Tuberculosis among people living with and without HIV in lower-income countries: Transmission, Resistance, Mortality

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PhD Thesis submitted by

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for the degree of

PhD in Biomedical Sciences

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Abstract

Tuberculosis (TB), an airborne disease caused by the bacterium *Mycobacterium tuberculosis (Mtb)*, is one of the most deadly infectious diseases, with an estimated 10 million newly diagnosed TB cases and 1.4 million deaths per year worldwide. The HIV pandemic and the emergence of drug-resistant *Mtb* strains are the main challenges to TB control, especially in low-and middle-income countries. HIV infection induces immunodeficiency and is a decisive risk factor for TB. With the significant scale-up of antiretroviral therapy (ART) for people living with HIV (PLHIV), their prognosis has improved and reduced TB incidence in this population. Nevertheless, the risk of TB in PLHIV on ART remains higher than in people without HIV. In addition, the diagnosis of TB in PLHIV is more challenging due to the reduced occurrence of lung cavitation and lower bacterial load in the sputum compared to HIV-negative people. The rising number of drug-resistant TB is another threat to the control of TB. To prevent drug-resistant TB, strategies such as surveillance, access to comprehensive drug susceptibility testing (DST), rapid treatment initiation and treatment completion with an appropriate regimen are still lacking in many low-and middle-income countries.

In this thesis, I explore different aspects of the epidemiology of TB, especially drug-resistant TB in PLHIV and HIV-negative people seeking care in low-and middle-income countries. I will focus on *Mtb* transmission, the management of TB in adults (age ≥16 years) from diagnosis to treatment and clinical outcomes, and the evolution of drug-resistant *Mtb* and the clinical consequences.

In chapter 1, I provide an introduction to TB. I describe the global burden of TB, the pathogen and the course of infection, the genetic diversity of TB, the diagnosis of TB including DST and the treatment of pan-susceptible and drug-resistant TB. In addition, I describe the current challenges to the control of TB.

Chapter 3 and chapter 4 focus on the transmission of *Mtb* at a primary care clinic in South Africa using a novel approach. Paper 1 is the study protocol, which describes the range of collected patient and environmental-associated data in detail. In paper 2, I showed that the risk of *Mtb* transmission increased with the presence of young adults and higher room humidity at the primary care clinic. During a clinic visit of one hour, the risk of infection was between 3-5% and increased to 9-29% for patients with monthly visits.

Chapter 5 and chapter 6 focus on the management of TB in adult PLHIV at ART clinics in low-and middle-income countries. In paper 3, I analysed clinical data of 2,695 adult PLHIV who developed TB, of whom 1,930 (72%) had pulmonary TB, and 765 (28%) had extra-pulmonary TB. I demonstrated the difficulties in obtaining bacteriological confirmation in PLHIV who also had pulmonary TB or extra-pulmonary TB (52% and 42%). I found no association between the increased mortality in PLHIV and the type of TB they developed (extra-pulmonary or pulmonary). I showed that bacteriological confirmation was associated with reduced mortality in PLHIV who had pulmonary TB or extra-pulmonary TB than PLHIV with a negative diagnostic result. Paper 4 used site-level data to study the integration of multidrug-resistant TB (MDR-TB) services at 29 ART clinics. I show that 14 (48%) ART clinics reported full MDR-TB services on-site, nine (31%) reported partial integration, and six (21%) had access to off-site MDR-TB services only. I demonstrated that the 22 clinics with on-site molecular DST could identify drug resistance to first-line drugs but rarely to second-line drugs. In addition, I showed that ART clinics with full integration of MDR-TB services were more likely to prescribe individualised MDR-TB treatment compared to off-site services.

Chapters 7 to 10 focus on the evolution of drug-resistant *Mtb* and clinical consequences. In paper 5 and 6, I examined mortality in adult PLHIV and HIV-negative adults who developed TB in seven low- and middle-income countries. I compared mortality by DST results done locally that were discordant or concordant with the results from the reference laboratory using culture-based phenotypic DST or WGS. In both studies, the degree of drug resistance, discordant DST results potentially leading to under-treatment and discordant DST results, which resulted in under-treatment according to WHO guidelines, led to increased mortality, especially among those with drug-resistant TB. Further, I demonstrate that DST for second-line drugs was rarely available locally. Paper 7 showed that mutation with low-fitness in *rpoB* variants is more likely in PLHIV who also had rifampicin-resistant TB than in HIV-negative people who had rifampicin-resistant TB. Finally, paper 8 demonstrated that drug resistance to delamanid occurred in people with TB who were drug naïve to delamanid.

In this thesis, I provide insights into different aspects of the epidemiology of TB, from *Mtb* transmission, management of TB, and drug resistance to death, but also identify the gaps in our current knowledge. I show that new approaches can be used to study *Mtb* transmission. I demonstrate the challenges of diagnosing PLHIV and pulmonary or extra-pulmonary TB at ART clinics in low-and middle-income countries. In addition, I show that DST to second-line drugs is limited in many low-and middle-income countries, and there is a need for more rapid and comprehensive DST testing to ensure appropriate treatment. My survey on integrated MDR-TB services also showed that 48% of ART clinics had full integrated MDR-TB services. I showed that low-fitness mutations in *rpob* are more likely in PLHIV who have rifampicin-resistant TB than in HIV-negative people who had rifampicin-resistant TB and that drug resistance can occur in people natïve to delamanid. In conclusion, to control TB, we need to strengthen the access and use of point-of-care diagnostic tests to diagnose TB disease and DST for first- and second-line anti-TB drugs and access to second-line anti-TB drugs in low-and middle-income countries. In addition, we need to improve surveillance of drug-resistant TB and better treatment options to ensure the completion of the treatment.

Contents

Introduction and Aim

1. Introduction

1.1. History of TB

Morphological changes such as spine lesions (Pott's disease), periosteal reactive lesions, or osteomyelitis and molecular studies suggest that tuberculosis (TB) was present in people who lived during prehistoric Eastern Mediterranean, prehistoric East Asia in ancient Egypt, and in Pre-Columbian South America (1-5). For millennia, TB has affected humans, and millions of people have died from TB (6).

The invention of the stethoscope by French physician René René Laënnec in 1816 changed the way of diagnosing various lung diseases, including TB (7). In 1865, the physician Jean-Antoine Villemin demonstrated that TB is an infectious disease by inoculating material from infected humans and cattle to rabbits. A few years later (1868), he hypothesised that TB is caused by inhaling infectious material (8). On 24 March 1882, Robert Koch showed in a lecture that TB was caused by the tubercle bacillus (9). Robert Koch continued his work on TB, for which he received the Nobel Prize in 1905 (6).

Since the mid-19th century, TB began to decline. Although not fully understood, this decline can partly be explained by improved living conditions, better nutrition, improved hygiene, and separating and sending people with active TB disease to sanatoria (6, 10-14). In 1921, Albert Calmette and Camille Guérin developed a vaccine against TB called “Bacillum Calmette-Guérin, or BCG” (15). The virulent BCG strain was subcultured 239 times for 13 years until the strain lost its virulence. Although several research groups are working on the development of new TB vaccines, the BCG vaccine remains the only vaccine available today with moderate protection against severe forms of TB (16). With the introduction of anti-TB drugs, such as streptomycin in 1943, isoniazid in 1952, and later other anti-TB drugs, TB declined further (6, 10, 17).

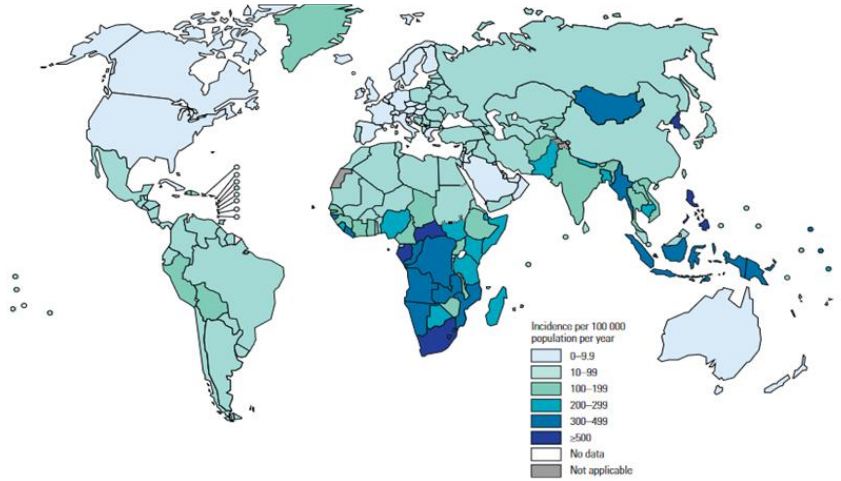
1.2. Global burden of TB

Worldwide, approximately one-quarter of people are infected with *Mtb*. In 2019, an estimated 10 million people developed active TB disease worldwide, of which an estimated 820 000 (8.2%%) were people living with HIV (PLHIV) (18). In the same year, 1.4 million people died from TB. Figure 1A shows the TB incidence was highest in sub-Saharan Africa (18).

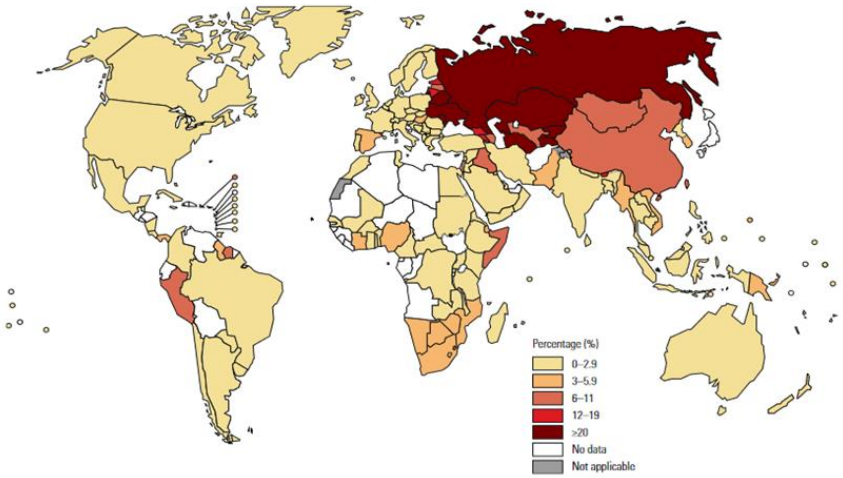
More than 80% of the global TB burden lies in the 30 high-burden countries. About 66% of people with TB live in eight of these countries, namely in India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%) (18, 19). Major challenges to TB control are the HIV pandemic and the emergence of drug-resistant TB, which fuelled the TB pandemic, especially in low-and middle-income countries. Recently (June 2021), the WHO released the new lists of the 30 high burden countries for TB, TB/HIV and multidrug resistance (MDR)–TB, with each category accounting for at least 80% of the global burden (20). Figure 1 shows that HIV and drug-resistant TB, and the current COVID-19 pandemic are the major challenges in the control of TB and are affecting similar regions. These challenges will be discussed in section 1.3.

Figure 1. Global burden of TB and challenges to the control of TB. Panel A) show the TB indicence in 2019, panel B) the percentage of multidrug resistant TB or rifampicin- resistant TB, panel C) the adult HIV prevalence and panel D) the cumulative confirmed COVID-19 cases. Figure A and B are from WHO (18), C from Unaids (21), and D from Ritchie et al. (22)

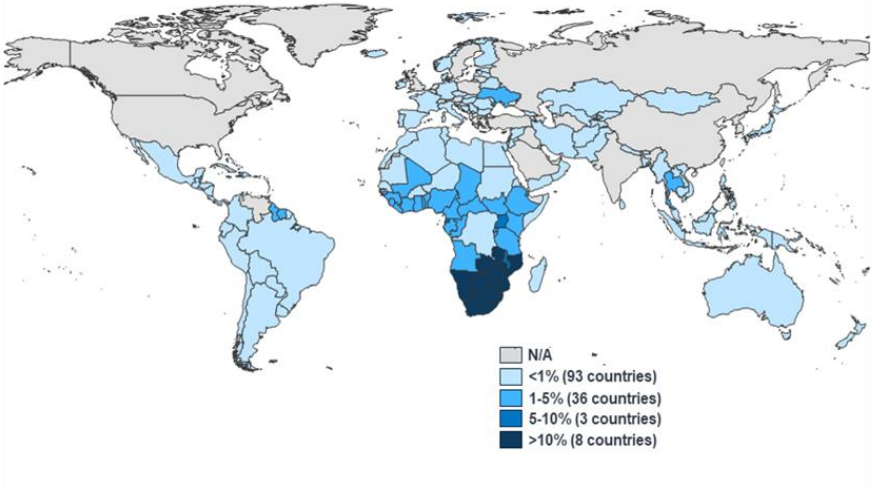
A. TB incidence in 2019



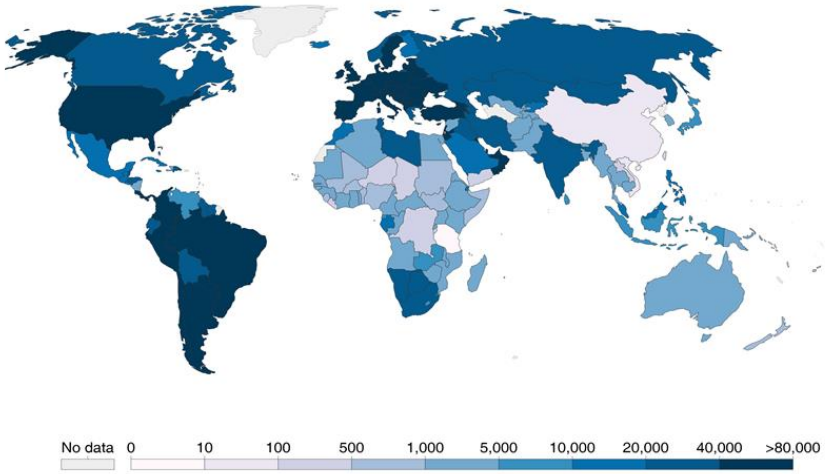
B. Percentage of new TB cases with MDR/RR-TB in 2019



C. Adult (aged 15-49) HIV prevalence in 2019



D. Cumulative confirmed COVID-19 cases per million people, 8 June 21



1.3. Current challenges in the control of TB

1.3.1. Drug-resistant TB

The emergence of drug-resistant TB is a central challenge to the control of TB. In 2019, an estimated 3.3% of people newly diagnosed with TB and 18% of previously treated people with TB worldwide had rifampicin-resistant or multidrug-reistant TB (MDR-TB). This results in an estimate of 465 000 people with rifampicin-resistant or MDR-TB (Figure 1B) (18).

Strategies to control and prevent drug-resistant TB include surveillance, drug susceptibility testing (DST), as well as ensuring an effective treatment regimen (18). There are different degrees of drug resistance ranging from mono-resistance to XDR-TB. Mono-resistant TB is defined as resistance to one first-line anti-TB drug (isoniazid, rifampicin, pyrazinamide, or ethambutol) (23). In contrast, MDR-TB is defined as resistance to at least both isoniazid and rifampicin (23). Pre-XDR-TB is defined as resistance to any fluoroquinolone in addition to MDR-TB. XDR-TB is defined as a resistance to any fluoroquinolone and at least one additional Group A drug (bedaquiline and linezolid) in addition to MDR-TB (24).

In addition, drug resistance can be classified into two groups based on the way resistance occurred, namely “primary resistance” or “acquired resistance”. Primary resistance refers to people infected with a drug-resistant *Mtb* strain who had no prior history of treatment with anti-TB drugs (25). In contrast, acquired resistance refers to people initially infected with a drug-susceptible strain and developed drug resistance during TB treatment. In addition, acquired resistance can occur in people who were treated with anti-TB drugs. Different factors can favour the acquisition of drug resistance in treated patients, such as poor adherence, sub-optimal treatment regimen or dosage, treatment interruption, or poor drug quality (25).

Drug resistance in *Mtb* is caused by genetic alterations such as indels, deletions or single nucleotide polymorphisms (SNPs), and consequently, changes in proteins involved in drug metabolism (26, 27). Single or combined mutations can result in low- or high-level drug resistance. For example, studies described different levels of phenotypic drug resistance to isoniazid caused by different mutations in different genes: mutations in the promotor region of the gene inhA causes mainly low levels of resistance, whereas mutations in the gene katG causes high level of resistance (28, 29). Also, for streptomycin, different levels of resistance have been associated with different mutations in different genes (30). In addition, within genes, different mutations have been associated with different levels of resistance. For example, the different levels of rifampicin resistance are associated with different polymorphisms in the gene *rpoB* (31, 32). The level of drug resistance not only results from drug-resistance-conferring mutations but depends on the combinations of mutations simultaneously being present, including drug-resistance-conferring mutations, compensatory mutations, and different strain genetic background (28, 33-35). This phenomenon is also called epistasis (28, 29). Many drug-resistance-conferring mutations in *Mtb* resulted in a reduction of bacterial fitness. They thus reduced transmission. Still, some mutations are associated with low or no fitness cost and maintain the ability of drug-resistant *Mtb* strains to replicate and transmit successfully (36-38). Compensatory mutations can be observed in the presence of drug-resistance-conferring mutations, which allows the bacteria to restore initial fitness loss (36). Such compensatory mutations have been described in the presence of isoniazid, aminoglycoside and rifampicin drug-resistance-conferring mutations (39-41). Understanding the level of phenotypic drug-resistant TB is important to decide whether an anti-TB drug can be prescribed and at which concentration. For example, people with low-level resistance, for example to isoniazid, could be treated with higher dosages, whereas high-level resistance indicates the need to prescribe alternative drugs.

1.3.2. HIV/AIDS and TB

With the HIV epidemic, TB became a new focus of WHO at the beginning of the 1990s, especially in low-and middle-income countries. HIV/AIDS induces immunodeficiency and is a very strong risk factor for the progression of active TB disease (42). In 2020, an estimated 38 million PLHIV worldwide, with the largest proportion in sub-Saharan Africa (35 million) (Figure 1C) (21). TB is the leading cause of death in PLHIV, and it is estimated that TB caused more than one third of all HIV-related deaths in 2019.

Studies showed that after HIV seroconversion, the risk of TB doubles and continues to increase as CD4-positive T lymphocytes (CD4 cells) drop (43-46). CD4-positive T lymphocytes are white blood cells that play a major role in protecting the body from infection. Therefore, PLHIV can not respond efficiently to TB (45) and put them at up to a 37-fold risk of progressing to active TB disease compared to HIV-negative people (47). The risk of TB remains high until the person initiates antiretroviral therapy (ART). With the wide availability of ART, the prognosis of PLHIV has substantially improved (48, 49). However, the risk of progressing to active TB disease is increased during the first months on ART because of the unmasking of subclinical TB (50-52). A study from South Africa showed that unmasking TB accounted for more than one third of people with active TB disease in the first four months of ART (50), but in the longer term, ART reduces the TB incidence by about two thirds (53). Nevertheless, the risk of TB among PLHIV on ART with a high CD4 cell count (>700 cells/ml^3) was four times higher compared to HIV-negative people from the same high burden setting in Cape Town, South Africa (50, 54, 55). This suggests that ART alone is not sufficient to prevent HIV-associated TB, and additional strategies are required to reduce the burden of TB in this vulnerable population.

In addition, TB disease is progressing faster in PLHIV compared to HIV-negative people. PLHIV who have TB are less able to control the *Mtb* infection since HIV destroys the CD4-positive T lymphocyte cells. These cells are necessary to activate macrophages and form granuloma to control the *Mtb* infection or prevent the progression to active TB disease (56-58).

1.3.3. SARS-CoV-2 and TB

The global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections in 2020 is another threat in the control of TB (Figure 1D) (18). Globally, as of the end of June 2021, SARS-CoV-2 infection was diagnosed in over 180 million people, and close to 4 million have died from COVID-19 (59). Both COVID-19 and TB are airborne diseases. A systematic review showed that TB is a risk factor for COVID-19 regarding disease severity and mortality (60). A modelling study based on data from the Philippines showed that the risk of death in people with TB who contracted COVID-19 was 2.17 times higher than TB patients without COVID-19 (61). In addition, TB patients with COVID-19 died more quickly than those without COVID-19 (61). A study from South Africa showed that PLHIV, people with current TB (drug-susceptible and drug-resistant) or people with a history of TB was associated with COVID-19 death (62).

The COVID-19 pandemic often stretches hospitals and health providers beyond their capacity due to a lack of infrastructure and equipment like hospital beds, ventilators or masks, and trained staff. In many low-and middle-income countries where TB notifications are high, additional pressure is placed on a already overburdened health systems, which often have to deal with other epidemics such as HIV. Studies from Brazil, China, India, Iran, Nigeria, and United States showed that the COVID-19 pandemic has led to a significant decrease in TB notifications (63-68). The full impact of the COVID-19 pandemic on the burden of HIV/TB and the clinical services has yet to be established.

