p.m. (2011)	13.7%
Housing indicator	
Informal dwellings (2014)	21%
Education indicators	
Basic education enrolment (2014)	32,076
Average school dropout rate (2012)	40.9
Literacy Rate (2011)	82.1%
Health indicators	
HIV prevalence (2010)67	4.6%
ART patient load	4,584
Mother–to–child HIV transmission rate	1.2%
Number of ART clinics/treatment sites	8
Maternal Mortality Ratio (per 100 000 live births)	27.0
Full immunisation coverage for infants	86%

Legend: Pm=per month. **ART**=Antiretroviral therapy. Literacy rate is calculated as the proportion of individuals aged ≥14 years who have successfully completed ≥7 years of formal education. Basic education refers to enrolment in first 12 years of formal education.

7.21. Appendix 5.4

Number of times spaces were sampled (either ddPCR air sampling or ambient carbon dioxide concentration measurement)

Type of space	Clinic A	Clinic B	Clinic C	School B	School D	Open air space	Total
General patient waiting area	5	5	NA	NA	NA	NA	10
Open air space	NA	NA	NA	NA	NA	5	5
TB clinic consultation room	5	NA	9	NA	NA	NA	14
TB clinic patient waiting area	5	5	5	NA	NA	NA	15
Classroom	NA	NA	NA	32	40	NA	72

Legend: NA=Not applicable

7.22. Appendix 5.5

ddPCR assay analytical methodology

The ddPCR assay was performed by the Desmond Tutu HIV Centre[54] of the University of Cape Town in collaboration with the laboratory of Professor Digby Warner (University of Cape Town).

Sample preparation for ddPCR assay

Polycarbonate track etch filters were processed as follows prior to ddPCR assay.

1. Double–bagged sterile 50 ml falcon tubes containing polyester felt filter of 47mm diameter and 1.0µm pore size (American Felt and Filter Company, New Windsor, New York; Lockheed Martin, Alexandria, VA, USA) filters samples were retrieved from storage (2–4° Celsius).
2. The bags were then surface–sterilized, opened inside a level III Biosafety cabinet (BSC), and the falcon tubes placed in a rack.
3. 10 ml of cold 1 X phosphate buffered saline with 0.5% Tween80 PBS.T was pipetted into each tube. Each tube was securely closed and placed in a rack. These were placed in a secondary container, or bagged and left to soak overnight, at room temperature.
4. The lid was securely fastened, and vortexed twice for 1 minute (with a pause in between).
5. The filter was squeezed against the side of the tube with a sterile serological pipette tip, before being discarded into the non–liquid waste bag.
6. Falcon tubes were loaded into centrifuge buckets inside the BSC, closed, decontaminated then placed into the centrifuge and centrifuged at 4500 rpm for 15 minutes.
7. Transport centrifuge buckets containing tubes back to the BSC. Transfer the tubes onto an appropriate rack. Decontaminate the buckets and put back in the centrifuge.
8. Discard the supernatant by pipetting it out into a liquid waste bottle. Leaving behind the last drop of liquid; the volume of which usually amounts to ±100 ul. Resuspend the pellets in the remaining liquid.

9. Transfer the resuspended pellets into 2 ml tubes and heat–kill at 80˚C for 1 hour in a water bath.
10. Remove the tubes containing the heat–inactivated bacterial pellets from the water bath. Secured in an appropriate rack; allow the tubes to cool down to room temperature for 10 minutes.
11. Spray the rack with 70% Ethanol and place it in a bag. Spray the bag and place it at the door for removal from the BSL 3. In the anteroom, place the bag containing the rack with the tubes within a decontaminated transport box. Transport the heat inactivated pellets to the BSL 2 for DNA extraction and ddPCR.
12. Decontaminate the transport box again and return to the anteroom, in the BSL3.

ddPCR assay

1) Preparation of 22 µL PCR reactions.

Stored field samples at 4°C containing gDNA (where applicable), controls and reagents were thawed on ice or preheated to 37–42°C. A master mix was prepared in the PCR–clean room using the following mastermix reagents (components) from BioRad Laboratories, Inc (Denver, Colorado, USA)[56] for each reaction: 11 µl of 2X Super Mix, 0.75 µl of Forward Primer 10 (µM), 0.75 µl of Reverse Primer 10 (µM), 0.5 µl of Probe 10 (µM), 1 µl of AmpErase 0.055U/µL, 1 µl of HINDIII 5U/µl. All reactions were pipetted in duplicates. The controls were positive *Mycobacterium tuberculosis* (H37RV) and No Template Controls (NTC) comprised of the master mix, with no added template. Enough mastermix was prepared for at least 3 extra reactions, to compensate for possible pipetting errors and the extra mastermix required to make 22 µl of NTC. Mastermix reagents were mixed in clean 1.5–2 ml PCR tubes, vortexed and span down. 30 µL of mastermix was added to each well in every second column on a 96–well plate to ensure accurate duplication after DNA–addition. The 96–well plate was put into sterile sealable bag, UV light turned on, heating block turned on and samples taken from DNA extraction room and moved to DNA–addition hood. 14 µL of DNA was added to each well containing mastermix. The sample was then mixed well, and duplicates created by pipetting 22 µL of PCR–mixture to adjacent, empty wells (using multichannel

pipette). The 96–well plate was sealed using the PX1 PCR Plate sealer and the pierceable foil (at 180℃ for 4 seconds). The 96–well plate was placed on ice, vortexed and briefly centrifuged using a plate spinner.

2) Generation of droplets

Bleach (using lint–free cloths) and ethanol (using alcohol wet wipes) were used to clean the interior of the Auto Droplet Generator (AutoDG) at least once a month. The following items were loaded; consumables, cartridges, tips, waste bin, and a new 96–well–plate on ice block (freeze for at least 2 hours at –20˚C prior to droplet generation) and samples. Correct loading was marked by color change from amber to green. The Auto DG was then run for droplet generation. The PCR plate containing droplets was sealed using the PX1 PCR plate sealer

3) Running ddPCR

The dd_UNG (at 40 µl) program on the Thermal Cycler was chosen. The following PCR program was followed sequentially: 50 °C for 2 min (incubation for Amperase); 95 °C for 10 minutes; 94 °C for 30 seconds; 60 °C for 1 minute; repeat 94 °C for 30 sec 40 times; 98 °C for 10 minutes; hold at 4 °C for between 5 hours to 24 hours before reading the plate after thermal cycling.

4) Reading fluorescence amplitude from ddPCR

The QX100 BioRad Droplet Reader was turned on for 30 minutes prior to PCR reading. The setting on the Auto DG was set to: Experiment – ABS (absolute quantification); Super Mix – (ddPCR Supermix for Probes); Target 1: Name – RD 9; Type – Ch1 Unknown; Target 2: Type: Unused; Save and run the experiment; Choose Fam/Vic (dyes) in the pop–up window. The QX100 BioRad Droplet Reader was run to read amplitude of droplets generated.

Quantification of *Mycobacterium Tuberculosis* DNA

The starting concentration of each target DNA molecule was calculated by modeling as a Poisson distribution; the formula used for Poisson modeling is; Copies per droplet=–ln(1–p) where p=fraction of positive droplets (units/ul).