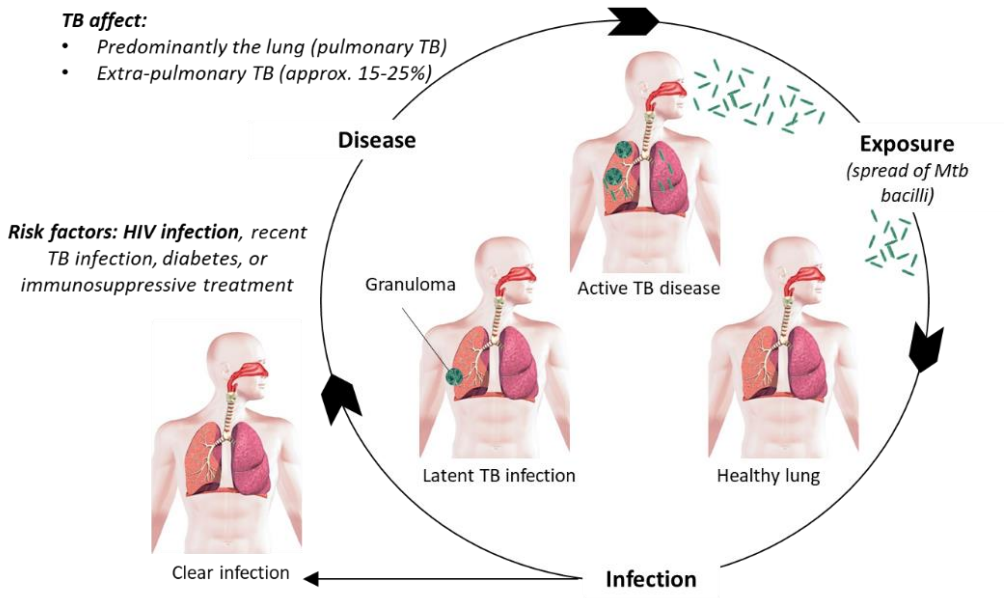
1.4. Pathogen and course of infection

Mtb is a member of the *Mycobacterium tuberculosis* complex (MTC). The MTC is a group of Gram-positive bacteria characterized by a high similarity of >99.95% and an identical 16S rRNA sequence. The MTC consists of seven closely related species with different hosts. *Mtb, M. africanum,* and *M. canettii* are pathogenic in humans, whereas the species *M. bovis* (cattle), *M. caprae* (goats), *M. pinnipedii* (seals) and *M. microti* (rodents) are adapted to animlas (69-72). All these species can infect humans but *Mtb* is responsible for most TB cases in humans. In this thesis, we will only refer to *Mtb*.

TB is an airborne disease transmitted from one person to the next when an uninfected person inhales air containing *Mtb* bacilli exhaled from an infectious person (Exposure, Figure 2). This is likely to occur when *Mtb* remain suspended in air for a sufficient amount of time for the next person to breathe in these infectious *Mtb* bacilli. Droplet nuclei with diameters ranging from 1µm to 5µm, are playing a key role of suspension of *Mtb* in air (73, 74). These small droplet nuclei, called aerosols, can remain suspended in the air for several hours, while larger nuclei aerosols generally drop to the ground more rapidly or, if inhaled, do not reach the alveoli (42, 75, 76).

For successful *Mtb* transmission, the droplets containing *Mtb* bacilli have to be small enough to reach the alveolus in the periphery of the lungs (42). After infection with *Mtb*, a person may spontaneously clear the infection (i.e. never gets sick) or contain the infection (i.e. latent TB infection, Figure 2). Individuals with latent TB have no symptoms and are not infectious. Around 5-15% of people with latent TB will progress to active and symptomatic TB disease within two years after infection (77). Active TB disease predominantly affects the lungs (pulmonary TB), but in in 15-25%, it affects other organs (extra-pulmonary TB) (77). Miliary TB is a form of disseminated disease (TB affecting the lungs and other organs) that results a spread of *Mtb* bacilli over the blood to the lungs and other organs. People with both pulmonary and extrapulmonary TB are classified as pulmonary TB (23). The typical symptoms of a person with active pulmonary TB disease are fever, night sweat, weight loss, and a cough. At this stage, the person can produce infectious aerosols, which are spread into the environment by talking, coughing, sneezing or singing and transmitted to other people, re-starting the cycle (42). At the same time, multiple immunological mechanisms are hypothesized to determine whether and how people progress to active TB disease. Immunosuppression, such as through HIV co-infection, is a strong risk factor (77-80).

Figure 2. Course of TB infection. Figure adapted from Pai et al. (2016) (77)



1.5. *Mtb* transmission

The transmission of airborne diseases, including TB, contains three stages: release of the infectious agent into the environment by coughing, talking, sneezing, singing and even breathing, transport of infectious agent from infectious person to another person, and inhalation of infectious aerosol by the other person (81-83). The risk of *Mtb* transmission depends on the infectiousness of the person with TB, the duration of infectiousness, the close proximity of the person with TB to susceptible people, and bacteriological factors of the *Mtb* pathogen that affect transmission (see section 1.6) (91-93). All these factors can differ by setting. For example, the prevalence of people with infectious TB can be affected by community-level access to diagnosis and care or by individual-level factors such as diabetes, silicosis, underweight, or HIV infection (84). The infectiousness and the ability to transmit *Mtb* has been extensively discussed in the context of HIV infection. Some studies found that PLHIV who also have TB are more likely to transmit to their close contacts than HIV-negative people and others found the reverse (85-90). A meta-analysis comparing the prevalence of tuberculin skin testing (TST) positivity among household contacts of the index case (person with TB) found lower rates of TST positivity among contacts of PLHIV who have TB compared to contacts of HIV-negative people who have TB (91). Similar a recent systematic review and meta-analysis (2018) showed that PLHIV and TB were around half as likely to transmit to their contacts than HIV-negative people who have TB (92).The current evidence indicates that PLHIV who have TB transmit *Mtb* less frequently than HIV-negative people who have TB due to fewer infectious *Mtb* bacilli in their sputum. Other factors include the higher risk of disseminated or extrapulmonary TB and faster TB disease progression, which results in earlier and higher mortality (93-97).

Environmental factors are also playing a role in *Mtb* transmission, such as ventilation, crowding or humidity. Poor ventilation in enclosed spaces favours and prolongs the dispersing of infectious aerosols and thus their transport from one person to another (81, 98). Crowded locations facilitate that aerosols from people with TB reach susceptible people (42). Relative humidity above 65% is associated with *Mtb* survival in the air (99). Therefore, *Mtb* is highly transmissible in overcrowded, poorly ventilated, and humide indoor locations like healthcare clinics (100-103). Studies of different settings showed that healthcare clinics can be drivers of *Mtb* transmission (104-107). In contrast, the risk of transmission at healthcare clinics can be mitigated by appropriate treatment and non-pharmaceutical interventions such as facial masks or sufficient room ventilation (108-111).

1.5.1. Approaches to measuring *Mtb* transmission

Mtb transmission is difficult to measure because only a small proportion of people infected with *Mtb* (5-15%) will progress to active TB disease. The period of latency can vary from weeks to decades. Transmission of *Mtb* does not always result in secondary cases as only some infected people with *Mtb* will progress to an infectious stage. There are multiple divers for disease progression like diabetes, or HIV infection (84). The exact time and location of the *Mtb* infection can often not be determined with certainty. In addition, no appropriate *in vitro* test assay exists to measure *Mtb* transmission (112).

The current knowledge of *Mtb* transmission is mainly from the *in vivo* guinea pig studies (113-115). In the 1950s, Richard Riley, William Wells and colleagues conducted several experimental airborne TB studies (42, 74, 114-116). For example, a TB ward with six single rooms occupied by people with TB was connected with a calibrated closed-circuit ventilation system chamber with healthy guinea pigs.

Monitoring of infections in these guinea pigs allowed the quantification of the number of infectious droplets in the air that resulted in successful transmission. The concept of infectious quanta was developed to estimate *Mtb* transmission (115). The study also showed that some people with TB are more infectious and more likely to transmit to guinea pigs than others (115).

One approach to estimate *Mtb* transmission in the population are TST surveys in schoolchildren (117-120). The estimated prevalence of TB infection can then be transformed into an annual risk of infection. Further repeated surveys or continuous measuring of the prevalence of infection in the same age group can allow estimates of the trend in *Mtb* transmission over time (117).

Traditional ways to measure *Mtb* transmission resulting in secondary cases are contact tracing, geo-temporal clustering analyses, and molecular genotyping methods (121-127). However, contact tracing data are difficult to obtain, particularly in low-and middle-income countries and high-risk groups (128-131). The limiting factor for geo-temporal clustering analyses is the data quality, which complicates subsequent analysis and interpretation of the data (121). Molecular genotyping methods allow to i) distinguish a new infection from a relapse, ii) identify chains of *Mtb* transmission, iii) identify multiple co-infections and iv) identify laboratory cross-contaminations. Since the early 1990s, several genotyping methods such as insertion element restriction fragment length polymorphism (RLFP), spacer oligonucleotide typing (spoligotyping), mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) or whole-genome sequencing (WGS) have provided insights into *Mtb* transmission (Table 1) (132). These genotyping methods require resource-intensive culturing of *Mtb* strains at centralized laboratories and are expensive.

Table 1. Methods used for the genotyping of *Mtb*. Apdapted from Kato-Maeda (2011) (132)

Method	Principle	Technical requirements	Advantages	Disadvantages
IS6110-RFLP	Based on the variability in IS*6110* number of copies and molecular weights of DNA fragments in which the insertions are found	- PCR amplification - 2-3 µg of pure DNA - Southern blotting - Visual of software-based comparison	- High discriminatory power - Widely used - Revolutionized the understanding of transmission.	- Resource-intensive - Needs a lot of high quality DNA - Difficult to reproduce and compare between laboratories.
Spoligotyping	Based on the presence of absence spacer in the direct repeat locus. Usually, a set of 43 spacer is used.	- PCR amplification - Specific blotter and membrane	- PCR-based - DNA extracted can be done directly from sputum - Exchangeable data format - Highly reproducible	- Limited discriminatory power - Can not detect mixed infections
MIRU-VNTR	Based on polymorphisms in MIRU-VNTR loci within the genome. Highest discrimination obtained with a 24 set locus.	- PCR amplification - Gel electrophoresis or sequencer	- PCR-based - High discriminatory power - Exchangeable data format	- Set of 12 loci less discriminatory than IS*6110* RFLP - Determination of band size is less reproducible with electrophoresis than with sequencer - Sequencer is expensive
WGS	Based on the analysis of the whole-genome sequence results	- Next-generation sequencing	- Gold standard for phylogenetic	- Expensive - Need specialized technology and software

In the early 2000s, studies on viral respiratory diseases reported a relation between environmental indoor carbon dioxide (CO_2) levels and airborne diseases (133-135). This novel approach can be used to study *Mtb* transmission resulting in infection or active TB disease (101, 135). CO_2 combined with social contact data has been used to estimate the proportion of exhaled air and the volumes of rebreathed air in a room, which can be used as a proxy for the potential risk for *Mtb* transmission (101, 133, 135-138). Over the years, mathematical models to estimate *Mtb* transmission have been developed. The modified Wells & Riley model takes into account the rebreathed air fraction (estimated from indoor and outdoor CO_2 levels), time at risk, the infectious quanta (infectious dose required to

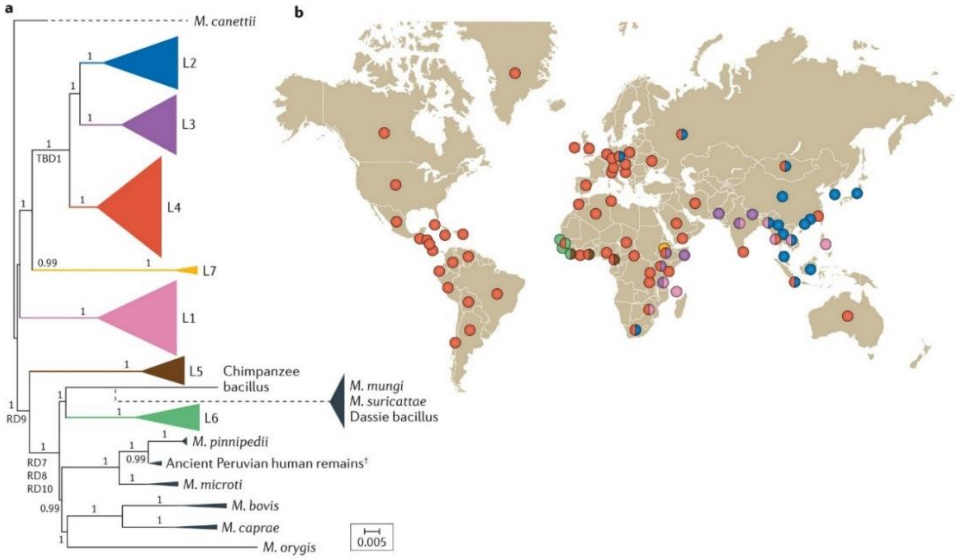
infect in an enclosed environment), and the number of people at the given location (133, 135, 136). We describe the Wells & Riley model in more detail in chapter 3. Several studies used this approach to estimated *Mtb* transmission and found that prisons, schools and public transportation are high-risk locations (135-139). One study from South Afrcia found an annual risk of *Mtb* transmission in public transport ranged from 3.5% to 5% being lower in buses and higher in taxis (136). A study from Tanzania found an annual risk of *Mtb* transmission of 42% among prison inmates and 20% among drivers in public transport (138).

1.6. Genetic diversity

The genetic diversity of *Mtb* was underestimated for a long time. Until the late 1980s, *Mtb* strains were considered "identical" (no/few genetic polymorphisms). In the early 1990s, the advent of molecular methods and the first whole-genome sequence of *Mtb* H37Rv in 1998, which is today the reference strain, opened the door for phylogenetic analysis. Small deletions and insertions, large duplications and insertion sequences (140), large genomic deletions (141) and SNPs (142) showed the genetic diversity in *Mtb*.

Phylogeny analysis showed that MTC could be classified into seven main phylogenetic lineages known to cause TB in humans (Figure 3). The seven lineages are Lineage 1 (Indo-Oceanic), Lineage 2 (East-Asian), Lineage 3 (East-African-Indian), Lineage 4 (Euro-American), Lineage 7 (occurs almost exclusively in Ethiopia); and the *M. africanum* lineages: Lineage 5 (West African 1), 6 (West African 2) (143-146). Figure 3 shows that the geographical spread of these lineages differs considerably. Lineages 2 and 4 a spread globally, and lineage 5, 6, and 7 are locally restricted. The remaining two lineages 1 and 3 show an intermediate spread.

Figure 3. Global phylogeny and geographical distribution of the *Mtb* complex. a.) Whole genome-based phylogeny of the *Mtb* complex rooted on *M. canettii*. b.) Global distribution of the seven *Mtb* lineages (145).



There is increasing evidence that the genetic differences between the MTC lineages impact their phenotypes including growth rates, gene expression profiles, infectiousness including the ability to transmit, and active TB disease progression. They may influence the clinical outcome. Further, this genetic diversity is one reason for the propensity of developing drug resistance (28, 143-145). For example the W-Beijing strains (Linage 2) is very virulent, highly transmissible to humans, and is associated with drug resistance and HIV (147-150).

1.7. Diagnosis of TB disease and drug susceptibility testing (DST)

Rapid diagnosis, including DST, to initiate appropriate treatment and reduce *Mtb* transmission is central to TB control. According to the WHO, TB can either be clinically diagnosed or bacteriologically confirmed (23). A clinical TB diagnosis is based on clinical symptoms or a chest X-ray without a laboratory confirmation (23). Bacteriological confirmation is by positive sputum smear microscopy, culture, rapid molecular tests such as the GeneXpert or the line probe assays (LPAs), or lateral flow urine lipoarabinomannan assay (LF-LAM) (23). The diagnostic pathway to detect active TB disease begins with (i) TB screening methods, such as symptom screening to evaluate people with presumptive TB, chest X-ray or other imaging techniques to identify pulmonary abnormalities that may be suggestive of TB, (ii) diagnostic methods for bacteriological confirmation of TB, and (iii) DST to identify resistance to TB drugs (151, 152).

Diagnosis of active TB disease is more challenging in PLHIV than HIV-negative people due to the reduced occurrence of cavitations, lower bacterial load in sputum (also called paucibacillary infection) and other pulmonary or systemic infections (57, 95, 97, 153). Furthermore, PLHIV who have TB are more likely to have disseminated or extra-pulmonary TB, especially in advanced immunosuppression (94). These challenges in diagnosing TB in PLHIV might lead to misdiagnosis or delayed diagnosis, consequently in high morbidity and mortality. This thesis focuses on TB in adults (age ≥16 years) and I will not discuss diagnosis or treatment in children.

1.7.1. Clinical diagnosis

1.7.1.1. Symptoms of TB

Suppose TB bacilli are not detected by a microbiological method. In that case, TB can be clinically diagnosed based on TB symptoms such as a prolonged cough (≥2 weeks), fever, night sweat, weight loss, or haemoptysis (151, 152, 154). As mentioned above, TB symptoms are used to screen people for TB. Both (TB screening and clinical diagnosis) are easy to perform and implement (151).

1.7.1.2. Chest X-ray

From the historical perspective, chest X-rays have been an essential method to diagnose pulmonary TB (155). Nowadays, chest X-rays are often used as an add-on diagnostic method if pulmonary TB cannot be bacteriologically confirmed. Typical TB-related abnormalities in the lung are infiltrations, nodes, and cavities. Chest X-rays and other imaging are central to diagnosing extra-pulmonary TB such as military or pericardial TB or tuberculous effusions (155). As mentioned above, chest X-rays and other imaging are used to screening for TB (151).

1.7.2. Bacteriological confirmation

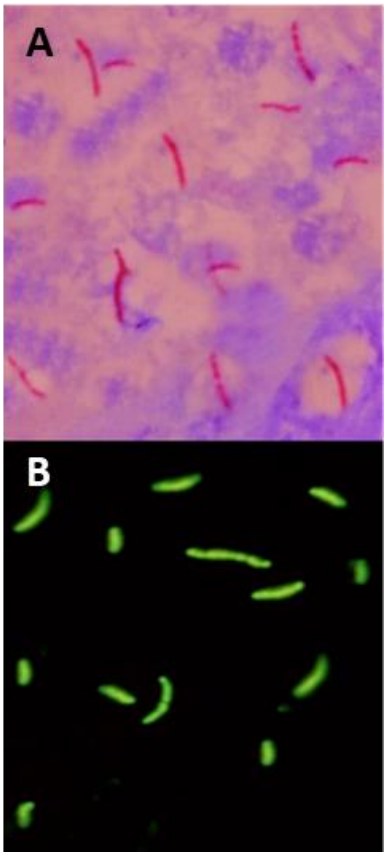
The traditional methods to diagnose active TB disease are sputum smear microscopy and mycobacterium culture. In recent years, several new diagnostic technologies based on DNA amplification, antigen or antibodies to MTC were developed (156). All these new technologies provide rapid detection of *Mtb,* and some technologies detect drug-resistant mutations. I will describe these methods in the next sections (1.7.2.1-1.7.2.7).

1.7.2.1. Sputum smear microscopy

The most frequently used diagnostic test to diagnose pulmonary TB disease in low-and middle-income countries is examining a stained sputum smear microscopy (157). Smear microscopy is simple, cheap and effective in identifying those with pulmonary TB who are most infectious. In addition, results are available within few hours (154). The techniques rely on the lipid-rich cell walls of the mycobacteria, which, when stained with carbol-fuchsin or fluorochromes, are not easily decolorized even with alcoholic-acidic solutions. Due to these characteristics, mycobacteria are often called "acid-fast bacilli (AFB)" (158). Currently, two methods of acid-fast stains are used, namely:

- Ziehl-Neelsen [ZN] or carbol-fuchsin staining method
- Fluorochrome (auramine or auramine-rhodamine) staining (Figure 4).

Figure 4. Ziehl-Neelsen staining (A) and fluorochrome staining (B) (158)



The ZN microscopy has a high specificity with 98% (95% CI 97 to 99%), but the sensitivity is imperfect, ranging from 50% to 70% compared to culture (159-161) (Table 2). Fluorescent microscopy has on average, a 10% higher sensitivity than ZN but slightly lower specificity (159).

Any smear microscopy staining is limited in sensitivity and can not distinguish between other members of the MTC. In addition, for a positive smear microscopy result, a high bacterial load in the sputum of at least 5 000 *Mtb* bacilli/ml of sputum is required. The sampling of sputum may be difficult in children or PLHIV (162, 163). In PLHIV the sputum smear microscopy results are more likely to be negative than HIV-negative people (164).

1.7.2.2. Mycobacterium culture

Culture can provide a bacteriological confirmation and remains the gold standard (Table 2) (156). Culture can be used to improve early-stage diagnosis in individuals with extrapulmonary TB and in individuals failing TB treatment. For initial growth, only a few *Mtb* bacilli are needed (approx. 10 bacilli/ml of sputum compared to at least 5,000 bacilli/ml of sputum for microscopy) (165). *Mtb* grows slowly. The generation time is between 18-24 hours depending on the growth medium, and it takes several weeks to get the result. Currently, different growth media for *Mtb* are available, including (i) solid media such as egg-based Löwenstein-Jensen (LJ) medium or agar-based media such as Middlebrook 7H10 and Middlebrook 7H11 and (II) liquid media such as Middlebrook 7H9 broth or BACTEC MGIT 960 system (BD [Becton, Dickinson and Company] Diagnostic Systems)(165, 166). The major advantage of solid media compared to liquid media is that colonies of mixed culture and contaminants can be identified, but in liquid media, *Mtb* grows faster, and results are available earlier (166). However, irrespective of the method, due to the slow growth of *Mtb*, the sample must be decontaminated before culture inoculation to prevent overgrowth by other, fast-growing microorganisms. Decontamination can damage the *mycobacteria*, and therefore culture-based methods are not 100% sensitive. In summary, mycobacterial cultures require high-level biosafety infrastructures, trained staff and take several weeks (Table 2). Further, there is always the risk of contamination (165). Culture can be used to perform phenotypic DST and to extract DNA for whole-genome sequencing (WGS) or next-generation sequencing (165).

1.7.2.3. Real-time polymerase chain reaction (PCR)

In 2010, WHO supported using the Xpert MTB/RIF® (Cepheid, USA) to diagnose TB (167). Currently, the Xpert is the most widely used rapid molecular diagnostic test in low-and middle-income countries. The Xpert MTB/RIF, an automated real-time PCR assay, detects *Mtb* and resistance to rifampicin within two hours (Figure 5) (168). After loading the sample into the Xpert MTB/RIF cartridge, PCR amplification and *Mtb* detection are performed in a single enclosed unit. A Cochrane review showed for the Xpert MTB/RIF a pooled sensitivity and specificity of 85% (95% Cl 79 to 90) and 98% (95% Cl 97 to 99) for *Mtb* detection compared to culture (Table 2) (169). For rifampicin resistance detection, the pooled sensitivity and specificity was 95% (95% Cl 90 to 98) and 99% (95% Cl 97 to 100) (169). The Xpert can be performed in decentralized clinics, as the assay uses agents to liquefy sputum, which can kill *Mtb* bacteria. Xpert MTB/RIF requires a stable electrical power supply, temperature control and yearly calibration of the instrument's modules (170).