7.23. Appendix 5.6

Duration of sampling by type of space

Type of space	n	Duration of sampling median (IQR)	Duration of sampling min, max
General patient waiting area	10 (8.8%)	86 (40, 120)	20, 300
Open air space	5 (4.4%)	81 (40, 120)	20, 300
TB clinic consultation room	13 (11.5%)	82 (39, 117)	15, 301
TB clinic patient waiting area	15 (13.3%)	82 (40, 122)	20, 306
Classroom	70 (61.9%)	40 (35, 54)	15, 149
Overall	113 (100%)	45 (35, 82)	15, 306

Legend: Duration of sampling is in minutes; n=number of times the space was sampled for classroom ambient carbon dioxide concentration measurement. 2 classrooms did not have classroom ambient carbon dioxide concentration measurements; min=minimum; max=maximum

7.24. Appendix 5.7

Number of people in room by type of space

Type of space	Number of people in room at start of sampling Median (IQR)	Number of people in room at end of sampling Median (IQR)
General patient waiting area	42 (27, 45)	20 (5, 24)
Open air space	0 (0, 0)	0 (0, 0)
TB clinic consultation room	3 (3,5)	3 (2,5)
TB clinic patient waiting area	5 (2,7)	5 (1,11)
Classroom	34 (29, 39)	34 (29, 38)

Legend: IQR=Interquartile range.

7.25. Appendix 5.8

Distribution of windows by type of space

Type of space	Number of windows Median (IQR)	Number of windows open at start of sampling Median (IQR)	Number of windows open at end of sampling Median (IQR)
General patient waiting area	12.5 (10, 17)	7.5 (6, 13)	7 (6, 13)
Open air space	NA	NA	NA
TB clinic consultation room	2 (2,2)	0 (0,2)	0 (0,2)
TB clinic patient waiting area	2 (0,8)	2 (0,6)	2 (0,6)
Classroom	7 (5, 7)	2 (0.5, 3)	2 (0, 3)

7.26. Appendix 5.9

Proportion of windows open by type of space

Type of space	Total number of windows* N	Proportion of windows open at start of sampling n (%)	Proportion of windows open at end of sampling n (%)
General patient waiting area	125	86 (69%)	82 (66%)
Open air space	NA	NA	NA
TB clinic consultation room	24	11 (46%)	9 (38%)
TB clinic patient waiting area	36	32 (89%)	31 (86%)
Classroom	501	137 (27%)	132 (26%)
Total	706	275 (39%)	258 (37%)

Legend: *=For clinic spaces, windows were counted for every instance of repeat sampling in the same space.

7.27. Appendix 5.10

Distribution of doors and proportion of doors open by type of space

Season (weather)	**Month**	**Average daytime temperature (Degrees Celsius)**	**Proportion of all available windows open in classrooms n/N (%)**	**Proportion of all available windows open in clinic spaces n/N (%)**	**carbon dioxide concentration Median (IQR)**
Summer	February	29	NA	25/35 (71%)	543 (392, 583)
Fall	March	27	18/24 (75%)	NA	835 (751, 893)
	April	25	18/79 (23%)	NA	933 (774, 1321)
	May	22	17/135 (13%)	NA	872 (761, 1448)
Winter	July	20	5/27 (19%)	NA	1297 (1235, 2100)
	August	20	40/110 (36%)	NA	860 (679, 1114)
Spring	September	21	39/126 (31%)	NA	935 (813, 1161)
	November	25	NA	104/150 (69%)	467 (408, 589)

Legend: NA=No sampling took place. No sampling occurred in January, June, October and December. Weather seasons in South Africa are approximately Summer (1 December – 28/29 February), Winter (1 June – 31 August), Spring (1 September – 30 November), Autumn/Fall (1 March – 31 May).

7.28. Appendix 5.11

Distribution of doors and proportion of doors open by type of space

Type of space	Number of doors per space* N	Total number of doors for all instances of sampling * N	Proportion of doors open at start of sampling n (%)	Proportion of doors open at end of sampling n (%)
General patient waiting area	4.5 (2,5)	51	13 (24%)	10 (20%)
Open air space	NA	NA	NA	NA
TB clinic consultation room	1 (1, 2)	27	5 (19%)	5 (19%)
TB clinic patient waiting area	4 (3, 9)	56	12 (21%)	12 (21%)
Classroom	1 (1, 1)	121	39 (54%)	34 (47%)
Total	1 (1, 2)	263	72 (27%)	63 (24%)

Legend: *=For clinic spaces, doors were counted for every instance of repeat sampling in the same space. Data on the volume of each space is not available.