Figure 5. A) GeneXpert MTB/RIF machine for detecting *Mtb* and resistance to rifampicin; B) GeneXpert MTB/RIF cartridge; C) Portable single GeneXpert devices; D) Curve of GeneXpert MTB/XDR assay, which resistance-conferring mutations for isoniazid, fluoroquinolones, and second-line injectables. Figure adapted from Lange et al. (2019) (170).

Compared to the Xpert MTB/RIF, the Xpert ultra has a larger chamber for DNA amplification and includes two additional molecular targets to detect *Mtb*. This modification explains the higher sensitivity of Xpert ultra to detect *Mtb* and allows the assay to detect a lower number of *Mtb* bacilli compared to Xpert MTB/RIF (16 bacilli/ml sputum vs 131/ml sputum) (156). For rifampicin resistance detection, the results were similar to Xpert MTB/RIF (169). In the latest WHO guidelines (2019), the Xpert MTB/RIF or Xpert ultra are both recommended as an initial diagnostic test for TB (156).

An add-on assay, the Xpert XDR, can be used after the Xpert MTB/RIF or Xpert ultra have confirmed an *Mtb* infection and resistance to rifampicin (171). Unfortunately, the Xpert XDR is not yet used in routine clinical care. The Xpert XDR detects resistance to isoniazid and the second-line drugs namely fluoroquinolones, injectable agents (amikacin, kanamycin, capreomycin), and ethionamide (Figure 5, Table 2, Table 3) (172).

The Truenat™ MTB, MTB Plus, and MTB-RIF Dx (Molbio Diagnostics, Goa, India) assays are automated chip-based real-time PCR to detect *Mtb* and MTB-RIF Dx detects drug-resistant mutations to rifampicin. These three tests are currently mainly used in India (152, 156). The Truenat™ MTB and MTB Plus have a lower sensitivity and similar specificity compared to culture (Table 2) (156, 173).

1.7.2.4. Line probe assays (LPAs)

The semi-automated tests include the first-line and second-line LPAs (GenoType® MTBDR*plus v1 and v2* and GenoType® MTBDR*sl*, Hain Lifescience, Nehren, Germany) (152, 165). Both assays are based on reverse hybridization between amplicons derived from a multiplex PCR and detect *Mtb* and perform DST. LPAs require more laboratory preparation steps than GeneXpert (Table 2).

The MTBDR*plus* is detecting *Mtb* infection and drug resistance to two first-line drugs, namely rifampicin and isoniazid (156, 165). A systematic review showed a pooled sensitivity and specificity of 94% (89 to 99) and 99% (95% CI 95 to 100) for *Mtb* detection for the MTBDR*plus* compared to culture. The pooled sensitivity and specificity was 90% (95% CI 88 to 92) and 99.2% (95% CI 98.7 to 100), respectively, for isoniazid resistance and 97% (95% CI 96 to 98) and 99% (95% CI 98 to 99) for rifampicin resistance (174). The result is available in 1-2 days (Table 2, Table 3).

The MTBDR*sl* detects resistances to second-line drugs namely fluoroquinolones and injectable agents (amikacin, kanamycin, capreomycin) (165). The result is available in 1-2 days. The Cochrane review showed a pooled sensitivity and specificity of 85% (95% CI 72 to 93) and 98% (95% CI 97 to 99) for fluoroquinolone resistance (175). For detection of resistance to injectable agents, the pooled sensitivity and specificity were 94% (95% CI 25 to 100) and 98% (95% CI 89 to 100), respectively (Table 2, Table 3) (175).

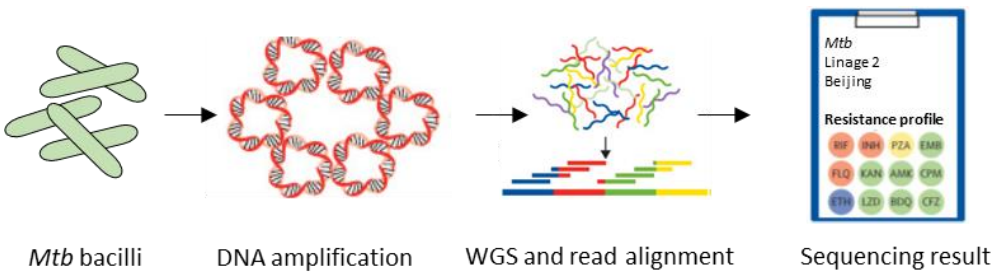
1.7.2.5. Loop-mediated isothermal amplification test (TB-LAMP)

The TB-LAMP (Eiken Chemical Co., Tokyo, Japan) is another rapid molecular test recommended by WHO since 2016 to diagnose pulmonary TB in adults (156, 176). A systematic review showed a pooled sensitivity and specificity of 78% (95% CI 71 to 83) and 98% (95% CI 96 to 99), respectively, compared to culture (Table 2) (177). As the TB-LAMP provides results within 1 hour and biosafety requirements are similar to sputum smear microscopy, the TB-LAMP could replace sputum smear microscopy (177). The TB-LAMP cannot detect drug-resistant mutations.

1.7.2.6. Whole-genome sequencing (WGS)

WGS is a method that provides the full nucleotide sequence of the *Mtb* DNA. This method can be used for TB diagnosis. It can simultaneously provide information about drug-resistant mutations to first and second-line anti-TB drugs, for which drug-resistance-conferring mutations are known (170) (Table 3). To perform WGS following steps are needed (i) DNA extraction from a TB sample, including enrichment and purification of the *Mtb* DNA to ensure high quality and quantity, (ii) DNA amplification of targeted genes and lineage-specific targets or randomly, (iii) library is sequenced, and sequences are aligned to the reference genome, (iv) data analysis provides information on drug-resistant mutations and *Mtb* linage (Figure 6)(178).

Figure 6. Steps to perform WGS. Figure adapted from Lange et al. (2019) (170).



Currently, WGS is mainly performed on *Mtb* strains grown in cultures and therefore a biosafety level 3 laboratory is needed. Further, WGS can not be achieved if the cultured isolates failed to grow or became contaminated. Therefore, there is much interest in performing WGS directly from sputum. Two studies showed that WGS directly from sputum is challenging due to the high level of contamination of human DNA (179, 180). Another study used biotinylated RNA baits to capture the whole genome of *Mtb* directly from sputum. It predicted resistant mutations in 32 of 43 (74%) people with drug-resistant TB. However, this method is not affordable in low-and middle-income countries (181). The study by Votintseva et al. showed that the results of WGS from a clinical *Mtb* sample using a low DNA concentration threshold (>0.05ng/ul) were comparable to those from culture-based WGS (130).

1.7.2.7. Lateral flow urine lipoarabinomannan assay (LF- LAM)

WHO recommended the LF-LAM (Alere Determine™ TB LAM Ag, Abbott, Palatine, IL, USA, previous Alere Inc., Waltham, MA, USA) as a rapid test to diagnose active TB disease in PLHIV with TB symptoms or in the advanced stage of HIV disease (182). LF-LAM is a lipopolysaccharide found in *Mtb* cell walls (183), released during metabolic processes or degenerating bacterial cells during active TB disease (184). LF-LAM is detectable in the urine and has a suboptimal sensitivity and specificity (Table 2) (185-187). Therefore, the LF-LAM should only be used in a targeted manner. The LF-LAM cannot detect drug-resistant mutations.

Table 2. Summary of TB diagnostic test (156, 159-161, 165, 169, 171, 173-175, 177, 186)

	Mtb detection			Laboratory	Drug susceptibility resistance		
	Sensitivity, 95% CI	Specificity, 95% CI	Time	Biosafety levels	Type of DST	Time	Drugs tested
Bacteriological confirmation							
Smear microscopy							
Ziehl-Neelsen	68% (65-71)	98% (97-99)	1 day	Basic	-	-	-
Fluorescence	73% (61-85)	98% (97-99)	1 day	Basic	-	-	-
Culture							
Liquid	reference	reference	8-16 days	3	phenotypic	14 days*	All drugs
Solid	reference	reference	16-29 days	3	phenotypic	42 days*	All drugs
Real-time PCR							
Xpert MTB/RIF	85% (79-90)	98% (97-99)	2 hours	Basic	genotypic	2 hours	RIF
Xpert ultra	91% (86-95)	96% (93-97)	2 hours	Basic	genotypic	2 hours	RIF
Xpert XDR	-	-	2 hours	Basic	genotypic	2 hours	INH, FLQ, INJ, ETH
Truenat MTB	73% (68-78)	98% (97-99)	1 hour	Basic	-	-	-
Trunat MTB Plus	80% (75-84)	96% (95-97)	1 hour	Basic	genotypic	1 hour	RIF
Line probe assay							
MTBplus	85% (70-93)	98% (96-99)	1-2 days	2 to 3	genotypic	1-2 days	INH, RIF
MTBsl	-	-	1-2 days	Basic	genotypic	1-2 days	FLQ, INJ
TB-LAMP	78% (71-83)	98% (96-99)	<1 hour	Basic	-	-	-
TB-LAM for PLHIV	42% (31-55)	91% (85-95)	<1 hour	Basic	-	-	-

* Additional days used to perform DST after the time used to detect *Mtb*
Abbreviation: 95% CI, 95% confidence interval; INH, Isoniazid; RIF, Rifampicin; FLQ, fluoroquinolone; ETH, ethionamide

1.7.3. Drug susceptibility testing (DST)

As discussed in section 1.7.2, some diagnostic tests also provide DST results. For an appropriate treatment of drug-resistant TB, it is crucial to obtain the drug resistance profile of *Mtb* strains (188). The gold standard method to detect drug resistance is a phenotypic, culture-based DST (189). An alternative to phenotypic DST is using molecular diagnostic tests or WGS to identify drug resistance profiles (168). Table 3 summarizes the anti-TB drugs, including the known genes with a high probability

of drug-resistant mutations covered by rapid molecular tests and WGS. Except for WGS, all rapid molecular tests only provide drug-resistant mutation for a limited number of anti-TB drugs.

Table 3. Summary of anti-TB drugs and the drug-resistant mutations detected by molecular test (170, 190).

Drug	Genes with common resistance mutations	Diagnostic tests
First-line anti-TB drugs		
Rifampicin	*rpoB*	Xpert MTB/RIF and Ultra; MTBDRplus; WGS
Isoniazid	*inhA*	Xpert XDR; MTBDRplus; WGS
	katG	Xpert XDR; MTBDRplus; WGS
	fabG1, ahpC	WGS
Ethambutol	*embB*	MTBDRSl v1.0, WGS
	embA, embC	WGS
Pyrazinamide	*pncA, clpC1, panD*	WGS
Group A		
Bedaquiline	*Rv0678, atpE, pepQ, mmpL5, mmpS5*	WGS
Levofkoxacin and moxiflocin	*gyrA*	Xpert XDR; MTBDRsl v1.0 and v2.0; WGS
	gyrB	Xpert XDR; MTBDRsl v2.0; WGS
Linezolid	*rplC, rrl*	WGS
Group B		
Clofazimine	*Rv0678, pepQ, mmpL5, mmpS5*	WGS
Cycloserine	*alr*	WGS
Group C		
Amikacin	*rrs*	Xpert XDR; MTBDRsl v1.0 and v2.0; WGS
	eis	Xpert XDR; MTBDRsl v2.0; WGS
	whiB7	WGS
Kanamycin	*rrs*	Xpert XDR; MTBDRsl v1.0 and v2.0; WGS
	eis	Xpert XDR; MTBDRsl v2.0; WGS
	whiB7	WGS
Capreomycin	*rrs*	Xpert XDR; MTBDRsl v1.0 and v2.0; WGS
	tlyA	WGS
Streptomycin	*rrs*	Xpert XDR; MTBDRsl v1.0 and v2.0; WGS
	whiB7, rpsL, gid, Rv1258c	WGS
Group D		
Delamanid	*ddn, fgd1, fbiA, fbiB, fbiC, Rv2983*	WGS
Ethionamide and prothionamide	*inhA*	Xpert XDR; MTBDRplus; WGS
	ethA	Xpert XDR; WGS
Imipenem and meropenem	-	WGS
Para-aminosalicylic	*folC, ribD*	WGS

Abbreviation: WGS, whole-genome sequencing

1.8. TB treatment

TB is a curable disease, but the treatment is long (6-24 months) compared to other bacterial diseases (where the treatment duration is typically ten days) (191-195). Currently, more than 27 anti-TB drugs are available (196). The anti-TB drugs are prescribed in different combinations depending on a person having pan-susceptible or drug-resistant TB.

In 2011, WHO categorized anti-TB drugs based on their efficacy and tolerability into first-line (most effective, less toxic) and second-line drugs (less effective, more toxic) (192). The first-line drugs are prescribed for pan-susceptible TB, while second-line drugs are used in drug-resistant TB, often combined with some first-line drugs to which the patient is susceptible. The second-line drugs can be categorized into different groups depending on their mode of action, route of administration, and potency (192-195). As shown in Table 4, the categorization of second-line drugs changed over time, as regimens changed and new evidence on drug-resistant TB became available.

Table 4. Changes of the hierarchy of recommended anti-TB drugs (192, 194, 195).

2011		2016		2019	
Group 1: first-line oral	Isoniazid Rifampicin Ethanbutol Pyrazinamide	Group 1: first-line oral	Isoniazid Rifampicin Ethanbutol Pyrazinamide	Group 1: first-line oral	Isoniazid Rifampicin Ethanbutol Pyrazinamide
Group 2: second-line injectable agents	Streptomycin Kanamycin Amikacine Capreomycin	Group A: second-line fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin	Group A: the second-line include all agents	Levofloxacin / moxifloxacin Bedaquiline Linezolid
Group 3: second-line fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin Ofloxacin	Group B: second-line injectable	Amikacin Capreomycin Kanamycin Streptomycin	Group B: second-line add one or both agents	Clofazimine Cycloserine / terizidone
Group 4: second line oral agents	Ethionamide / prothionamide Cycloserine / terizidone P-aminosalicylic acid	Group C: second-line other core drugs	Ethionamide / prothionamide Cycloserine / terizidone Linezolid Clofazimine	Group C: add-on agents (not core MDR-TB regimen)	Ethambutol Delamanid Pyrazinamide Imipenem-cilastatin / meropenem Amikacin / streptomycin Ethionamide or prothionamide P-aminosalicylic acid
Group 5: second-line drugs with limited data on efficacy or long term safety	Bedaquiline Delamanid Linezolid Amoxicillin / clavulanate Imipenem / cilastatin Meropenem High-dose isoniazid Thioacetazone Clarithromycin	Group D: add-on agents (not core MDR-TB regimen)	Pryazinamide Ethambutol High-dose isoniazid Bedaquiline Delamanid P-aminosalicylic acid Imipenem-Cilastatin Meropenem Amoxicillin-clavulanate Thioacetazone		

Abbreviation: MDR-TB, multidrug resistant TB

1.8.1. Treatment of pan-susceptible TB

Pan-susceptible TB is treated with the standard regimen over six months. The treatment can be divided into an intensive phase of two months, during which the patient takes all four first-line drugs (isoniazid, rifampicin, pyraminazid, and ethambutol) and a subsequent phase of four months where the patient continues with isoniazid and rifampicin (191). The treatment duration can be extended for up to seven months in patients with cavitations on their initial chest X-ray or with positive cultures after two months on TB treatment (191). Treatment success (including cured and completed treatment) of pan-susceptible TB is possible in 95% or more of patients. However, the treatment success rate is often lower due to challenges of daily treatment for six months, adverse events and non-adherence to treatment (188).

The TB treatment is similar in PLHIV and HIV-negative people but more complicated in PLHIV due to drug interactions of antiretroviral drugs and anti-TB drugs. It may require the substitution of rifampicin with other rifamycins (197, 198).

1.8.2. Treatment of drug-resistant TB

Sub-optimal treatment, treatment interruption, or incomplete treatment can result in mutations and drug-resistant *Mtb* strains (198, 199), leading to treatment failure and relapses. The accumulation of mutations leads to drug-resistant TB (198-200). However, a person can also be infected with a resistant *Mtb* strain. The treatment of drug-resistant TB is more challenging in PLHIV because of poor drug absorption due to vomiting or diarrhoea, immune reconstitution inflammatory syndrome (IRIS), and toxicity. Adverse drug interactions may occur between antiretroviral drugs and anti-TB drugs, leading to adverse reactions such as seizures, peripheral neuropathy, depression, hepatitis, gastrointestinal intolerance, renal toxicity, hearing loss and bone marrow toxicity (201-203).

The WHO drug-resistant TB treatment guidelines have changed over time. The first WHO guidelines entitled “The programmatic management of drug-resistant TB” were released in 2006 and updated in 2011 (192, 204). The updated guidelines recommended combining at least four drugs for the treatment of rifampicin-resistant TB or MDR-TB, including pyrazinamide, an injectable agent, and a late generation fluoroquinolone (moxifloxacin or levofloxacin) ethionamide or prothionamide, and cycloserine or para-aminosalicylic acid. The treatment duration was between 18 and 24 months. This lengthy treatment was expensive, often toxic, and the success rate was unsatisfactory, between 56% and 62% (205-207).

In 2016, new evidence supported a standardized short-course regimen of nine to eleven months, also known as the “Bangladesh regimen” to treat rifampicin-resistant TB or MDR-TB treatment. This regimen consists of an initial 4-6 months of kanamycin, moxifloxacin, ethionamide/prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol, followed by five months of moxifloxacin, clofazimine, pyrazinamide, and ethambutol (208, 209). Studies reported success rates with the "Bangladesh regimen" (sometimes with minor modifications) of 80.2% to 95.5% (209-212). The WHO recommended this short course regimen in 2016 under specific conditions. MDR-TB patients are excluded if exposed to second-line drugs for more than one month; there is resistance to fluoroquinolones or injectable agents, a severe form of extra-pulmonary TB or pregnancy (194).

A meta-analysis confirmed the effectiveness of the short course regimen (213). Furthermore, the STREAM trial showed similar treatment outcomes comparing the short-course regimen to the longer regimen (212). The long regimen prescribed in the STREAM trial was based on the WHO guidelines from 2011, so very different from the current recommended all-oral long regimen. The latest 2019 WHO guidelines for MDR-TB recommend a slightly modified standardised short-course regimen of nine to eleven months and a long all-oral regimen of 18-20 months (195, 196). The long all-oral regimen uses a hierarchy of recommended anti-TB drugs and is, therefore, to some extent, standardized (Table 4). The standardized short-course regimen includes 6 months of bedaquiline combined with 4–6 months of levofloxacin or moxifloxacin, clofazimine, pyrazinamide, ethambutol, high-dose isoniazid and ethionamide, followed by 5 months of levofloxacin or moxifloxacin, clofazimine, pyrazinamide, and ethambutol. People with MDR-TB are not eligible if exposed to any of the mentioned drugs for more than one month or have fluoroquinolone resistance (195, 196). The long all-oral regimen combines all three drugs in group A (levofloxacin or moxifloxacin, bedaquiline, linezolid) plus at least one from group B (clofazimine or cycloserine, terizidone) to ensure that four drugs are effective (Table 4). Drugs from group C can be added if a regimen cannot be built with group A and B drugs (195, 196). The recommendation of this new all-oral long regimen is based on a large individual patient data meta-analysis (214). According to all WHO treatment guidelines, drug-resistant TB can be treated with an individualised regimen based on the drug resistance profile (192-196, 204).

2. Aims and outline of the thesis

2.1. Aims and objectives

The overall aim of this thesis is to examine the epidemiology of TB, especially drug-resistant TB in PLHIV and HIV-negative people seeking care at health care clinics in low-and middle-income countries. In particular, it focuses on the transmission of *Mtb*, on the management of TB from diagnosis of TB to treatment and clinical outcomes during treatment, on drug-resistant TB, and mortality. The overall aim can be divided into three sub-aims:

Sub-aim 1: To describe the risk of *Mtb* transmission in a health care clinic

Sub-aim 1 focuses on a novel approach to measure the risk of *Mtb* transmission. The classical ways to investigate *Mtb* transmission are contact tracing, analyses of geo-temporal clustering, and molecular typing methods. However, these methods are resource-intensive and only measure transmission resulting in secondary cases. We propose a novel approach to study transmission resulting in infection or disease. Specific objectives of sub-aim 1 include:

> **Paper 1:** to develop a study protocol, which describes a novel approach to quantify the risk of TB transmission at a primary care clinic in Southern Africa.
>
> **Paper 2:** to estimate *Mtb* transmission in a primary care clinic in South Africa using novel approaches.

Sub-aim 2: To describe the management of TB at ART clinics

Sub-aim 2 focuses on the capacity/availability and routine practices of ART clinics related to diagnosis, treatment and clinical outcomes in PLHIV with pan-susceptible TB or MDR-TB. We used site-level data, patient-level data and clinical scenarios. Specific objectives of sub-aim 2 include:

> **Paper 3:** to study diagnostic modalities and clinical outcomes of extra-pulmonary TB compared to pulmonary PTB in adult PLHIV at ART clinics.
>
> **Paper 4:** to study capacity and routine practices of ART clinics related to the diagnosis and treatment of MDR-TB.

Sub-aim 3: To study the evolution of drug-resistant TB and clinical consequences

Sub-aim 3 focuses on the evolution of drug resistance in *Mtb* in the context of HIV co-infection using phenotypic drug susceptibility testing and whole-genome sequencing of clinical *Mtb* strains collected in low-and middle-income countries, where both diseases co-exist. We examine the association of HIV with drug-resistant TB and mortality. Specific objectives of sub-aim 3 include:

> **Paper 5:** to study the accuracy of drug susceptibility testing performed at ART clinics or TB clinics compared to phenotypic drug susceptibility testing performed at the Swiss reference laboratory and examine mortality by treatment appropriateness.

Paper 6: to study the accuracy of drug susceptibility testing performed at ART clinics or TB clinics compared to whole-genome sequencing and examine mortality by treatment appropriateness.

Papers 7: to explore the association between HIV-coinfection and the fitness effect of different drug resistance-conferring mutations in *Mtb*.

Paper 8: Natural polymorphisms in *Mtb* conferring resistance to delamanid in drug-naïve patients.