7.29. Appendix 5.12

Summary of shape of distribution (skewness) of carbon dioxide values

Distribution of carbon dioxide values	Clinic	Classroom
High negative skew	4 (19%)	8 (12%)
High positive skew	10 (47%)	13 (21%)
Moderate negative skew	1 (5%)	13 (21%)
Moderate positive skew	4 (19%)	5 (8%)
Symmetrical	2 (10%)	24 (38%)
Total	21 (100%)	63 (100%)

7.30. Appendix 5.13

Distribution of carbon dioxide concentration by episode of sampling

Space ID	Type of space	Median carbon dioxide	Nadir carbon dioxide	Peak carbon dioxide	Kurtosis	Skewness	Distribution/ skew
WORC–093	classroom	2100	1929	2100	21.03	–4.35	High Neg
WORC–079	classroom	2100	1375	2100	6.34	–2.11	High Neg
WORC–056	clinic space	555	447	591	7.66	–1.86	High Neg
WORC–122	classroom	984	753	1020	5.46	–1.8	High Neg
WORC–052	clinic space	619	392	702	6.53	–1.73	High Neg
WORC–071	classroom	2034	1488	2100	4.42	–1.65	High Neg
WORC–118	classroom	906	813	938	5.05	–1.4	High Neg
WORC–066	classroom	1162	664	1250	3.69	–1.37	High Neg
WORC–050	clinic space	616	491	671	4.01	–1.17	High Neg
WORC–057	clinic space	584	390	676	3.4	–1.11	High Neg
WORC–091	classroom	1242	816	1320	2.7	–1.1	High Neg
WORC–102	classroom	2100	572	2100	2.39	–1.01	High Neg
WORC–008	clinic space	408	384	526	3.6	1.04	High Pos
WORC–074	classroom	761	690	904	6.82	1.2	High Pos
WORC–010	clinic space	411	381	524	3.64	1.22	High Pos
WORC–076	classroom	595	524	821	3.98	1.26	High Pos
WORC–009	clinic space	595	437	1128	5.25	1.26	High Pos
WORC–126	classroom	788	679	1210	4.26	1.32	High Pos
WORC–108	classroom	638	585	969	3.79	1.32	High Pos
WORC–124	classroom	816	792	1167	4.01	1.46	High Pos

WORC–073	classroom	1452	1243	2100	5.24	1.68	High Pos
WORC–095	classroom	753	650	1131	6.19	1.83	High Pos
WORC–020	clinic space	459	408	640	12	1.85	High Pos
WORC–007	clinic space	591	391	1085	16.68	1.97	High Pos
WORC–098	classroom	1018	915	1320	8.54	2.02	High Pos
WORC–083	classroom	838	775	1282	7.06	2.11	High Pos
WORC–018	clinic space	379	368	515	10.19	2.24	High Pos
WORC–053	clinic space	385	372	616	7.61	2.35	High Pos
WORC–120	classroom	554	532	626	9.12	2.35	High Pos
WORC–101	classroom	761	651	1350	9.04	2.39	High Pos
WORC–014	clinic space	400	389	434	10.38	2.44	High Pos
WORC–077	classroom	617	566	919	10.48	2.58	High Pos
WORC–086	classroom	759	605	1303	11.7	2.95	High Pos
WORC–012	clinic space	384	377	562	16.6	3.82	High Pos
WORC–017	clinic space	379	372	484	25.72	4.48	High Pos
WORC–096	classroom	994	659	1127	2.54	–0.92	Mod Neg
WORC–080	classroom	2080	1191	2100	2.23	–0.9	Mod Neg
WORC–117	classroom	2100	983	2100	2.19	–0.89	Mod Neg
WORC–107	classroom	1097	895	1179	2.73	–0.83	Mod Neg
WORC–111	classroom	945	731	992	2.18	–0.8	Mod Neg
WORC–062	classroom	815	501	1013	2.84	–0.67	Mod Neg
WORC–110	classroom	1447	973	1615	2.18	–0.66	Mod Neg
WORC–059	clinic space	556	439	623	3.57	–0.64	Mod Neg
WORC–106	classroom	699	647	714	2.07	–0.61	Mod Neg
WORC–099	classroom	1814.5	654	2100	1.79	–0.6	Mod Neg
WORC–119	classroom	1254	705	1458	2.01	–0.6	Mod Neg