2.2. Outline of the thesis

My thesis consists of six first-author papers (papers 1-6) and two co-author papers (papers 7 and 8), to which I made substantial contributions. Papers 1-6 are covered in chapters 3-8, and papers 7 and 8 in chapter 9 and chapter 10. In the concluding chapter, chapter 11, I discuss the findings and the implication and provide an outlook for future research.

First author papers

3. Paper 1 – Study protocol on *Mtb* transmission

Novel approach to estimate tuberculosis transmission in primary care clinics in sub-Saharan Africa: protocol of a prospective study

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The article has been accepted on the July 6, 2020 and has been published in BMJ Open.
DOI: http://dx.doi. org/10.1136/bmjopen-2019- 036214

Contribution: I contributed to the conception and study design. I wrote the first draft of the manuscript and all subsequent versions – addressing comments from co-authors and reviewers- until the final published version.

Abstract

Introduction: Tuberculosis (TB) transmission is difficult to measure, and its drivers are not well understood. The effectiveness of infection control measures at healthcare clinics and the most appropriate intervention strategies to interrupt transmission are unclear. We propose a novel approach using clinical, environmental and position-tracking data to study the risk of TB transmission at primary care clinics in TB and HIV high burden settings in sub-Saharan Africa.

Methods and analysis: We describe a novel and rapid study design to assess risk factors for airborne TB transmission at primary care clinics in high-burden settings. The study protocol combines a range of different measurements. We will collect anonymous data on the number of patients, waiting times and patient movements using video sensors. Also, we will collect acoustic sound recordings to determine the frequency and intensity of coughing. Environmental data will include indoor carbon dioxide levels (CO_2 in parts per million) and relative humidity. We will also extract routinely collected clinical data from the clinic records. The number of *Mycobacterium tuberculosis* particles in the air will be ascertained from dried filter units using highly sensitive digital droplet PCR. We will calculate rebreathed air volume based on people density and CO_2 levels and develop a mathematical model to estimate the risk of TB transmission. The mathematical model can then be used to estimate the effect of possible interventions such as separating patient flows or improving ventilation in reducing transmission. The feasibility of our approach was recently demonstrated in a pilot study in a primary care clinic in Cape Town, South Africa.

Ethics and dissemination: The study was approved by the University of Cape Town (HREC/REF no. 228/2019), the City of Cape Town (ID-8139) and the Ethics Committee of the Canton Bern (2019-02131), Switzerland. The results will be disseminated in international peer-reviewed journals.

Strengths and limitations of this study

- We describe the protocol for a prospective study design to studying tuberculosis (TB) transmission in primary care clinics in high TB/HIV-burden settings.
- This rapid approach will combine a wide range of different measurements, including patient waiting times and movements, acoustic recording of coughing, measurement of carbon dioxide levels as a natural tracer gas, air humidity and semiquantitative detection of *Mycobacterium tuberculosis* (*Mtb*) particles in the air.
- We will develop a mathematical model which will integrate the collected data to estimate the risk of TB transmission, identify key drivers of transmission and evaluate the impact of infection control measures such as improved ventilation or wearing masks.
- The main limitation of this study design is the lack of direct observation of transmission events and the reliance on the number of *Mtb* particles in the air as a proxy for TB transmission.
- Study limitations pertain to the need for stable electricity and WiFi for data collection.

Introduction

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis (Mtb)*, remains a major global public health problem, particularly in the context of HIV and drug resistance. Sub-Saharan Africa is one of the most heavily burdened regions globally, although control measures have been in place since the beginning of the 20th century.[1] Over a century of investment in TB control has reduced TB mortality, but effective strategies are urgently needed to reduce TB transmission.[2] Drivers of the TB epidemic in sub-Saharan Africa are HIV-infection and the resulting immunodeficiency (the strongest risk factor),[1] delayed diagnosis and treatment as well as undetected and untreated cases of TB or drug-resistant TB. These factors allow patients with infectious TB to transmit *Mtb* to the community.[3–5] There are still many gaps in our knowledge on TB transmission such as the factors and locations associated with the risk of transmission, the effectiveness of infection control measures at clinics in high-burden settings and the most appropriate intervention strategies to interrupt transmission.[5–7]

For TB transmission to occur, infected individuals must expel *Mtb* bacilli from their respiratory tract, and an uninfected individual must inhale aerosols containing live bacilli to become infected. Transmission of *Mtb* is difficult to measure due to the lack of an in vitro test assay. The preferred approach is to measure presumptive transmission resulting in secondary cases as determined by molecular/genomic epidemiology. TB transmission has traditionally been investigated using contact tracing, analyses of geo-temporal clustering and molecular typing.[8 9] However, molecular methods require resource-intensive culturing of strains and measure only transmission resulting in secondary cases. Furthermore, contact tracing is difficult to implement in resource-limited settings and may not be an effective control strategy in endemic areas where casual contacts are increasingly recognised as an important contributor to transmission.[10] New approaches are therefore urgently needed.

Mtb is carried in airborne particles (called infectious droplets), which are generated when people with TB cough, sneeze or shout.[5] Indoor carbon dioxide (CO_2) levels can be used to assess the amount of exhaled air in a room and the amount of rebreathed air.[11–13] Humidity is associated with *Mtb* survival in the air.[14] Viable *Mtb* particles have been captured from contaminated air.[11 15–17] We describe a unique study design to assess risk factors for airborne TB transmission in primary care clinics in high TB/HIV-burden settings. The approach combines a range of relevant measurements, including data on patient and infrastructure, movements of patients through the facility, coughing, environmental indoor CO_2 levels and concentration of *Mtb* particles in the air.

We hypothesise that (1) exposure to *Mtb* particles at the clinic can be estimated by studying the patient flow and CO_2 levels; (2) the number of individuals present, the rebreathed fraction and the frequency of coughs influence exposure to *Mtb*; (3) clinics with small and crowded waiting rooms, low ventilation and suboptimal patient separation have an increased risk of TB transmission.

Methods

Study design

Longitudinal study with data collection at the levels of the patients, the clinic and the environment. Data collection will take place for over 4 weeks. Figure 1 shows the floor plan of an exemplary primary clinic with the planned study activities, including the recording of patient movements, coughing, CO_2 levels and *Mtb* particles in the air.

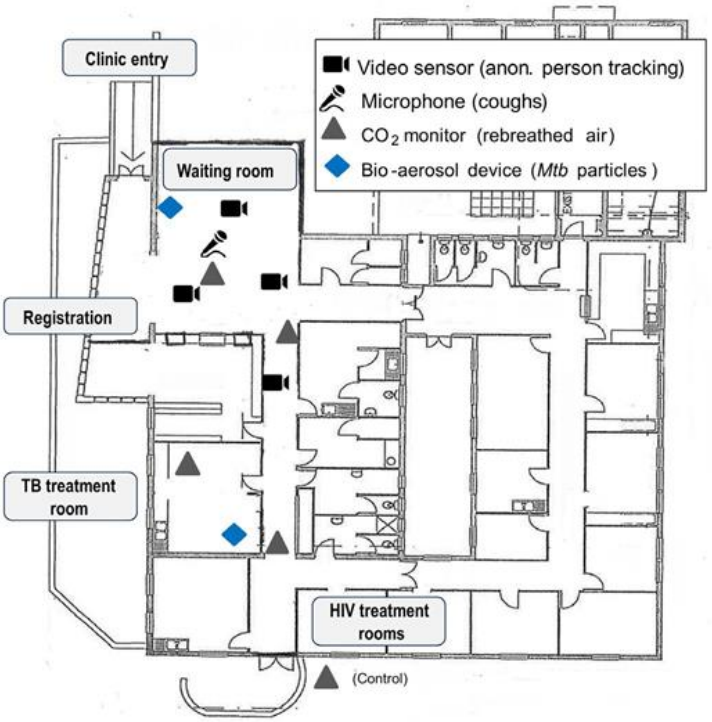

Figure 1. Floor plan of the pilot primary care clinic in Cape Town, South Africa, indicating study measurements. *Mtb*, *Mycobacterium tuberculosis*; TB, Tuberculosis.

Study setting and study population

The study will take place at several antiretroviral therapy (ART) or primary care clinics in countries participating in the International Epidemiology Database to Evaluate AIDS (IeDEA) in Southern Africa collaboration with a high TB and HIV burden.[18] These clinics are located in urban and peri-urban communities with predominantly young and black African residents. TB and HIV are both prevalent in these communities.

Patient-level variables

Video sensor data

A person-tracking sensor system developed by Xovis (Zollikofen, Switzerland and Cambridge, Massachusetts, USA; see www.xovis.com) will be used to monitor the clinic attendees' movements. The data will be used to calculate waiting times, the number of people in different locations and the average distance between people, and to identify highly frequented areas. Several sensors will be installed to cover the clinic area, calibrated and validated. Sensors with overlapping ranges will be combined for the seamless coverage of people's movements over large areas (figure 2). The raw data consist of the person's height, time, date and the position (x–y coordinates) for each unique

individual during the duration of their stay within the clinic. The data are captured every 0.25 s. The raw data are then parsed by a python script to calculate the height, total movement and observation time at different locations, and to visualise hotspots.

The sensors have four levels of privacy. In our study, privacy will be set to level 2, which means that the data are a fully anonymised stream of the coordinates of moving dots. These dots will be linked probabilistically to the anonymised clinical data thus excluding any risk of re-identification. Further, the images of tracked individuals taken by the sensors will not be stored (see also the data privacy and security statement by Xovis).[19 20]

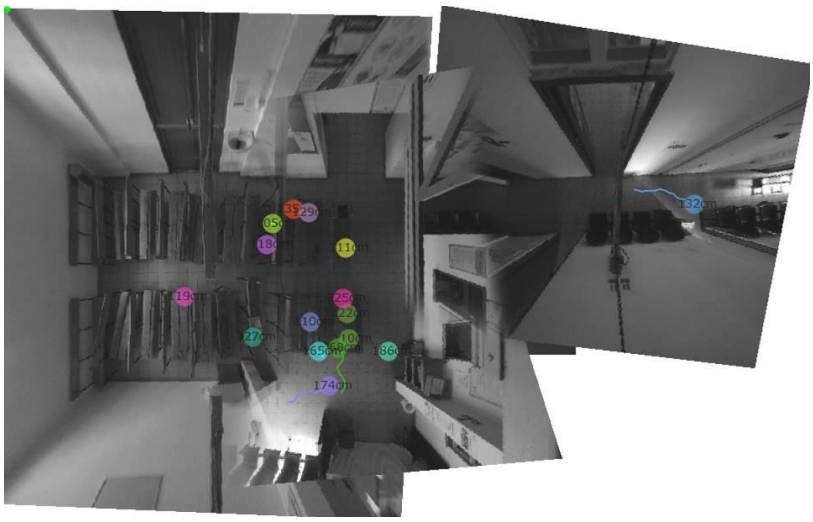

Figure 2. Output from video sensors with moving dots showing the tracked persons. The numbers in the dots indicate the height of patients. The different sectors coveredby the sensors are merged images from the pilot study.

Patient and clinic data

We will extract clinical data from the electronic patient registry for all patients who visited the clinics during the 4 weeks of data collection. The data will include date and time of registration, age, sex, height (used for linkage with video sensor data), HIV status, presumptive TB ('TB suspects'), mode of TB diagnosis (sputum smear microscopy, Xpert MTB/RIF, mycobacterium culture), TB diagnostic test results, date of TB treatment start and current anti-TB treatment regimen. We will not extract any personal data, such as names or social security numbers. The clinic-level data will include information such as the setting (urban or rural), level of care, the number of patients seen each year, availability of adult and paediatric care, TB control measures (natural ventilation, use of masks, separation of patients with TB or coughers) and the floor plan of the clinics. The data will be collected using the web-based REDCap Data Entry System (www. redcap.org).[21]

Definitions

Presumptive TB refers to a patient who presents with clinical symptoms or signs suggestive of TB. A bacteriologically confirmed TB case is a person for whom a biological specimen is positive by smear, microscopy, culture or rapid diagnostic such as Xpert MTB/RIF or line probe assay.[22]

Environmental variables

CO_2, relative humidity and temperature monitoring

We will use CO_2 monitors (Digital CO_2 Monitor Carbon Dioxide Metre XE-2000, XEAST, Guangdong, China) which include the COZIR-A sensor for ambient CO_2 levels of 0%–1% (Gas Sensing Solutions, Cumbernauld, Scotland). The monitors will record indoor CO_2 concentrations (in parts per million

(ppm)), temperature and relative humidity at minute intervals. Data from the five monitors are stored and exported in serial digital format.[15 16] At each clinic we will install five monitors to cover the most visited spaces and a control area outside the clinic (figure 1). The monitors auto-calibrate over time to a standard minimum value of 400 ppm. This value is close to the average monthly outdoor carbon dioxide concentration measured at Cape Point, South Africa (405.48 ppm in December 2018).[23] Monitors will be run for 1 week before the start of the study to allow them to calibrate and to confirm that they delivered comparable data.

Cough monitoring

We will install a microphone (RØDE NT-USB, Sydney, Australia) near the ceiling to continuously record the sounds in the waiting room (figure 1). CoughSense, a deep learning cough detection algorithm based on MXNet, an open-source deep-learning software framework, was developed to classify audio signals as coughing or other sounds.[24] The algorithm uses spectrograms extracted from the raw audio for classification. The model was trained and tested using multiple audio recordings obtained through clinical and ambulatory deployments. We will identify audio records with cough sounds and calculate the cough frequency, intensity and duration. The cough data will be linked with the CO_2 data and video sensor data by time and date. Finally, we will probabilistically link the video sensor data (ie, the moving dots) with the clinical data by the height of the patient, time and date of the visit.

Bio-aerosol sampling and molecular detection

We will collect *Mtb* particles from the air using mobile bio-aerosol sampling devices (Dry Filter Unit (DFU) 1000, Lockheed Martin Integrated Systems, Gaithersburg, Maryland, USA). The DFU 1000 is a portable biological air sampler. Ambient air is drawn through 1 µm polyester felt filters at a rate of ~1000 L/min via an electrical blower. The DFU is a useful collection system for low concentration aerosols, capturing particles of 1 µm or larger, and allowing easy access for the retrieval of filters. One DFU will be placed in the waiting room and the other in the TB treatment room (figure 1). Each DFU collects air for 7 hours (two periods of 3.5 hours) every day onto two filters. Due to logistical and human resource limitations, we will be able to change filters only two times per day. Therefore, we decided to run the mobile bio-aerosol sampling devices during the busiest time at the clinic, between 07:00 and 14:00. All other data will be collected from 07:00 to 16:00.

As previously described,[17] duplicate filters from each sampling session will be transferred to 50 mL Falcon tubes and vortexed in sterile phosphate buffered saline (with 0.05% Tween 80). Following centrifugation at 3750 rpm for 15 min, filters will be removed and the pellet subjected to DNA extraction. DNA from *Mtb* cells will be extracted using an in-house lysis buffer with subsequent pelleting (centrifugation 13 000 rpm for 10 min) of DNA and resuspension in 50 µl of Tris-EDTA buffer (1 mM Trist, 1 mM EDTA, pH 8.0). Given that droplet digital PCR (ddPCR) is relatively robust against inhibitors, no further DNA purification will be required. The primer/probe combinations and reaction conditions for *Mtb*-specific ddPCR have been described.[16] Samples with known amounts of purified *Mtb* DNA (0.01 ng and 0.001 ng) will be included to serve as positive and nuclease-free water as negative control. The data generated from the ddPCR reaction will be analysed via the Umbrella pipeline,[25] using wells with a minimum of 10 000 droplets.

Statistical analyses

We will describe the data and examine associations between sources of data and then use results to parameterise a mathematical model (table 1). The statistical analysis will quantify the joint association between the clinical and environmental variables and the number of *Mtb* particles measured by the mobile aerosol sampling. To this end, we will use Poisson regression, with the number of *Mtb* particles by the period of time as the dependent variable, considered as a proxy measure for the risk of TB transmission. As this variable is measured by periods of 3.5 hours, the other variables will be aggregated over the same periods. We will consider all combinations of independent variables, including second-order interactions, and compare the model using standard model selection methods. We will use variable selection methods in a Bayesian framework, including the deviance information criterion and the leave-one-out information criterion.[26]

Table 1. Description of the measurements

Data source	Parameter	Description	Unit	Measurement taken by:
CO_2 monitor	CO_2	Observed CO_2 concentration in the indoor air per minute and a control in the outdoor air. Based on CO_2 levels (parts per million [ppm]) and people density, we will calculate rebreathed air volume (RAV), which is used as a proxy for airborne TB transmission.[15]	Ppm	minute and date
	Relative humidity	Data on the effects of relative humidity on the survival of airborne bacteria are inconsistent.[24 33] However, a recent study found that relative humidity above 65% is associated with *Mtb* survival in the air.[34]	%	minute and date
	Temperature	Temperatures above 24°C are required to reduce airborne bacteria survival.[24 33]	°C	minute and date
Cough recording	Frequency	One of the typical symptoms of TB is coughing; coughing is also the main way of transmission.	N	minute or day and date
	Duration	Duration of each cough is different from healthy and people with TB or other lung diseases [26]	Sec	cough by minute and date
	Intensity	Intensity of each cough is different from healthy and people with TB or other lung diseases. [26]	Decibel	cough by minute and date
Mobile aerosol sampling	*Mtb* DNA copies	Detection of *Mtb* particles in the air by filter or per day (7am to 2pm).[16]	Copies per microliters	filter (ca.3.5h sampling) or per day
Video sensor	Number of people	From the raw data (x-y coordinates) we can calculate the number of people at a given location by 0.25 seconds and by minute.	n of people	0.25 seconds or minute and by date
	Time spent at a given location	From the raw data (x-y coordinates) we can calculate for each person their time spent at different locations.	Minutes	minute and date
Patient charts	Number of registered patients	All patients who are visiting the clinic are registered.	n of registered patient	minute and day
	Number of presumptive TB and of TB patients	From all registered patients we will know the number of presumptive TB and of TB patients.	n of presumptive TB and TB patients	minute and day

dB, decibel; Mtb, *Mycobacterium tuberculosis*; n, number; ppm, parts per million; s, second; TB, tuberculosis.

We will operationalise the variables as follows:

1. Patient data: Numbers and characteristics of patients consulting the clinic overall will be summarised using descriptive statistics.
2. Video sensor data: Raw data about individuals' movements will be transformed into waiting times until medical consultation, number of individuals in the different locations, highly frequented areas in the clinic and the average distance between patients (ie, clustering). We will link each tracked individual to the clinical data collected from the clinic's database (ie, TB/HIV diagnosis), using the order of arrival and time of registration.
3. CO_2 data: Measurements of CO_2 concentration at the different locations in the clinic (waiting room, registration desk, TB treatment room), together with estimates in outdoor air, will be

used to estimate the proportion of air in the different locations that was expired by individuals, the rebreathed fraction.[12] We will use a modified Wells-Riley model appropriate for non-steady states conditions of ventilation and number of individuals to describe and calculate the shared rebreathed air in the different locations in either litre/minute or litre/day and the air exchange (litre/hour per person). Table 2 describes the parameters to calculate the shared rebreathed air and the air exchange.[13 16 27 28]

The proportion of rebreathed air (f) will be calculated from the excess CO_2 measured indoors, divided by the exhaled CO_2 (Ca):

Equation 1:

$$\mathrm{f} = \frac{(\mathrm{C} - \mathrm{Co})}{\mathrm{Ca}}$$

(see table 2 for definitions of parameters in this and the following equations).

Before we can calculate the rebreathed air volume (RAV) we will need the rebreathed proportion from other people (ƒo). Therefore, we need to know the number of people (n) present at each time point at the clinic:

Equation 2:

$$fo = f \times \frac{(n-1)}{n}$$

Finally, we can calculate the RAV for each minute by multiplying fo and the minute respiratory volume (8 L/min, (p)) as:

Equation 3: $Rebreathed\ air\ volume\ (RAV) = (pfo)$

In the next step, we aim to calculate the ventilation rate (air exchange). The indoor CO_2 generated rate is the product of the average volume of gas exhaled per person (0.13 L/s per person, (V)) and the CO_2 concentration of the exhaled air:

Equation 4: $Indoor\ CO_2\ generation\ rate\ in\ l/s\ per\ person\ (G) = VxCa$

The ventilation rate is expressed as:

Equation 5:

$$Ventilation\ rate\ in\ l/s\ per\ person\ (Q) = \frac{G}{C - Co}$$

To calculate the air exchange per hour (Equation 6) we need to know the volume of air in a given location.

Equation 6:

$$Air\ exchange\ per\ hour\ (ACH) = \frac{3600xQxn}{vol}$$

4. Relative humidity and temperature: We will describe changes in relative humidity and temperature over the day.
5. Cough sounds: Frequency, intensity, duration of recorded coughs per time period.[29 30]
6. Detection of *Mtb* in bio-aerosol sampling: The number of *Mtb* genome copies present in each sample.