WORC–075	classroom	1003	701	1114	1.77	–0.55	Mod Neg
WORC–092	classroom	1189.5	577	1449	1.91	–0.55	Mod Neg
WORC–085	classroom	856	566	947	2.54	–0.54	Mod Neg
WORC–081	classroom	1383	887	2100	3.5	0.52	Mod Pos
WORC–013	clinic space	682	519	1026	2.54	0.62	Mod Pos
WORC–025	clinic space	615	548	777	2.48	0.62	Mod Pos
WORC–065	classroom	1327	1288	1384	2.15	0.63	Mod Pos
WORC–072	classroom	835	641	1435	1.96	0.67	Mod Pos
WORC–115	classroom	925	855	1269	1.8	0.71	Mod Pos
WORC–089	classroom	861	818	978	3.14	0.8	Mod Pos
WORC–027	clinic space	557	422	810	3.15	0.83	Mod Pos
WORC–023	clinic space	444	408	528	4.97	0.87	Mod Pos
WORC–112	classroom	829	603	867	1.58	–0.5	Symmetrical
WORC–088	classroom	1313	425	1944	2.71	–0.49	Symmetrical
WORC–103	classroom	1699	731	2100	1.79	–0.46	Symmetrical
WORC–067	classroom	1290	970	1472	3.31	–0.46	Symmetrical
WORC–113	classroom	1064	648	1202	1.71	–0.45	Symmetrical
WORC–078	classroom	1688	991	2100	1.83	–0.44	Symmetrical
WORC–116	classroom	807	651	906	2.01	–0.41	Symmetrical
WORC–097	classroom	597.5	553	629	2.27	–0.33	Symmetrical
WORC–121	classroom	993	627	1206	1.86	–0.31	Symmetrical
WORC–082	classroom	822	750	882	2.98	–0.28	Symmetrical
WORC–104	classroom	694	609	764	3.15	–0.28	Symmetrical
WORC–125	classroom	871	821	902	2.15	–0.16	Symmetrical
WORC–109	classroom	637	594	669	1.58	–0.12	Symmetrical
WORC–069	classroom	585	529	638	1.84	–0.12	Symmetrical

WORC–064	classroom	847	532	1128	2.04	0.01	Symmetrical
WORC–100	classroom	867	758	964	1.97	0.03	Symmetrical
WORC–114	classroom	1257	564	2100	1.7	0.09	Symmetrical
WORC–068	classroom	842	745	967	2.8	0.17	Symmetrical
WORC–084	classroom	768	713	850	2.89	0.17	Symmetrical
WORC–070	classroom	816	672	978	2.72	0.2	Symmetrical
WORC–094	classroom	619	575	668	2.25	0.21	Symmetrical
WORC–030	clinic space	582	445	787	2.09	0.24	Symmetrical
WORC–063	classroom	874	820	946	2.11	0.26	Symmetrical
WORC–105	classroom	874	572	1282	3.19	0.32	Symmetrical
WORC–011	clinic space	657	522	861	2.52	0.38	Symmetrical
WORC–123	classroom	1018	865	1218	2.03	0.42	Symmetrical

Legend: Mod=Moderate. Pos=positive. Neg=Negative. Carbon dioxide concentration is in parts per million (ppm). Notes on interpretation of skewness and kurtosis;[69, 70]

1) If skewness is less than −1 or greater than +1, the distribution is highly skewed.