Table 2. Description of the variables to calculate the shared rebreathed air volume and air exchange as well as the parameters to construct the mathematical transmissionmodel

Parameter	Description	Value
C	Observed CO_2 concentration in the indoor air per minute	Observed
Co	CO_2 concentration in the outdoor air per minute	400-420ppm
Ca	CO_2 concentration in the exhaled air	38000-40000ppm
f	Proportion of rebreathed air	equation
n	Number of people recorded at the location	Observed
fo	Rebreathed proportion from other people	Equation
p	Minute respiratory volume	8 l/per minutes
RAV	Rebreathed air volume	Equation
V	Average volume of gas exhaled per person	0.13 l/s per person
G	Indoor CO_2 generation rate (l/s per person)	equation
Q	Ventilation rate (l/s per person)	equation
vol	Volume of the room	calculated

Mathematical modelling

A mathematical model (figure 3) will integrate all sources of data to model the risk of TB transmission and identify key drivers of transmission.[13 27 31 32] We hypothesise that the number of individuals present, the rebreathed fraction and the frequency of coughs will have the greatest influence on the risk. As we do not observe transmission events, we will use yu, the number of *Mtb* genomes counted by the bio-aerosol sampling for each 6 hour period u, as the dependent variable. The model will describe yu as a Poisson process with time-dependent intensity λt (also called a Cox process). This intensity can be interpreted as a proxy for the (unobserved) risk of TB transmission. We will model λtu using 10 min time periods t within u. In a first simple model, λtu will integrate multiple independent variables xtu: the number of individuals in the waiting room during time t, the rebreathed fraction and the number of coughs. The model will be estimated in a Bayesian framework using Stan, a probabilistic programming language.[33]

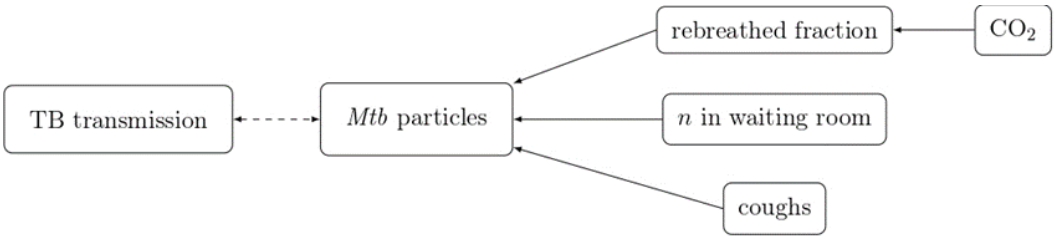

Figure 3. Model structure. *Mtb*, *Mycobacterium tuberculosis*; TB, tuberculosis

We will use several metrics to assess the goodness of fit of the model. We will then add more complexity to the model by integrating other independent variables such as the characteristics of the patients, shared rebreathed air (CO_2 monitors) [12 13 15 16], patient flow (clustering of individuals, movements of patients) and other potential drivers (intensity/frequency/duration of coughs).[29 30]

Having developed the model of the risk of TB transmission, we will evaluate the possible effect of interventions such as limiting the number of patients in the waiting room, separating coughers or increasing ventilation. All analyses will be performed in R (V.3.6.0) or Stata (V.15.1, Stata Corporation, Texas, USA).

Reporting

The results from this study will be reported following the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology statement.[34]

Pilot study

We conducted a pilot study in Cape Town (South Africa) to examine the feasibility of our approach (figure 1). The pilot study took place at a primary care clinic, which incorporates HIV counselling and testing, and a TB clinic for the diagnosis and management of TB. The clinic is situated in Masiphumelele, a large settlement of formal and semiformal housing in Cape Town, which has previously been described.[35 36] The clinic is open on workdays from Monday till Friday from 07:00 till 16:00. We studied clinic activities for over 4 weeks on workdays between July 25 and August 23 2019. Data collection was successful overall, however, we experienced several power cuts during the pilot study. An important lesson learnt is that power banks for laptops and WiFi routers are needed, as well as Uninterruptible Power Supply (UPS) and access to a generator as back up for the DFU and CO_2 monitors.

Patient and public involvement

We discussed the aims and study design with local clinic staff, colleagues at the University of Cape Town and public health specialists early on in the planning phase and developed the specific objectives and data collection procedures in collaboration with them. Patients were not involved in the design, recruitment or conduct of the study. We will make the results of this study available to the participating clinics and the public health authorities.

Discussion

TB control is particularly relevant for sub-Saharan Africa, which carries a disproportionally large portion of the global burden of both TB and HIV. There is an urgent need to understand the drivers of TB transmission to reduce TB incidence using new intervention approaches.[1] A better understanding of transmission, coupled with a rapid test system are likely to contribute to improving TB control in clinic settings. This project will provide new insights into the complex TB transmission framework at a primary care clinic in an endemic setting using clinical data, CO_2 levels, cough analyses, video tracking and *Mtb* particles sampled from the air. Although the large scale implementation and evaluation of interventions are beyond the scope of this study, the results will generate new hypotheses and opportunities for public health intervention studies (eg, randomised controlled or cluster randomised trials).

Importantly, with mathematical models based on real-life data, we can evaluate the likely effect of interventions thus improving intervention studies and inform help with logistical and infrastructural planning of primary care clinics to reduce the transmission risk. Having established feasibility in one clinic in South Africa, we are planning to collect data in several countries in sub-Saharan Africa. The results from this broader study have the potential to inform national and international guidelines to reduce TB transmission at healthcare centres.

Strengths and limitations

This is a novel and rapid approach to studying TB transmission combining a wide range of different measurements, which goes beyond the traditional methods such as contact tracing, geo-temporal clustering or molecular genotyping. It will lead to a comprehensive transmission model to measure the effects of various interventions in the clinic setting, paving the way for future studies. Study limitations pertain to the need for stable electrical power and WiFi for over 24 hours for data

collection at the primary care clinic, which is often an issue in low income and middle-income countries. We will address this problem by using UPS power stabilisers with access to generator back-up power.

Ethics and dissemination

The University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC/REF: 228/2019), the City of Cape Town (Project ID 8139) and the Ethics Committee of the Canton of Bern (2019-02131), Switzerland approved the pilot study. Expansion of the study will include other clinics in the greater Cape Town metropolitan area, but also clinics in Zambia, Zimbabwe or Malawi, including urban and rural sites to ensure representative and generalisable results. Separate ethical approval will be sought from the relevant ethics committees or institutional review boards. The results will be disseminated in international peer-reviewed journals and presented at national and international conferences.

Acknowledgements

We would like to thank Mbali Mohlamonyane for setting up the bio-aerosol sample collection system, Zeenat Hoosen and Ronnett Seldon for developing the laboratory protocols. We are also grateful to the clinical manager, the staff members at the clinic and all patients who contributed to this study. Finally, we would like to thank the City of Cape Town for the support they gave us for the use of one of their clinic facilities. Research findings and recommendations do not represent an official view of the City of Cape Town.

Author's contributions

Overall concept: KZ, CM, MB, RW, ME, LF. Medical informatics: SB, JR. Cough sound concept: XL, MS, CM, RW. Video sensor concept: KZ, JR, SB, ME, LF. Patient data concept: CM, KM, RW. Laboratory work concept: CM, ASK, KM, DW, RW. Modelling work concept: KZ, JR, SB, ME, LF. Pilot study coordination: CM, KM, RW. KZ, MB, ME, LF wrote the first draft of the paper, which was reviewed by all authors and revised on the basis of the comments received by co-authors. All authors approved the final version of the manuscript.

Funding

The rollout of this study is supported by a grant from the Swiss National Science Foundation (grant no. CRSK-3_190781). KZ and LF are supported by the National Institute of Allergy and Infectious Diseases (NIAID) through grant no. 5U01-AI069924-05, ME by special project funding (grant no. 174281) from the Swiss National Science Foundation.

Competing interests

None declared.

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4. Paper 2 – Estimating *Mtb* transmission

Estimating tuberculosis transmission in a primary care clinic in South Africa using novel approaches

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The article has been submitted to the Journal of Infectious Diseases on the July 16, 2020 and is in revision.

Contribution: I contributed to the conception and study design. I visited the clinic and installed all measurements. I coordinated the data collection. I cleaded the data and Julien helped me with the data analysis. I wrote the first draft of the manuscript and all subsequent versions – addressing comments from co-authors and reviewers – until the final published version.

Abstract

Background: Congregate settings, such as healthcare clinics, may play an essential role in *Mycobacterium tuberculosis (Mtb)* transmission. Using patient and environmental data, we studied transmission at a primary care clinic in South Africa.

Methods: We collected patient movements, cough frequency, and clinical data, and measured indoor carbon dioxide (CO_2) levels, relative humidity, and *Mtb* genomes in the air. We used statistical models to investigate associations.

Results: We analyzed 978 unique patients who contributed 14,795 data points. The median patient age was 33 years ([IQR] 26-41), 757 (77.4%) were female. Overall, median CO_2 levels were 564 ppm (IQR 495-646), highest in the morning. The median number of coughs/day was 466 (368-503), overall median *Mtb* DNA copies/μL/day 4.2 (IQR 1.2-9.5). We found an increase of *Mtb* genomes in the air of 32% (95% credible interval 7%-63%) per 100 additional young adults (aged 15-29) and 1% (0%-2%) more *Mtb* DNA per 10% increase of relative humidity. Estimated cumulative transmission risks for patients attending the clinic monthly for at least 1 hour range between 9%-29%.

Conclusions: We identified young adults and relative humidity as potentially important factors for transmission risks in healthcare clinics. Our approach should be used to evaluate interventions to halt transmission.

Introduction

Caused by *Mycobacterium tuberculosis (Mtb*), tuberculosis (TB) remains a global public health problem and one of the deadliest infectious diseases worldwide. Understanding TB transmission at primary care clinics is of particular public health importance in high TB/HIV burden settings, such as South Africa, and in places with a risk of transmission of multidrug-resistant (MDR) and extensively drug-resistant *Mtb* in clinics (1). Sub-Saharan Africa is one of the most heavily burdened TB regions. *Mtb* is transmitted by droplet aerosols generated when people infected with TB cough, sneeze, shout, speak, or breathe (2, 3). For TB transmission to occur, an infected person must expel *Mtb* bacilli from their respiratory tract, and an uninfected person must inhale *Mtb* bacilli-containing aerosols. Although TB control measures have been in place since the beginning of the 20th century, *Mtb* transmission is difficult to measure. Currently, the preferred approach is to measure presumptive transmission by determining secondary cases through molecular and genomic epidemiology (4, 5). This approach is expensive and not feasible in all settings. Therefore, new approaches to measure TB transmission are needed.
This study piloted a novel approach to estimate transmission risk based on environmental measurements and patient data at a South African primary care clinic. We measured indoor carbon dioxide (CO_2) levels, which indicate the proportion of exhaled, rebreathed air in a room (6-8). We also captured aerosol droplets containing viable *Mtb* bacilli from contaminated air (6, 9-11) and measured humidity, which is associated with the survival of airborne *Mtb* (12). We obtained clinical data on patient diagnoses, visit frequency from electronic medical records, and cough counts in waiting areas, and we tracked people's movements through the primary care clinic. Combining the different data allowed us to assess risk factors for airborne *Mtb* transmission in a high TB/HIV burden setting (13).

Methods

Study design

We previously described the study design in detail (13). We collected environmental and patient data over four weeks from July 25 to August 23, 2019 at a primary care clinic in Cape Town, South Africa.

Study setting

The primary care clinic offers both TB and HIV services and reproductive health and childhood immunization services, Monday to Friday, from 7 am to 4 pm. The clinic is situated within a large settlement of formal and semiformal housing where both TB and HIV are highly prevalent (14, 15). We delineated three areas within the clinic: the registration area, the waiting room, and the TB treatment room (Figure 1). Further we defined three time periods: morning (7am-10:30am), midday (10:30am-2pm), and afternoon (2-4pm).

Patient data

Tracking data

We used an anonymized movement tracking system (Xovis; Zollikofen, Switzerland; see www.xovis.com) to monitor people's movements (staff members, patients, and other visitors) throughout the clinic. The resulting date- and time-stamped movement data consisted of a person's height, their position recorded as x-y coordinates, and a unique signal for each person while in the clinic. The tracking data were captured every 0.25 seconds (Table S1) (13). If individuals went out of a sensor's range and subsequently returned, they could contribute multiple signals. Thus, the number of captured signals is higher than the number of unique persons. While in the waiting room, close contacts were defined as other persons passing within a radius of 1 meter.

Clinical data

We extracted clinical data from the electronic patient registry for all patients who visited the clinic during the study period. These data included the date and time of arrival for the clinic visit and when the patient passed by the registration desk and their age, sex, TB diagnostic results, and date of TB treatment start (if applicable).

Environmental data

CO_2 monitoring

Three CO_2 monitors (Digital CO_2 Monitor Carbon Dioxide Meter XE-2000, XEAST; Guangdong, China) covered the clinic's most crowded spaces. The monitors recorded indoor CO_2 concentrations (in parts per million [ppm]), temperature, and relative humidity at one-minute intervals (Table S1) (9, 10). Before data collection, the monitors were regularly auto-calibrated to a standard minimum value of 400 ppm (16).

Cough monitoring

We installed a microphone (RØDE NT-USB; Sydney, Australia) near the clinic's waiting room ceiling to continuously record sounds. We used a cough detection algorithm based on MXNET's open-source deep learning software framework to classify audio signals as coughing or other sounds (CoughSense; Seattle, Washington, USA) (17). In addition, we developed a cough counting algorithm to test for cough in the recorded coughs automatically. We trained, tested, and validated the algorithm model using multiple audio recordings obtained during the study period. The algorithm extracted the frequency and duration of coughs per minute (Table S1).

Bioaerosol sampling and molecular testing

Air was sampled using mobile bioaerosol sampling devices. The number of *Mtb* genomes was ascertained from dried filters using highly sensitive droplet digital polymerase chain reaction (PCR) (11). We placed one bioaerosol sampling device in the clinic's waiting room and the other in the TB treatment room. During data collection, each bioaerosol sampling device collected air through two filters over two time periods (morning and midday). Each day both devices collected air for about 3.5 hours, totalling approximately 7 hours per day (Table S1).

Linkage of people tracking data with clinical patient data

We applied several criteria to link the movement tracking system data with the clinical data. We included people who (1) passed by registration and (2) had a height of at least 140cm according to the tracking data to exclude children; we included clinical visits of patients aged 15 years and older from the clinical data. We then combined the datasets using the time-stamp of when a person was recorded by the tracking system in the registration area and the time a patient was registered in the electronic patient registry. We identified 2,355 adult patients (≥15 years) whose visits were recorded in the clinic's electronic patient registry from the clinical data. After linking with the movement tracking data, we included 978 unique adult patients, resulting in 1,135 clinical visits.

Statistical analyses and modelling

We used descriptive statistics for the environmental and patient data obtained in the different clinic areas. We calculated the number of individuals in the three clinic areas, the time spent in the waiting room, and the number of contacts an individual had during this time period, thus enabling the identification of highly frequented areas.

As previously described, we calculated the rebreathed air volume and ventilation rates from CO2 and clinic presence (10, 13). We summarized the coughs per minute in the waiting room over the three time periods (18, 19). We described the number of *Mtb* genome copies present in each filter by time period and clinic area.

We used a negative binomial regression model to assess clinical and environmental factors associated with the number of *Mtb* genome copies measured in the waiting room air (Table 1). Using the mean for the environmental data (CO_2, rebreathed air volume, and relative humidity), the total

number of people present in the clinic, and the total number of coughs, we aggregated the data by the minute to the exact time period of the bioaerosol sampling devices. The results are unadjusted and adjusted risk ratio per unit increase with 95% credible intervals. The model was adjusted for sex, age group (15-29 years, 30-44 years, 45-59 years, and >60), relative humidity, rebreathed air volume, and frequency of cough (Table 1).
Finally, we calculated the risk of *Mtb* transmission per hour during the day and per each clinical visit as previously described (20). Briefly, we used the modified Wells-Riley formula considering the work of Rudnick Milton on non-steady state situations to estimate the annual risk of TB transmission, taking into account the re-breathed air volume, time at risk, the infectious quanta of contagion, and the number of people occupying the confined space (6, 8). The parameters we used to calculate the risk of *Mtb* transmission are given in Table S2.
All analyses were performed in R (version 3.6.0) (21).

Ethics statement
The University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC/REF: 228/2019); the City of Cape Town (Project ID: 8139), South Africa; and the Ethics Committee of the Canton of Bern (2019-02131), Switzerland approved the study.

Results

Patient data

Movement of patients
The movement tracking system captured 14,795 unique data points corresponding to people in the clinic between July 25 and August 23, 2019. The median number of unique signals per day was 706 (interquartile range [IQR] 622-803). Most individuals visited the clinic in the morning when the highest density of individuals was found in the waiting room (Figure 2A). The median time spent in the waiting room was 24 minutes (IQR 23-27 minutes).

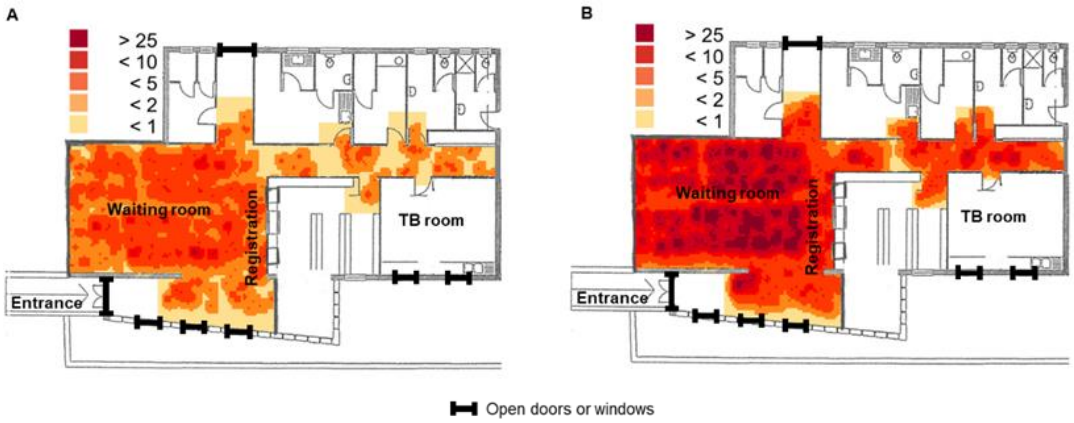

Figure 1: Density of (A) potentially infectious TB patients (defined as being diagnosed with TB [clinically or bacteriologically confirmed] one week before the study started or up to three months after the monitoring period [July 18- November 25, 2019]); and (B) all other people visiting the primary care clinic over the study period. Data from the movement tracking system were linked with clinical data from the electronic register.

Patient characteristics
After data linkage, we included 978 unique patients. Their median age was 33 years ([IQR] 26-41), and 757 patients (77.4%) were female. Overall, 171 (17.5%) had a TB diagnosis at some time, among whom 153 (90.6%) had a clinical history of TB, and 16 (9.4%) had active pulmonary TB and were potentially infectious at the time of their clinic visit (Table S3). The density of potentially infectious TB patients and all other people was highest in the waiting room (Figure 1). These potentially infectious

TB patients were more likely HIV-positive males who had three or more visits during the four weeks (Table S3).

Time in the waiting room

The median time a patient spent in the waiting room was 41 minutes (IQR 17-85 minutes) with a median of 62 (IQR 16-173) close contacts (within a radius of 1 meter). There were no significant differences between potentially infectious TB patients and all other patients in the time spent in the waiting room (41 vs 43) or in the number of contacts (67 vs 66).

Coughing

The median number of coughs per day in the waiting room was 466 (368-503). The total number of coughs was higher at midday than in the morning (495 vs. 421, Table 2). The median length of coughs was 0.67 seconds (IQR 0.47-0.91).

Environmental data

CO_2 levels

The median CO_2 level in the clinic was 564ppm (IQR 495-646). It was higher in the morning than at midday and in the afternoon (639 vs 568.7 vs 477ppm). We measured the highest CO_2 levels in the waiting room (Table 2, Figure 2B). The share of time people experienced CO_2 levels at or above 1,000 ppm of the opening hours was 4.7%.

Table 1. **Environmental data collected at a primary care clinic in Cape Town, South Africa, overall and by location.**

	Overall median, (IQR)	**Registration area** median, (IQR)	**Waiting room** median, (IQR)	**TB treatment room** median, (IQR)
CO_2 levels				
Per day	564.3 (495-646)	564 (494-686)	646 (531-765)	471 (447-516)
Time				
Morning	639 (551-753.7)	669.5 (551-823)	747 (623-852)	497 (460-572)
Midday	568.7 (514.5-624)	570 (502-659)	655 (564-742)	468 (445-504)
Afternoon	477 (455.7-517.3)	487 (461-524)	491 (458-565)	453 (437-477)
Rebreathed air volume in litres/day				
Per day	46.5 (22.7-74.8)	42.3 (22.1-74.0)	68.1 (33.0-102.2)	9.5 (0-18.6)
Time				
Morning	46.5 (44.2-98.6)	67.2 (38.1-107.6)	97.1 (61.9-127.2)	13.4 (0-25.0)
Midday	47.7 (30.6-70.3)	39.1 (22.1-63.1)	69.5 (42.1-93.3)	9.5 (0-15.9)
Afternoon	11.6 (0-24.5)	16.7 (0-26.5)	15.9 (0-33.4)	5.8 (0-13.1)
Relative humidity				
Per day	60.6 (53.6-65.8)	60.9 (54.1-66.2)	57.3 (49.9-63.3)	63.6 (57.2-67.7)
Time				
Morning	66.2 (61.6-68.6)	66.7 (62.9-69.4)	63.9 (58.9-66.6)	67.4 (63.5-70.7)
Midday	58.9 (52.1-62.9)	58.8 (52.5-63)	55.2 (48.3-59.9)	62.1 (55.8-66.3)
Afternoon	54.1 (48.2-59.4)	53.7 (48.2-59.4)	50.3 (45.1-56.3)	58.7 (51.2-62.8)
Number of coughs				
Per day	466 (368-503)	-	466 (368-503)	-
Time				
Morning	421 (350.5-487.5)	-	421 (350.5-487.5)	-
Midday	495 (392-514)	-	495 (392-514)	-
Number of *Mtb* copies/µL				
Per day	4.2 (1.2-9.5)	-	4.2 (1.8-9.4)	4.7(0.5-9.5)
Number of observations	*79*	-	*38*	*41*
Time				
Morning	3.6 (0.4-7.4)	-	4.2 (1.4-8.0)	2.1 (0.30-6.3)
Number of observations	*39*	-	*19*	*20*
Midday	5.6 (2.2-11.8)	-	6.2 (1.8-10.9)	5.5 (2.7-12.2)
Number of observations	*40*	-	*19*	*21*

CO_2, carbon dioxide; IQR, interquartile range; *Mtb, Mycobacterium tuberculosis*

Rebreathed air volume

The overall median rebreathed air volume was 46.5 L/day (IQR 22.7-74.8), and it decreased over the day (Table 2, Figure 2C). The rebreathed air volume was highest in the waiting room compared to the registration area and TB treatment room (68.1 vs 42.3 vs 9.5 L/day). The ventilation rate in the waiting room was at 12.2 L/h per person, compared to the minimum recommended ventilation rate of 6.0 L/h for the treatment room (22).

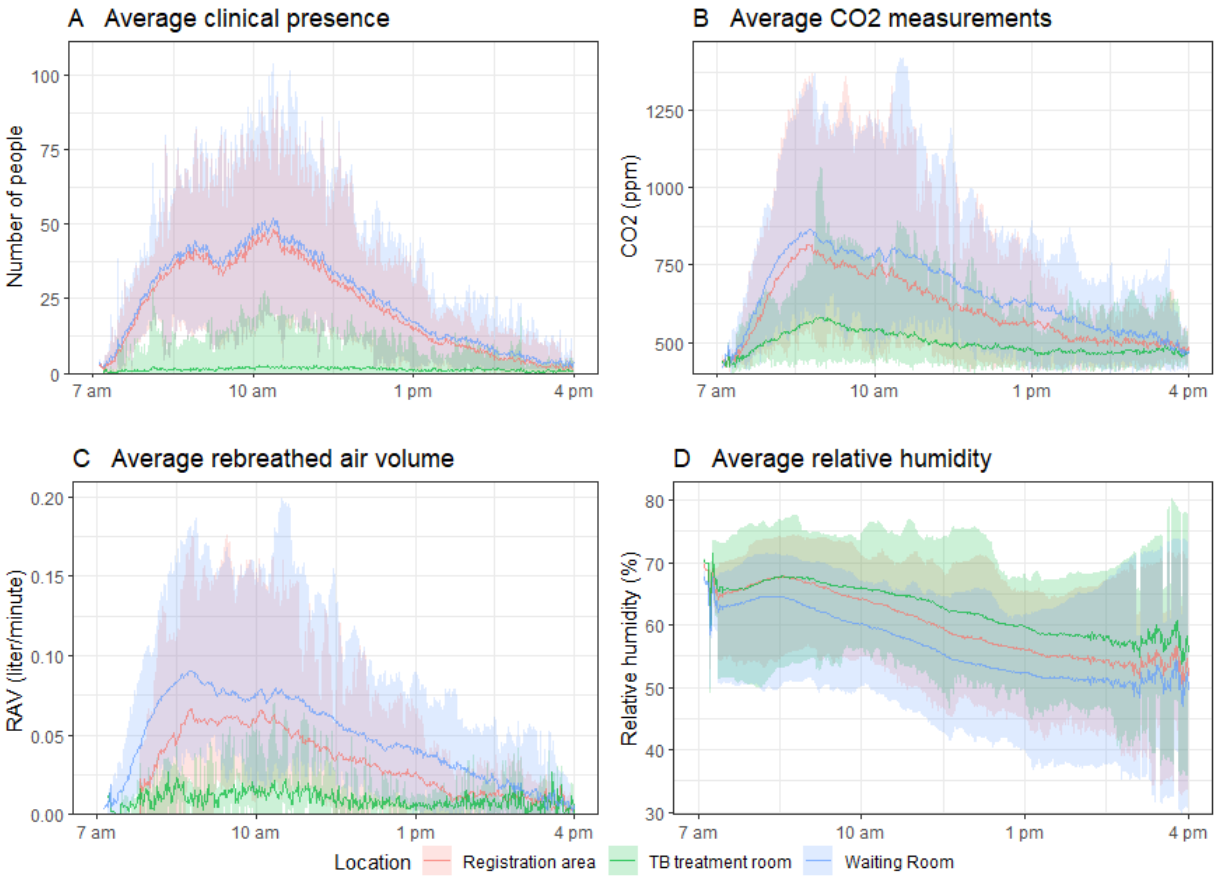

Figure 2. Environmental data collected at the primary care clinic. Average clinic presence, CO_2, rebreathed air volume (RAV), and relative humidity, over time and by location. The solid line is the mean with pale fill the recorded values from the minimum to the maximum.

CO_2, carbon dioxide; RAV, rebreathe air volume; ppm, parts per million

Relative humidity

The overall median relative humidity was 60.6% (53.6-65.8%). It was higher in the morning compared to midday and afternoon (66.2% vs 58.9% vs 54.1%). The relative humidity was highest in the TB treatment room followed by the registration area and the waiting room (63.6% vs 60.9% vs 57.3%) (Table 2, Figure 2D).

Mtb particles in the air

The overall median number of *Mtb* DNA copies/µL per day was 4.2 (1.2-9.5). The median *Mtb* DNA copies/µL throughout the day was slightly higher in the waiting room than in the TB treatment room (Table 2, Figure S1A). In both locations, the median *Mtb* DNA copies/µL were higher in the afternoon than in the morning.

Risk factors for potential transmission

In the univariate analysis, we found an increased level of *Mtb* DNA copies in the air of 15% (95% credible interval 3-32%) per 100 incremental young adults (aged 15-29 years) visiting the clinic. No other variables were associated with an increase of *Mtb* DNA copies (Table 1).

Table 2. Factors associated with transmission risk (indicated by *Mtb* genome copies in the air) presented as risk ratio per 100 incremental persons with the corresponding 95% credible interval.

Variable	Unit	Unadjusted risk ratio, 95% credible interval	Adjusted risk ratio, 95% credible interval
Sex			
Female	Per 100 incremental persons	1.04 (0.97-1.11)	0.92 (0.75-1.15)
Age groups			
15-29	Per 100 incremental persons	1.15 (1.03-1.32)	1.32 (1.07-1.63)
30-44	Per 100 incremental persons	0.96 (0.87-1.08)	0.80 (0.61-1.03)
45-59	Per 100 incremental persons	1.08 (0.85-1.43)	1.35 (0.89-2.09)
>60	Per 100 incremental persons	0.86 (0.40-2.15)	1.19 (0.22-6.57)
Environmental factors			
Average RH per day	Per 10% incremental increase RH	0.99 (0.99-1.01)	1.01 (1.00-1.02)
Average RAV per day	Per 0.01 incremental increase RAV	0.0 (0.0-0.97)	0.0 (0.0-1.08)
Sum coughs	Per 100 incremental coughs	0.99 (0.83-1.20)	1.11 (0.89-1.34)

RH, relative humidity; RAV rebreathed air volume

In the multivariate analysis, we found an increased level of *Mtb* DNA copies in the air of 32% (95% credible interval 7%-63%) per 100 incremental young adults (aged 15-29 years) visiting the clinic. For a 5% incremental increase of relative humidity, 1% (95% credible interval 0%-2%) more *Mtb* DNA copies were in the air (Table 1). Figure 3 shows the standardized risk ratio per one standard deviation with the 95% credible interval.

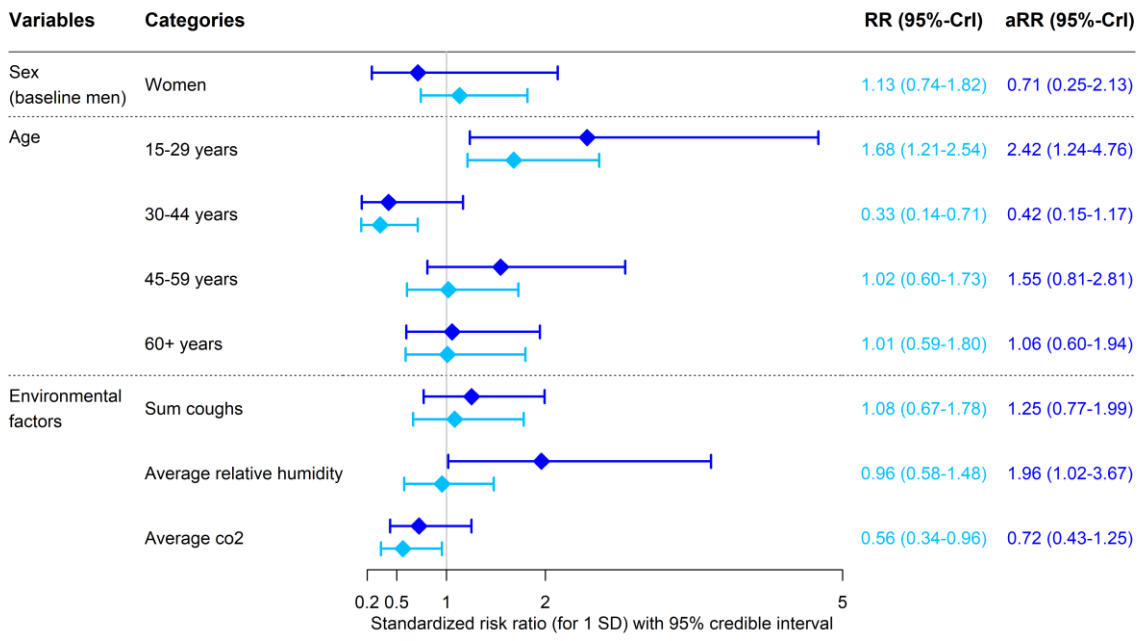

Figure 3. Patient and environmental factors associated with Mtb genome copies in the air, presented as standardized risk ratio from a multivariate analysis.

CrI, credible interval; CO_2, carbon dioxide

Risk of infection

We modelled different scenarios using the observed TB prevalence at the clinic and the estimated TB prevalence of 737 per 100 000 people for South Africa by the National Department of Health with

varying infectious quanta [23] (5.5 and 8.2 infectious quanta per hour, Table S2). The observed TB prevalence at the clinic suggested that the risk of *Mtb* transmission during the day was about 3% per hour using 5.5 infectious quanta per hour. It was about 6% per hour using 8.2 infectious quanta per hour (Figure 4A). The risk of infection was lower when using the TB prevalence estimated by WHO (Figure 4B).

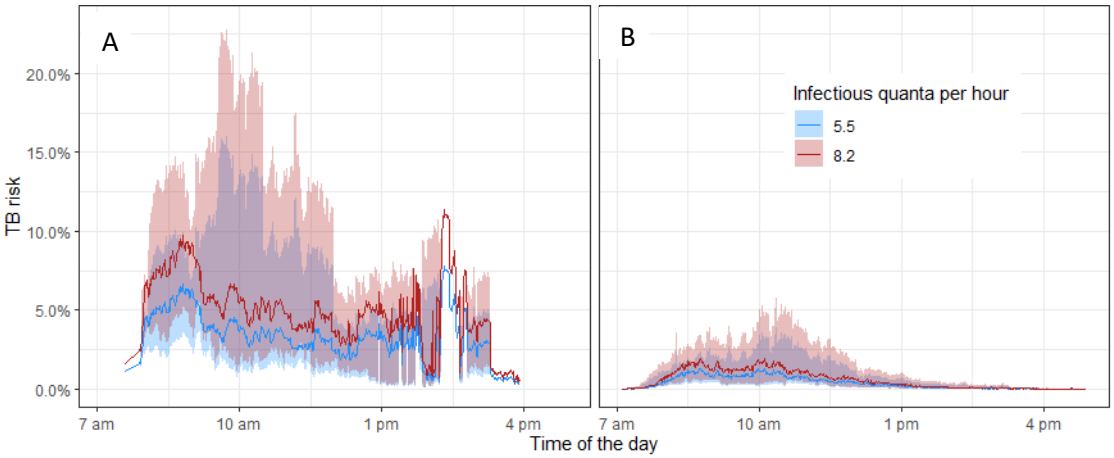

Figure 4. The risk of TB infection during a day at the primary care clinic estimated based on a mathematical transmission model (20). Panel A shows the risk of infection based on the observed TB prevalence at the clinic. Panel B shows the risk based on the TB prevalence in the general population as estimated by WHO. The solid line is the mean with pale fill the recorded values from the minimum to the maximum. Estimations for two different definitions of the infectious quanta are shown. The parameters and assumptions for the transmission model are described in Table S2 or described in Hella et al. (20)(138).

To put this in perspective, a patient coming each month to the clinic for 1 hour (12 visits per year) would have a cumulative risk of *Mtb* transmission ranging from 9% to 29% depending on the scenario (Figure 5). The cumulative risk was higher for observed TB prevalence at the clinic compared to the TB prevalence estimated by WHO. In an extreme scenario assuming a weekly visit to the clinic of 1 hour (52 visits per year), a patient would have a cumulative risk ranging from 33% to 78%, depending on the scenario.

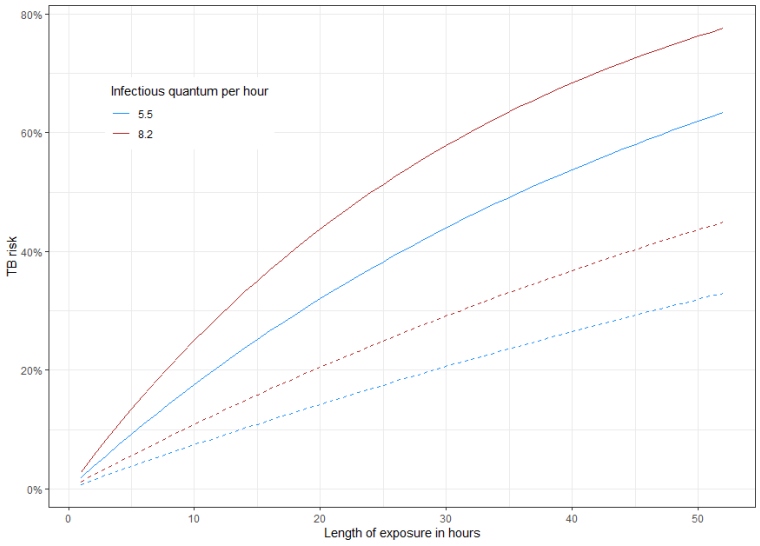

Figure 5. Cumulative risk of TB infection according to the time spent at the primary care clinic. The solid line present the observed prevalence and the hashed line the estimated TB prevalence by WHO.

Discussion

At this South African primary care clinic, an increased risk of *Mtb* transmission was associated with the presence of young adults and higher room humidity. We estimated the risk of transmission during a clinic visit of one hour to be 3% to 6%, increasing to 9% to 29% for patients making regular

monthly visits. Our study suggests that multiple environmental measures and clinical data can be used to assess indoor ventilation quality and evaluate airborne disease transmission control measures in primary care and similar settings.

Our study observed more copies of *Mtb* DNA in the air when young adults visited the clinic than when clinic visitors were older. Several factors might account for this. Behavioral and social contact patterns differ by age, and they might play a role in the risk of *Mtb* transmission (23, 24). Young index cases (<40 years) have been shown to have more close contacts and contacts with all age groups than older index cases who have fewer contacts (and mainly within their own age group) (24). And as adolescents and young adults' transition from child to adult health services, they face specific age-related challenges accessing appropriate healthcare (25, 26). These challenges might result in delayed HIV or TB diagnoses and treatments. A study from Cape Town, South Africa, showed that TB notification was highest among young adults. Among those aged 25-45 years, 63% were HIV-associated TB patients. The study also showed that TB notification rates among HIV-negative people peaked between 20-24 years and a second peak between 45-54 years (27). We observed that increasing relative humidity was associated with increased copies of *Mtb* DNA in the air, only a few other studies have investigated this relationship. Relative humidity was shown to play an important role in the presence of *Mtb* genome copies in the air (28), and our finding is also in line with results from a more recent study which showed that *Mtb* genome copies were more likely to be found in health facilities when the relative humidity was above 65% (12).

Studies of different settings have reported that healthcare clinics may be drivers of *Mtb* transmission (29-33). In low-and middle income countries, resource constrained care clinics are often crowded with people sitting close together on benches or standing in passageways. In these clinics, the waiting times are often long and the ventilation is poor. These kinds of conditions favour *Mtb* transmission from infected to uninfected people (9, 34, 35). Because of these conditions, exposure to *Mtb* might be prolonged. People with undiagnosed TB or delayed TB diagnoses pose risks of *Mtb* transmission to other individuals at the clinic. In addition, those diagnosed with TB who continue to receive care at a clinic may pose a risk to uninfected people and reinfection in people with a *Mtb* infection (34). Furthermore, HIV coinfection plays a major role as disease progression is faster in people living with HIV compared to HIV-negative individuals (29, 30). Therefore, it is important to screen people regularly for TB symptoms at ART clinics. Infection control measures are needed, such as improved ventilation and, for presumptive TB cases or anyone who is coughing, wearing masks. Because of the COVID-19 pandemic, wearing masks is likely an easy and familiar intervention to implement. Finally, and relevant to the measurement of airborne *Mtb,* the detection of *Mtb* particles exhaled by newly diagnosed TB patients by ppPCR (molecular method) has been shown to be more sensitive than detection by aerosol using traditional culture techniques (11).

High indoor CO_2 levels (above 1000 ppm) are indicators of poor ventilation. We found CO_2 levels above 1000 ppm, mainly in the morning in the waiting room area of the clinic. Levels in the TB treatment room were kept lower through measures to minimize occupancy and keeping the doors and windows open to allow ventilation. Studies have measured CO_2 levels at different locations and combined these environmental data with social interaction data to model the risk of *Mtb* transmission (9,10, 20). The highest annual risk for *Mtb* transmission in another Southern African setting was found in prisons, with descending lower risks for persons in schools, riding public transport, and social halls (20). These findings complement other studies of high-burden settings, which found that only a small proportion of *Mtb* transmission occurs between household members (36-38). Using the observed prevalence at the clinic, we found that the risk for *Mtb* transmission during the day was 3 to 5% per hour. A modelling study showed that the annual risk of *Mtb* infection in the waiting room at a clinic with closed windows and doors ranged from 23-34% for chronic patients with monthly visits and from 2.2-3.4% per patient visit (39). Further, they showed that with good ventilation (open windows and doors), the risk of *Mtb* infection was reduced 50-fold.

The mathematical models showed that the duration and frequency of clinic visits increased the risk of *Mtb* transmission. However, this could be addressed effectively by relatively simple infection control interventions: improved ventilation through opening windows and decreased room presence, which resulted in very low rebreathed air volume for the room. In settings where airborne transmission is possible, both *Mtb* bacilli and the SARS-CoV-2 virus are transmissible via aerosols (40-43). In the COVID-19 pandemic, primary care clinics have implemented infection control measures such as increased hand hygiene and physical distancing, and all attendees and clinical staff members are wearing face masks. A systematic review has shown that at least 1 meter of physical distancing, face masks, and eye protection for health care workers and the general population reduce the risk of SARS-CoV-2 infection (44). These infection control measures would likely also decrease the risk of *Mtb* infection and other airborne transmitted diseases at healthcare clinics.

The collection of environmental data had several limitations. The video sensor system assigned a new ID whenever a seated person stood up. Therefore we had challenges in tracking people, and we cannot exclude incorrect assignments in these cases. Furthermore, the bioaerosol sampling devices collected data over about 3.5 hours, whereas the other data were collected by the minute. By aggregating these data, we lost some information, which may explain why we did not find an association between *Mtb* counts and CO_2 levels. The highly sensitive ddPCR assay we applied detects *Mtb* genome DNA but does not distinguish between viable, *Mtb* bacilli causing infections and dead or noninfectious bacilli and DNA fragments. Moreover, the assay could conceivably be detecting DNA fragments present in the clinic over a long time, and efforts in our laboratory are underway to develop improved analysis and assay approaches that can address this. These caveats notwithstanding, we used a novel and rapid system to study *Mtb* transmission that combined various environmental and clinical measurements, which goes beyond traditional methods such as geo-temporal clustering or molecular genotyping.

Our approach to assessing *Mtb* transmission risks using various environmental and clinical data is novel. It identified young adults and relative humidity as potentially important factors in TB transmission in these settings. A global study using the WHO TB notification database that showed about 17% of all new TB cases were among people aged 10-24 years (45). Therefore, TB research and public health interventions should have increased focus on young adult health (27, 45, 46). However, we should not only understand the drivers of transmission, but also identify and evaluate the most promising interventions (47). Our multiple measures approach can be used in health care clinics and other congregate settings to evaluate interventions to halt transmission, including the evaluation of infection control measures such as improved room ventilation, increased hand hygiene, or wearing of masks.

Author's contribution

KZ, CM, RW, ME, and LF wrote the concept. KZ and LF wrote the first draft of the paper, which was reviewed by all authors and revised based on the comments received by all coauthors. KZ, CM, KM, and RW coordinated data collection. CM, AK, KM, DW, and RW were involved in laboratory work, and they were involved in extracting the clinical data from the electronic registry. SB did the medical informatics and cough extraction AI. JR and KZ completed the statistical analyses. All authors approved the final version of the manuscript.

Acknowledgements

We thank Mbali Mohlamonyane for setting up the bioaerosol sample collection system, and Zeenat Hoosen and Ronnett Seldon for developing the laboratory protocols. We are also grateful

to the clinical manager and the staff members at the primary care clinic. Finally, we would like to thank the city of Cape Town, South Frica for the use of one of their clinic facilities and their support. Our research findings and recommendations do not represent an official view of the city of Cape Town. We thank Kristin Marie Bivens and Christopher Ritter for editorial assistance.

Funding

The rollout of this study is supported by a grant from the Swiss National Science Foundation (grant no. CRSK-3_190781). KZ and LF are supported by the National Institute of Allergy and Infectious Diseases (NIAID) through grant no. 5U01-AI069924-05, and ME is supported by a special project funding (grant no. 174281) from the Swiss National Science Foundation.

Competing interests

All authors declare that they have no conflicts of interest.

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Supplementary material

Table 1: Description of the measurements.

Data source	Parameter	Unit	Measurement taken by	Reference
CO_2 monitor	Carbon dioxide	ppm	minute and date	(10)
	Relative humidity	%	minute and date	(12, 28)
	Temperature	°C	minute and date	(12, 28)
Cough recording	Frequency	n	minute or day and date	
	Duration	sec	cough by minute and date	(19)
	Intensity	decibel	cough by minute and date	(19)
Mobile aerosol sampling	*Mtb* DNA copies	copies per microliters	filter (ca. 3.5 hours sampling) or per day	(11)
Video sensor	Number of people	n of people	0.25 seconds or minute and by date	
	Time spent at a given location	minutes	minute and date	
Patient charts	Number of registered patients	n of registered patients	minute and day	
	Number of presumptive TB and TB patients	n of presumptive TB and TB patients	minute and day	

CO2, carbon dioxide; *Mtb*, *mycobacterium tuberculosis*; ppm, parts per million; n, number

Table 2: Parameters used to estimate *Mtb* transmission at the primary care clinic.