2) If skewness is between −1 and −½ or between +½ and +1, the distribution is moderately skewed.

3) If skewness is between −½ and +½, the distribution is approximately symmetrical.

4) For kurtosis, the reference standard is a normal distribution, which has a kurtosis of 3.

7.31. Appendix 5.14

Sociodemographic characteristics of students screened for TB disease by ventilation risk status

Variable	**Low ventilation risk classrooms**	**High risk ventilation classrooms**
Number of participants (n(%))	980 (57.8%)	716 (42.2%)
Age (median (IQR)	16.3 (15.0; 17.4)	15.9 (15.1; 17.4)
Sex (male)	393 (45.4%)	297 (44.3%)
Race		
African	486 (54.2%)	449 (65.1%)
Mixed race	401 (44.6%)	235 (34.1%)
White	10 (1.1%)	4 (0.6%)
Indian/Asian	1 (0.1%)	1 (0.2%)

Legend: Numbers are n (%) or n (IQR).

7.32. Appendix 5.15

Description of measurement of proportion of rebreathed fraction, absolute rebreathed air volume, classroom ventilation rate and volume of air through the classroom

To calculate the proportion of rebreathed fraction from other people, we adjusted the rebreathed fraction for the number of people in the defined space, as follows.[32]

$$f_o = f^{*}((n-1)/n) \quad (2)$$

Where n is the total number of people in the defined space and f is the rebreathed fraction.

To obtain the absolute rebreathed air volume, we obtained the product of proportion of rebreathed air from other people (f_o) and respiratory minute volume ((p) and summed over all observations (termed 'summation' in statistics). i.e.[32]

$$\text{Rebreathed Air Volume} = \sum p\, f_o\, (t) \quad (3)$$

Where p is the respiratory minute volume, estimated from literature as 8.0 litres/minute.[53]

Ambient carbon dioxide concentration values can thus be used to estimate rebreathed (shared) air in an enclosed space.[32]

Calculation of classroom ventilation rate

The conversion between indoor carbon dioxide concentration and ventilation rate (in litres per person per second) was obtained using the following equation;[71]

$$Q = G/(C_{in} - C_{out}) \quad (4)$$

Where: Q=Ventilation rate per person (l/p/s); C_{in}= indoor carbon dioxide concentration in ppm; C_{out}= outdoor carbon dioxide concentration in ppm; G=carbon dioxide generation rate in classroom (in litres per person per second) which was obtained using the formulae:[28]

$$G = \bar{V} C_{ex} \quad (5)$$

Where C_{ex}= carbon dioxide concentration in exhaled breath (38,000 from literature);[53] $\overline{V}$=average volume of gas exhaled by adolescents per second (0.13 litres/second/person from literature).[53] Therefore, G=5067.

The ventilation rate per person obtained above was used as one of the parameters in the equation for obtained the air flow or ventilation rate to a room.

Classroom ventilation in air changes per hour (ACH) was obtained by the following equation from Robertson *et al.*[28]

$$ACH = \frac{3600Qn}{Volume} \quad (6)$$

Where; ACH= Air Changes per Hour; Q= per person ventilation rate (l/p/s); n=Number of students in classroom and Volume=Estimated volume of classroom in litres.

Volume of air through the classroom

The total volume of air that was in the classroom for the duration of ddPCR air sampling, for calculation of RD9 *Mycobacterium tuberculosis* DNA concentration per volume of air was obtained by the following equation;

Total volume=ACH * Estimated volume of classroom * Duration of sampling in hours (7)

Where; *ACH*= Air Changes per Hour.

See reference list of Chapter 4 for bibliographies of references in appendix 15.