Parameter description	Value	Reference
Infectious quanta (*q*)		
Smear-positive TB patient co-infected with HIV	5.5 q/hour	(48)
Smear-positive TB patient co-infected with HIV (average)	8.2 q/hour	(20, 48, 49)
Infectious individuals in space (*I*)		
TB prevalence at the clinic	Mean per day or per hour	This study
TB prevalence estimates from National Department of Health in South Africa	737 (95%CI 580-890) per 100,000	(50)
Time spent in each location (*t*)	Mean per day or per hour	This study
Number of contacts at each time point (*n*)	Mean per day or per hour	This study
Rebreathed fraction (*f*)	Mean per day or per hour	This study

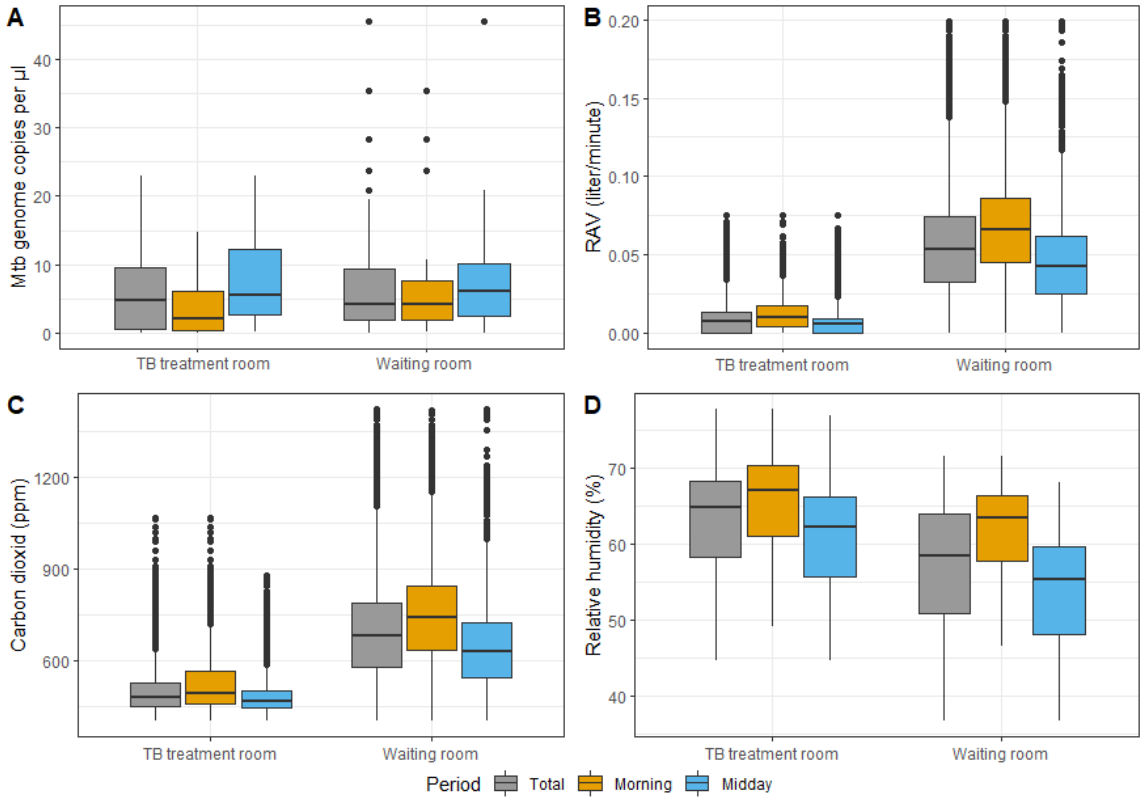
Mtb, *mycobacterium tuberculosis*; q, infectious quanta

Table 3: Patient characteristics after linkage of patient's clinical data from the electronic registry with movement tracking data from video sensors at a primary care clinic in South Africa.

	Total patient visits (n=978, %)	**Other patient visits** (n=962, %)	**Potentially infectious TB patient visits** (n=16, %)	**p-value**
Age, median (IQR)	33 (26-41)	33 (26-41)	34 (25.5-39.5)	0.56
Sex				<0.001
Male	221 (22.6)	210 (21.8)	11 (68.8)	
Female	757 (77.4)	752 (78.2)	5 (31.2)	
HIV status				<0.001
Positive	167 (17.1)	152 (15.8)	15 (93.8)	
Unknown	811 (82.9)	810 (84.2)	1 (6.2)	
CD4 count at TB treatment start, median (IQR), cells/µl	185.5 (69-290)	185.5 (69-300)	201.5 (69-252)	0.49
No. of observations	116	110	6	
TB diagnosis				
Yes	171 (17.5)	155 (15.9)	16 (100)	<0.001
Past TB	155 (15.9)	155 (16.1)	0	
Potentially infectious TB	16 (1.6)	0	16 (100)	
No	807 (82.5)	807 (83.9)	0	
Resistant TB				<0.001
Yes	68 (7.0)	61 (6.3)	7 (43.8)	
No	42 (4.3)	38 (4.0)	4 (25.0)	
Missing	868 (88.7)	863 (89.7)	5 (31.2)	
Number of visits				<0.001
1 visit	890	886 (92.2)	4 (25.0)	
2 visits	57	54 (5.6)	3 (18.8)	
3 or more visits	30	21 (2.2)	9 (56.2)	
Average time spent in the waiting room	41 (17-85)	43 (17-85)	41 (32-60)	0.75
Average number of contacts	62 (16-173)	66 (17-176)	67 (45-96)	0.91

IQR, interquartile range; TB, tuberculosis; No, numbers

Figure 1: Environmental data collected at a primary care clinic in South Africa, overall and by time period (morning and midday).



Mtb, *mycobacterium tuberculosis*; RAV, rebreathed air volume; ppm, parts per million

5. Paper 3 – Diagnosis of TB and clinical outcomes

Diagnosis and clinical outcomes of extrapulmonary tuberculosis in antiretroviral therapy programmes in low-and middle-income countries: a multicohort study

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The article has been published in the Journal of the International AIDS Society in 2019.
DOI: https://doi.org/10.1002/jia2.25392

Contribution: I performed the data cleaning, data analysis and made all figure and tables. I wrote the first draft of the manuscript and all subsequent versions – addressing comments from co-authors and reviewers- until the final published version

Abstract

Introduction: Extrapulmonary tuberculosis (EPTB) is difficult to confirm bacteriologically and requires specific diagnostic capacities. Diagnosis can be especially challenging in under-resourced settings. We studied diagnostic modalities and clinical outcomes of EPTB compared to pulmonary tuberculosis (PTB) among HIV-positive adults in antiretroviral therapy (ART) programmes in low-and middle-income countries (LMIC).

Methods: We collected data from HIV-positive TB patients (≥16 years) in 22 ART programmes participating in the International Epidemiology Databases to Evaluate AIDS (IeDEA) consortium in sub-Saharan Africa, Asia-Pacific, and Caribbean, Central and South America regions between 2012 and 2014. We categorized TB as PTB or EPTB (EPTB included mixed PTB/EPTB). We used multivariable logistic regression to assess associations with clinical outcomes.

Results and Discussion: We analysed 2695 HIV-positive TB patients. Median age was 36 years (interquartile range (IQR) 30 to 43), 1102 were female (41%), and the median CD4 count at TB treatment start was 114 cells/lL (IQR 40 to 248). Overall, 1930 had PTB (72%), and 765 EPTB (28%). Among EPTB patients, the most frequently involved sites were the lymph nodes (24%), pleura (15%), abdomen (11%) and meninges (6%). The majority of PTB (1123 of 1930, 58%) and EPTB (582 of 765, 76%) patients were diagnosed based on clinical criteria. Bacteriological confirmation (using positive smear microscopy, culture, Xpert MTB/RIF, or other nucleic acid amplification tests result) was obtained in 897 of 1557 PTB (52%) and 183 of 438 EPTB (42%) patients. EPTB was not associated with higher mortality compared to PTB (adjusted odd ratio (aOR) 1.0, 95% CI 0.8 to 1.3), but TB meningitis was (aOR 1.9, 95% CI 1.0 to 3.1). Bacteriological confirmation was associated with reduced mortality among PTB patients (aOR 0.7, 95% CI 0.6 to 0.8) and EPTB patients (aOR 0.3 95% CI 0.1 to 0.8) compared to TB patients with a negative test result.

Conclusions: Diagnosis of EPTB and PTB at ART programmes in LMIC was mainly based on clinical criteria. Greater availability and usage of TB diagnostic tests would improve the diagnosis and clinical outcomes of both EPTB and PTB.

Introduction

In low-and middle-income countries (LMIC), tuberculosis (TB) accounts for approximately 40% of HIV/AIDS-related deaths among adults, and half of those TB cases are undiagnosed at the time of death [1]. TB predominantly affects the lungs (pulmonary tuberculosis [PTB]), but can affect extrapulmonary sites as well (EPTB). Globally, about 25% of all TB cases are estimated to be EPTB [2]. EPTB is a common presentation in HIV-positive individuals, particularly in those with low CD4 cell counts [3,4]. EPTB is most frequently identified in the lymph nodes, pleura, bones and joints, abdomen, meninges and genitourinary tract [5]. TB meningitis is considered the most severe form of EPTB with mortality as high as 70% in low-income countries [6].

The diagnosis of EPTB is particularly difficult and is often solely based on clinical signs and symptoms. A bacteriological confirmation of EPTB often requires invasive specimen collection by biopsy or fine needle aspiration [7,8], followed by use of adequate diagnostic tests [9,10]. Much progress has been made in developing new diagnostic tests for TB, including the Xpert MTB/RIF and other nucleic acid amplification tests (NAAT), which have higher sensitivity than smear microscopy and can also be used to diagnose both PTB and EPTB [11]. This study assessed diagnostic modalities and TB treatment outcomes of EPTB compared to PTB in HIV-positive adults in clinical care in antiretroviral therapy (ART) programmes in six International epidemiology Databases to Evaluate AIDS (IeDEA) regions in sub-Saharan Africa, Asia-Pacific, Caribbean, Central and South America Central and South America.

Methods

Study setting and study population

IeDEA (www.iedea.org) is a large consortium of ART programmes predominantly located in LMIC [12]. ART programmes in six IeDEA regions participated in this study are mostly public but often supported by NGOs or academia: East Africa; Central Africa; West Africa; Southern Africa; Asia-Pacific; Caribbean, Central and South America.

We reviewed records of consecutive 3165 HIV-positive patients diagnosed with any form of TB between January 1, 2012 and December 31, 2014 in participating ART programmes. Patient records missing data on sex, date of birth or site of disease were excluded from the analysis (35 records). We studied only adults and excluded paediatric cases (age <16 years, 396 records). In case of multiple TB episodes, only the patient's first episode was included in the study (39 duplicate records deleted). This resulted in the inclusion of 2695 adult HIV-positive TB patients from 22 ART programmes (Figure 1).

Data collection

Standardized electronic case report forms (CRFs), available in English or French, were used to record age, sex, date of TB diagnosis, site of TB disease, site of EPTB manifestation (predominant organ), start date of TB treatment, body mass index (BMI) at start of TB treatment, ART status at TB diagnosis, CD4 cell count at start of TB treatment, previous history of TB, TB drug resistance, results from TB diagnostic tests (smear microscopy, culture, Xpert MTB/RIF and other NAAT) and TB treatment outcomes [13]. All data were collected using REDCap (www.project-redcap.org) [14]. Local IeDEA site investigators completed CRFs for TB patients. Data were entered between January 2012 and January 2016. During data collection, routine audits were made to ensure data quality [15]. Furthermore, we used programme-level data previously collected in the same ART programmes in 2012 [16].

Definitions

We categorized TB as PTB (involving the lungs only), mixed PTB/EPTB and EPTB only [5]. For binary outcome analyses, we used the categories PTB and EPTB (includes mixed PTB/EPTB cases) as previously defined [17]. Miliary TB was categorized as EPTB. Furthermore, we categorized bacteriological confirmation as "test performed" if at least one of the tests (smear microscopy, culture, Xpert MTB/RIF and/or other NAATs) was performed regardless of whether the result was positive or negative; "positive" bacteriological confirmation (any positive test result); "negative" (all performed tests were negative); and "no test performed." TB treatment outcomes were categorized as cured, treatment completed, treatment failed, died, lost to follow-up (LTFU) and not evaluated [5]. The category "not evaluated" included patients who were still on treatment, transferred out and those whose treatment outcome was unknown. The category "treatment success" included cured patients and patients who completed TB treatment.

Statistical analyses

We used descriptive statistics to characterize both programme-level and patient-level data, as well as diagnostic modalities. Differences between groups were assessed using chi-square, Fisher's exact or Wilcoxon rank-sum tests as appropriate. We used univariate and multivariate logistic regressions to assess risk factors for mortality and LTFU during TB treatment. These associations were presented as unadjusted odds ratios (ORs) and ORs adjusted (aORs) for age, sex, BMI at start of TB treatment, previous history of TB, ART status at TB diagnosis and CD4 cell counts at start of TB treatment, taking into account heterogeneity across regions (clustering by treatment programmes). In separate models, we obtained the estimates for EPTB sites, (adjusted for the same co-variates). Patients without documented treatment outcomes were excluded from the primary analysis. However, we performed a sensitivity analysis considering patients LTFU as having died. To account for missing data we used multiple imputations by chained equations to impute missing BMI, ART status at TB diagnosis, CD4 cell counts at start of TB treatment and previous history of TB. The quality of the imputation can be improved by adding variables outside the analysis [18], therefore in addition to the outcome and the covariates used in the analysis, we also considered the date of TB treatment start, IeDEA region, setting, level of care for imputation. We ran the model on 20 imputed datasets for each analysis and used the Rubin rule to pool the estimates. All analyses were performed in STATA (version 14.1, Stata Corporation, Texas, USA).

Ethics statement

Local institutional review board or ethics committee approval was obtained at all local study sites. Informed consent was obtained where requested per local regulations. The Vanderbilt University Medical Center Institutional Review Board, Nashville, Tennessee (USA), and the Cantonal Ethics Committee Bern (Switzerland) approved the analyses for this specific project.

Results

Study sites and patient characteristics

The 2695 HIV-positive TB patients participating in this study were treated at 22 ART programmes in 19 countries (Figure 1). Eighteen sites were urban, three were peri-urban and one site was rural. The level of care was mostly tertiary, at 17 sites, followed by secondary at four sites and primary at one site. For TB diagnostics a free-of-charge cost model was available at 11 of the 22 sites, a cost sharing model was available at 10 of the 22 sites and one site had a mixed cost model.

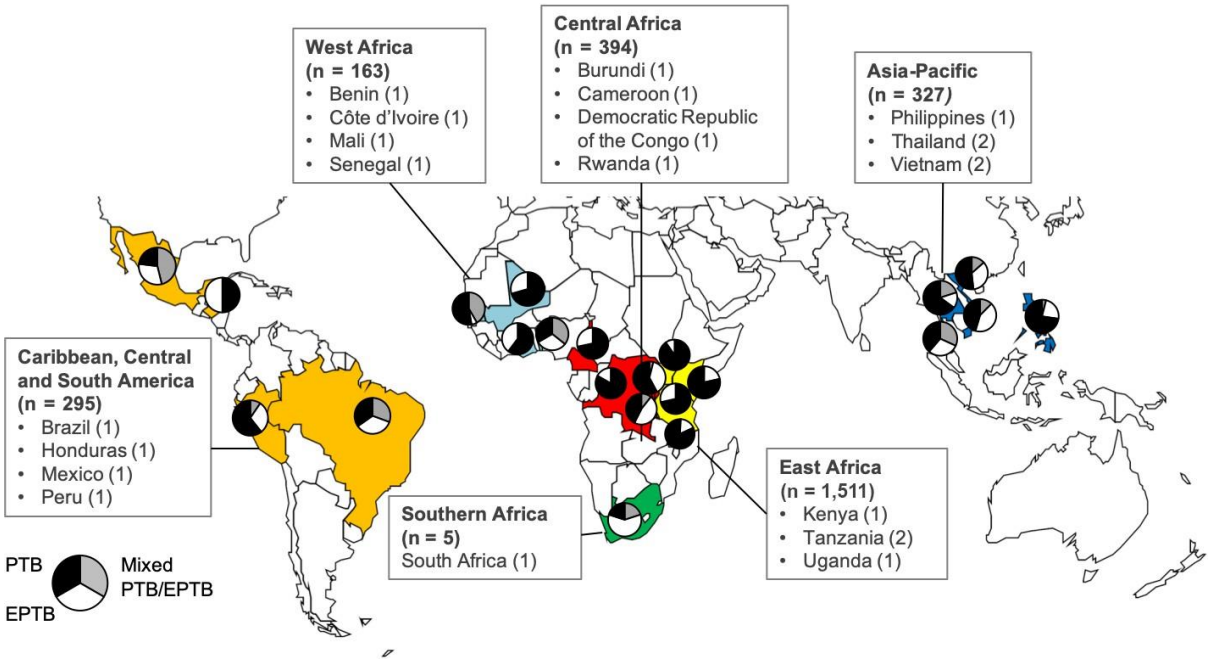

Figure 1. Geographical distribution of 22 antiretroviral treatment (ART) programmes treating HIV-positive patients (≥16 years) in low-and middle-income countries. The proportions of pulmonary (black), mixed pulmonary/extrapulmonary (grey), and extrapulmonary tuberculosis cases (white) diagnosed at each site are indicated in the pie charts; n indicates the number of patients included in the study by IeDEA region; the numbers in parentheses following the country names indicate the number of ART programmes participating in the study by country.
EPTB, extrapulmonary tuberculosis; IeDEA, International Epidemiology Databases to Evaluate AIDS; PTB, pulmonary tuberculosis.

The median patient age was 35.5 years (interquartile range (IQR) 29.9 to 42.8), and 1102 patients (40.9%) were female. Among the 2695 patients, 1930 had PTB (71.6%) and 765 had EPTB (28.4%); 131 patients (4.9% overall) had both. At the time of TB diagnosis, 1270/2965 (47.1%) TB patients had not started ART and 763/2695 (28.3%) TB patients were on ART; the ART status of the remaining patients was unknown. Of the TB patients on ART, 342/763 (44.8%) were more than six months on ART before TB diagnosis and 421/763 (55.2%) were six or less months on ART. Among the 765 EPTB patients, the most frequent sites of disease were the lymph nodes (24.4%), pleura (14.2%), abdomen (11.1%), and meninges (6.3%). Complete patient characteristics are given in Table 1. When stratifying CD4 cell counts at the time of TB treatment (0 to 49 cells/lL; 50 to 199 cells/lL; ≥200 cells/lL; missing CD4 values), the frequencies of sites of EPTB manifestations remained similar over all groups.

Table 1. Characteristics of HIV-positive patients diagnosed with pulmonary tuberculosis and extrapulmonary tuberculosis at the start of TB treatment in 22 antiretroviral treatment programmes from lower income countries.

	All	PTB	EPTB		
			EPTB	Mixed PTB/EPTB	EPTB only
Total, n (%)	2,695 (100)	1,930 (71.6)	765 (28.4)	131 (4.9)	634 (23.5)
Age, year					
16-29	588 (21.8)	414 (21.4)	174 (22.8)	28 (21.4)	146 (23.0)
30-39	1,105 (41.0)	793 (41.1)	312 (40.8)	59 (45.0)	253 (39.9)
40-49	696 (25.8)	503 (26.1)	193 (25.2)	35 (26.7)	158 (24.9)
≥50	306 (11.4)	220 (11.4)	86 (11.2)	9 (6.9)	77 (12.2)
Sex, n (%)					
Male	1,593 (59.1)	1,134 (58.8)	459 (60.0)	90 (68.7)	369 (58.2)
Female	1,102 (40.9)	796 (41.2)	306 (40.0)	41 (31.3)	265 (41.8)
BMI at start of TB treatment kg/m², , median (IQR)	18.7 (16.8-20.9)	18.6 (16.7-20.8)	18.8 (16.9-21.1)	18.1 (16.4-19.8)	18.9 (17.0-21.4)
No. of observations (%)	2,115 (78.5)	1,553 (80.5)	562 (73.5)	82 (62,6)	480 (75.7)
CD4 count at TB treatment start, median (IQR), cells/µl	114 (40-248)	124 (45-263)	92 (32-212)	55 (19-129)	105 (34-228)
No. of observations (%)	2,196 (81.5)	1,575 (81.6)	621 (81.2)	108 (82.4)	513 (80.9)
ART status at TB diagnosis					
Not on ART	1,270 (47.1)	936 (48.5)	334 (43.7)	51 (38.9)	283 (44.6)
On ART	763 (28.3)	599 (31.0)	164 (21.4)	12 (9.2)	152 (24.0)
6+ months at TB diagnosis	342 (12.7)	268 (13.9)	74 (9.7)	4 (3.1)	70 (11.0)
< 6 months at TB diagnosis	421 (15.6)	331 (17.2)	90 (11.8)	8 (6.1)	82 (12.9)
Missing	662 (24.7)	395 (20.5)	267 (34.9)	68 (51.9)	199 (31.4)
Previous history of TB, n (%)					
Yes	55 (2.0)	37 (1.9)	18 (2.4)	3 (2.3)	15 (2.4)
No	2,304 (85.5)	1,705 (88.3)	599 (78.3)	82 (62.6)	517 (81.5)
Unknown	336 (12.5)	188 (9.7)	148 (19.3)	46 (35.1)	102 (16.1)
TB treatment outcomes, n (%)					
Treatment success[1]	1,908 (70.8)	1,383 (71.7)	525 (68.6.9)	75 (57.3)	450 (71.0)
Treatment failed	15 (0.6)	13 (0.7)	2 (0.3)	1 (0.8)	1 (0.2)
Died	281 (10.4)	194 (10.1)	87 (11.4)	13 (9.9)	74 (11.7)
Lost to follow-up (default)	136 (5.0)	100 (5.2)	36 (3.2)	16 (12.2)	20 (3.2)
Not evaluated[2]	355 (13.2)	240 (12.4)	113 (14.0)	24 (18.3)	89 (14.0)
Organs involved in EPTB, n (%)					
Lymph nodes[3]	187 (6.9)	-	187 (24.4)	31 (23.7)	156 (24.6)
Pleura	109 (4.0)	-	109 (14.2)	15 (11.5)	94 (14.8)
Abdomen	85 (3.2)	-	85 (11.1)	13 (9.9)	72 (11.4)
Meninges	48 (1.8)	-	48 (6.3)	10 (7.6)	38 (6.0)
Miliary[4]	32 (1.2)	-	32 (4.2)	10 (7.6)	22 (3.5)
Joints and/or bones	22 (0.8)	-	22 (2.9)	4 (3.1)	18 (2.8)
Pericardium	13 (0.5)	-	13 (1.7)	3 (2.3)	10 (1.6)
Genitourinary tract	3 (0.1)	-	3 (0.4)	1 (0.8)	2 (0.3)
Larynx	1 (<0.1)	-	1 (0.1)	-	1 (0.2)
Unknown	265 (9.8)	-	265 (34.6)	44 (33.6)	221 (34.9)
IeDEA region, n (%)					
Caribbean/C-S America	295 (11.0)	160 (8.3)	135 (17.6)	42 (32.1)	93 (14.7)
Asia-Pacific	327 (12.1)	176 (9.1)	151 (19.7)	45 (34.4)	106 (16.7)
West Africa	163 (6.0)	87 (4.5)	76 (9.9)	30 (22.9)	46 (7.3)
Central Africa	394 (14.6)	262 (13.6)	132 (17.3)	9 (6.9)	123 (19.4)
East Africa	1,511 (56.1)	1,244 (64.5)	267 (34.9)	4 (3.1)	263 (41.5)
Southern Africa	5 (0.2)	1 (<0.1)	4 (0.5)	1 (0.8)	3 (0.5)

ART, antiretroviral therapy; BMI, body mass index; Caribbean/C-S America, Caribbean, Central and South America; EPTB, extrapulmonary tuberculosis; IQR, interquartile range; MDR, multidrug-resistant; n, number; PTB, pulmonary tuberculosis; TB, Tuberculosis.
[a] Treatment success includes cured patients and patients who completed TB treatment; [b]not evaluated includes on treatment, transfer out, and unknown; [c]extra- and intrathoracic; [d]miliary TB defined as EPTB.

Diagnostics of EPTB and PTB

Diagnostic capabilities varied according to sites. Sputum smear microscopy was available at all sites. Culture was not available at one site each in East Africa (1/4) and Central Africa (1/4), and Xpert MTB/RIF was not available at half of the sites: two in East Africa (2/4), three in Central Africa (3/ 4), two in West Africa (2/4), one in Asia-Pacific (1/5), and three in Caribbean, Central and South America (3/4) and other NAATS were not available in half of the sites: two in East Africa (2/4), two in Central Africa (2/4), two in West Africa (2/4), three in Asia-Pacific (3/5), and two in Caribbean, Central and

South America (2/4). Bacteriological confirmation of PTB, EPTB only, and PTB/EPTB was sought in varying proportions in the three groups, and test results in groups varied as well. A confirmatory bacteriological test was performed in 438 of 765 EPTB (including mixed PTB/EPTB) patients (57.3%) and 1557 of 1930 PTB patients (80.7%). Bacteriological confirmation by any test (positive test result) was obtained in 183 of those 438 EPTB patients (41.8%), 103 of 334 patients with only EPTB (30.8%) and 807 of the 1557 PTB patients (51.8%). The diagnoses of the remaining EPTB and PTB patients who were not tested or whose test results were negative were based on clinical criteria (Table 2).

Table 2. Diagnostic testing (smear microscopy, culture, Xpert MTB/RIF and/or nucleic acid amplification tests) of PTB and EPTB in HIV-positive patients: proportion of bacteriologically confirmed results (any positive result/any confirmatory test performed (positive and negative), and proportions by specific diagnostic tests (smear microscopy, culture and Xpert MTB/RIF)

	Total	**Bacteriological confirmation[1]**	**Smear microscopy confirmation**	**Culture confirmation**	**Xpert MTB/RIF confirmation**
	n (%)	Proportion n/n, (%)	Proportion n/n, (%)	Proportion n/n, (%)	Proportion n/n, (%)
Site of disease					
PTB	1,930 (100)	807/1,557 (51.8)	805/1,531 (52.6)	95/191 (49.7)	53/77 (68.8)
EPTB	765 (100)	183/438 (41.8)	118/416 (28.4)	75/133 (56.4)	23/35 (65.7)
EPTB only	*634 (100)*	*103/334 (30.8)*	*58/317 (18.3)*	*43/84 (51.2)*	*12/20 (60.0)*
Mixed PTB/EPTB	*131 (100)*	*80/104 (76.9)*	*60/99 (60.6)*	*32/49 (65.3)*	*11/15 (73.3)*
Organs involved in EPTB					
Lymph nodes	187 (100)	77/131 (58.8)	46/115 (40.0)	17/32 (53.1)	15/18 (75.0)
Meninges	48 (100)	14/29 (48.3)	5/22 (22.7)	4/7 (57.1)	4/4 (100)
Abdomen	85 (100)	18/58 (31.0)	9/45 (20.0)	3/8 (27.5)	1/1 (100)
Pleura	109 (100)	14/49 (28.6)	7/47 (14.9)	3/6 (50.0)	-
Joint/bones	22 (100)	6/12 (50.0)	4/11 (36.4)	3/4 (75.0)	0/1 (0)
Miliary	32 (100)	5/20 (25.0)	3/17 (17.6)	5/5 (100)	1/1 (100)
Other	17 (100)	3/10 (30.0	2/9 (22.2)	1/1 (100)	1/1 (100)
Unknown	265 (100)	58/154 (37.7)	42/150 (28.0)	39/70 (55.7)	1/9 (11.1)

EPTB, extrapulmonary tuberculosis; n, numbers; PTB, pulmonary tuberculosis.

[a] Bacteriological confirmation was defined as confirmed if any diagnostic test result was positive (smear microscopy, culture, Xpert MTB/RIF and/or nucleic acid amplification tests).

Among EPTB patients, smear microscopy was the most frequently performed diagnostic test (in 416 of 765 patients, 54.4%) and Xpert MTB/RIF was the least frequently performed diagnostic test (in 35 of 584 patients, 6.0%), but had the highest proportion of bacteriological confirmation (in 23 of 35 patients, 65.7%). The highest proportion of bacteriological confirmation was found among patients with lymph node TB, (in 77 of 131 patients, 58.8%).

Table 3. Risk factors for lost to follow-up and mortality during tuberculosis treatment in HIV-positive patients diagnosed with extrapulmonary and pulmonary TB

Variable	No. of patients	Lost to follow-up (LTFU)					Mortality				
	n	No. LTFU (%)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value	No. of deaths (%)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Age, years				0.15		0.007			0.01		<0.001
16-29	494	38 (7.7)	1		1		43 (8.7)	1		1	
30-39	966	58 (6.0)	0.77 (0.50-1.17)		0.79 (0.54-1.16)		109 (11.3)	1.33 (0.92-1.94)		1.20 (0.85-1.71)	
40-49	617	29 (4.7)	0.59 (0.36-0.97)		0.65 (0.49-0.85)		90 (14.6)	1.79. (1.22-2.63)		1.80 (0.84-3.87)	
≥50	263	11 (4.2)	0.57 (0.29-1.12)		0.61 (0.24-1.56)		39 (14.8)	1.83 (1.15-2.90)		1.95 (1.26-3.00)	
Sex				0.39		0.55			0.95		0.68
Female	953	51 (5.4)	1		1		114 (12.0)	1		1	
Male	1,387	85 (6.1)	1.17 (0.82-1.67)		1.14 (0.74-1.75)		167 (12.0)	1.00 (0.78-1.29)		0.89 (0.50-1.56)	
BMI at start of TB treatment, kg/m²	2,340	-	0.91 (0.85-0.97)	0.004	0.88 (0.81-0.96)	0.003	-	0.96 (0.93-1.01)	0.13	0.98 (0.94-1.01)	0.19
History of TB				0.57		0.71			0.47		0.87
No	2,293	132 (5.8)	1		1		274 (11.9)	1		1	
Yes	47	4 (8.5)	1.37 (0.47-3.94)		1.30 (0.32-5.23)		7 (14.9)	1.36 (0.59-3.12)		0.91 (0.29-2.87)	
ART status at TB diagnosis				0.13		0,20			0.94		0.28
Not on ART	1,660	104 (6.3)	1		1		200 (12.0)	1		1	
On ART	680	32 (4.7)	0.73 (0.49-1.10)		0.75 (0.48-1.17)		81 (11.9)	0.99 (0.75-1.30)		1.21 (0.85-1.74)	
CD4 count at TB treatment start, cells/µL				0.19		0.024			<0.001		0.008
0-49	646	40 (6.2)	1.36 (0.84-2.21)		1.26 (0.94-1.69)		115 (17.8)	2.27 (1.61-3.19)		2.60 (1.46-4.64)	
50-199	829	59 (7.1)	1.51 (0.97-2.34)		1.46 (1.09-1.97)		89 (10.7)	1.25 (0.89-1.75)		1.38 (0.99-1.93)	
≥200	865	39 (4.5)	1		1		77 (8.9)	1		1	
Site of disease				0.82		0.86			0.21		0.75
PTB	1,688	100 (5.9)	1		1		194 (11.5)	1		1	
EPTB	652	36 (5.5)	0.96 (0.65-1.41)		0.92 (0.36-2.32)		87 (13.3)	1.19 (0.90-1.56		1.03 (0.84-1.27)	
EPTB only [1]	*546*	*20 (3.7)*	*0.60 (0.37-0.99)*	*0.044*	*0.58 (0.30-1.13)*	*0.12*	*74 (13.6)*	*1.21 (0.91-1.61)*	*0.19*	*1.04 (0.87-1.71)*	*0.69*
Mixed PTB/EPTB [1]	*106*	*16 (15.9)*	*3.03 (1.74-5.29)*	*<0.001*	*2.59 (1.06-6.32)*	*0.037*	*13 (12.3)*	*1.07 (0.59-1.96)*	*0.81*	*1.03 (0.62-1.71)*	*0.92*
Organs involved [2]				0.72		0.67			0.14		<0.001
Lungs	1,772	116 (6.5)	1		1		207 (11.7)	1		1	
Meninges	43	1 (2.3)	1.71 (0.60-4.89)		1.76 (0.46-6.67)		10 (23.3)	2.28 (1.10-4.69)		1.85 (1.00-3.10)	
Miliary	26	2 (7.7)	1.39 (0.32-5.98)		1.52 (0.38-6.02		2 (7.7)	0.63 (0.15-2.67)		0.74 (0.31-1.74)	
Other	449	18 (4.0)	1.12 (0.73-1.68)		1.10 (0.36-3.41)		62 (13.8)	1.05 (0.77-1.42)		0.87 (0.64-1.17)	

ART, antiretroviral therapy; BMI, body mass index; 95% CI, 95% confidence interval; ART, antiretroviral therapy; EPTB, extrapulmonary tuberculosis; LTFU, lost to follow-up; OR, odds ratio; PTB, pulmonary tuberculosis; TB, tuberculosis.

The main logistic regression model was adjusted for age, sex, BMI at start of TB treatment, previous history of TB, CD4 cell count at TB treatment start, ART status at TB diagnosis, and site of disease, taking into account heterogeneity across regions (clustering by treatment programmes). The model was based on 2340 patients since patients with TB treatment outcome defined as "not evaluated" (n = 355) were excluded from the analysis. [1] These estimates were obtained from a separate model (n = 2340) comparing PTB versus EPTB only and mixed PTB/EPTB and was adjusted for age, sex, BMI at start of TB treatment, previous history of TB, CD4 cell count at TB treatment start, ART status at TB diagnosis, and taking into account heterogeneity across regions (clustering by treatment programmes). [2] these estimates were obtained from a separate model (n = 2340) comparing involved organs lungs versus meninges, miliary, and other organs and was adjusted for age, sex, BMI at start of TB treatment, previous history of TB, CD4 cell count at TB treatment start, ART status at TB diagnosis, and taking into account heterogeneity across regions (clustering by treatment programmes). The reference category is indicated with 1.

Patient factors associated with LTFU and mortality

In a multivariate model, LTFU during TB treatment was equivalent in EPTB patients' (including both PTB/EPTB) compared to PTB patients (aOR 0.92, 95% CI 0.36 to 2.32). It was also equivalent in EPTB patients' only compared to PTB patients (aOR 0.58, 95% CI 0.30 to 1.13). However, patients with both PTB/EPTB had at higher odds for LTFU compared to PTB patients (aOR 2.59, 95% CI 1.06 to 6.32, Table 3). EPTB mortality was similar to that of PTB (aOR 1.03, 95% CI 0.84 to 1.27; Table 3). However, TB meningitis was associated with increased mortality (aOR 1.85, 95% CI 1.00 to 3.10) compared to PTB, and overall mortality was also higher in patients with CD4 cell counts <50 cells/lL compared to those with CD4 cell counts ≥200 cells/lL (aOR 2.60, 95% CI 1.46 to 4.64; Table 3). Sensitivity analyses (Table 3) considering patients LTFU as having died showed similar results. From a separate model, bacteriological confirmation was associated with reduced mortality among PTB patients (aOR 0.68, 95% CI 0.61 to 0.76) and EPTB patients (aOR 0.32 95% CI 0.13 to 0.79) compared to all other TB patients with a negative test result.

Discussion

The lymph nodes, pleura, abdomen, and meninges were the most frequently involved organs in this large, multicohort study of HIV-positive EPTB patients treated in ART programmes in sub-Saharan Africa, Asia-Pacific, and Caribbean, Central and South America. Diagnosis were mainly based on clinical criteria, and bacteriological confirmation was seen less frequently in EPTB than PTB patients. Mortality was reduced among EPTB patients and PTB patients with a positive diagnostic test result compared to all other TB patients with a negative result. The observation that CD4 cell count in patients with EPTB was frequently lower than that of PTB patients was similar to the report of a South African study, that found that EPTB was generally more common in HIV-positive patients with lower CD4 cell counts, and three times more frequent among those with HIV and a CD4 count <50 cells/lL than among HIV-negative individuals [4]. Similarly, the predominating involvement of the lymph nodes that we observed is consistent with previous publications on the presentation of EPTB in HIV-positive adults [19-24].

The diagnosis of TB is more challenging in HIV-positive than in HIV-negative patients. In PTB, this is due to reduced lung cavitation and lower bacterial load in sputum [25-28]. In line with previously published results, we observed that smear microscopy was the most commonly used diagnostic tool, even when other diagnostic modalities were available [16]. We further observed that bacteriological confirmation (positive smear microscopy, culture or Xpert MTB/RIF result) was associated with reduced mortality in PTB and EPTB patients compared to TB patients with a negative result. A study from Malawi showed similar results, but also found increased mortality among EPTB patients with a smear-negative result [29]. A systematic review explained the reduced mortality in bacteriologically confirmed PTB cases by showing that smear- and culture-negative disease is typical of advance HIV immunosuppression compared to smear-positive TB patients with a less compromised immune system [30].

Among the EPTB patients for whom a bacteriological confirmation test was performed, only 42% were confirmed positive. Bacteriological confirmation is challenging due to EPTB's paucibacillary nature, in tissue, body fluid, or cerebrospinal fluid and the need for invasive specimen collection for microbiological diagnosis by biopsy or fine needle aspiration [7-9]. Mycobacterial culture and Xpert MTB/RIF have been shown to reliably diagnose EPTB, but are still rarely used in resource-limited settings, even when available [13,16]. From a programmatic perspective, the introduction of new diagnostics can indeed increase the proportion of bacteriological confirmed TB patients, as shown by a study from Cape Town, South Africa [31], but this may depend on the clinical setting [32]. The

newly developed, next generation Xpert MTB/RIF Ultra assay has a higher sensitivity and similar specificity than the first generation Xpert MTB/RIF assay [33], and seems to be particularly useful in EPTB and paediatric TB [34].

While overall mortality was similar in PTB and EPTB patients, the mortality of HIV-positive patients with TB meningitis was greater than that of HIV-positive patients with PTB. This is in accord with a review that reported mortality up to 69% for TB meningitis in low-income countries [6]. We observed no difference in the odds of being LTFU during treatment among PTB compared to EPTB patients, which is in line with a Nigerian study [35]. However, we found that patients with only EPTB showed lower and mixed PTB/EPTB cases slightly increased risk of LTFU compared to PTB. This could be explained by the fact that the patients with mixed PTB/EPTB diagnosis are too sick to return to the clinic, or might even have died at home. A recent study from Botswana also reported an increased risk for LTFU during treatment in EPTB patients (including mixed PTB/EPTB cases) compared to PTB patients [36].

The main limitation of our study was its potential for misclassification bias. EPTB might have been underestimated due to the limited availability of diagnostic capacities [16] and the clinical practice of not pursuing further diagnoses once a PTB diagnosis has been established. Another limitation of the study was the heterogeneity of the participating ART programmes in terms of TB and HIV treatment, availability of supportive care [16] as well as lack of data on opportunistic infections other than TB. Further analysis of treatment outcomes by diagnostic method was not possible due to the small numbers. Although we could not assess the vital status of those LTFU, the sensitivity analysis that we conducted to assess the potential impact of misclassification of death as lost to follow-up did not show any differences in the main outcomes. In spite of these potential limitations, our study provides important evidence on the limited diagnostic capacities available for EPTB at ART programmes in LMIC, and is one of few studies investigating EPTB in this context.

Conclusions

Diagnosis of EPTB in ART programmes in LMIC is based mainly on clinical symptoms, and the introduction of molecular assays is still challenging despite major efforts. We conclude that greater access to diagnostic services could improve diagnosis, increase the number of diagnosed EPTB and improve clinical management of EPTB as well as treatment outcomes.

Competing interests

All authors have no competing interests.

Author's contribution

KZ, MB, ME and LF involved in conception and design. KZ and MB analysed the data. KZ and LF completed the final draft of the manuscript. MB, SK, HC, MY, BG, DM, TRS, KMN, AMM, ACP and ME provided input into the study design, analyses and drafting of the paper. All authors reviewed and approved the final version of the manuscript.

Acknowledgements

We thank the sites that participated in the survey and the patients whose data were used in the study. We are indebted to the IeDEA Tuberculosis Working Group for following the project's progress. We also thank the regional data centers, which contributed not only to coordinating the study but also to recording and entering data. The authors thank Christopher Ritter for editorial assistance.

Funding

The International Epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, and the National Institute on Drug Abuse: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919; Pettit K08 AI104352. This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

Participating sites

The sites participating in this project: Benin, CNHU Cotonou; Brazil, INI-Fiocruz; Burundi, CHUK Bujumbura; Cameroon, Military Hospital of Yaoundé; Côte d'Ivoire, CIRBA Adultes; Honduras, IHHS; Kenya, AMPATH; Mali, Point G, Bamako; Mexico, INCMNSZ; Peru, IMTAvH, CoVIHS; Philippines, Research Institute for Tropical Medicine; République Démocratique du Congo; Rwanda, Military Hospital; Senegal, Dakar; Tanzania, National Institute for Medical Research, Mwanza Research Centre, Kisesa Clinic, Mwanza; Tanzania, National AIDS Control Programme (NACP), Tumbi Regional Hospital; Thailand, HIV-NAT; Thailand, Faculty of Medicine Ramathibodi Hospital; Uganda, Masaka Regional Hospital; Vietnam, Bach Mai Hospital; Vietnam, National Hospital for Tropical Diseases. Membership of the IeDEA collaboration for participating programmes is shown in Additional File 3.

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6. Paper 4 – Integrated services of HIV and MDR-TB

Integrating services for HIV and multidrug-resistant tuberculosis: a global survey in low-and middle-income countries

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The article has been submitted and is currently under review.

Contribution: I contributed to the conception and study design. I designed the questionnaire and coordinated the data collection. I performed the data cleaning, data analysis and made all figure and tables. I wrote the first draft of the manuscript and all subsequent versions – addressing comments from co-authors and reviewers – until the final published version.

Abstract

Background: Tuberculosis (TB) is the leading cause of death among people living with HIV and multidrug-resistant-TB (MDR-TB) is associated with high mortality. We examined the integration of services for adults living with HIV and co-infected with MDR-TB at antiretroviral therapy (ART) clinics in low-and middle-income countries.

Methods: Between 2019 and 2020, we surveyed 29 ART clinics, all members of the International epidemiology Databases to Evaluate AIDS (IeDEA) consortium. Clinics in WHO-defined high TB burden countries were eligible. We used structured questionnaires to collect clinic-level data on the integration of TB and HIV services and the availability of diagnostic tools and treatment for MDR-TB. To assess the treatment practices of health care workers, we used hypothetical clinical scenarios presenting cases of adult patients co-infected with HIV-TB.

Results: Of 29 ART clinics, 20 (69%) were in Africa, 6 (21%) were in Asia-Pacific, and 3 (10%) were in South America; 25 (86%) were in urban areas and 19 (66%) were tertiary care clinics. Integrated HIV-TB services were reported at 25 (86%) ART clinics for pan-susceptible TB, and 14 (48%) ART clinics reported full MDR-TB services on-site, i.e. drug susceptibility testing [DST] and MDR-TB treatment. Some form of DST was available on-site at 22 (76%) clinics, while the remainder referred testing off-site. All clinics with on-site DST used rapid molecular DST for identifying resistance to first-line drugs (22, 76%). On-site DST for second-line drugs was available at 9 (31%) clinics. MDR-TB treatment was delivered on-site at 15 (52%) clinics, with 10 individualising treatment based on DST results and 5 using standardised regimens alone; all recommended directly observed therapy during the entire MDR-TB treatment course. Bedaquiline was routinely available at 5 (17%) clinics and delamanid at 3 (10%) clinics.

Conclusions: Although most ART clinics reported having integrated HIV and TB services, few had fully integrated MDR-TB services. Access to advanced diagnostic tools and newer treatment options for MDR-TB was limited. There is a continued need for increased access to diagnostic and treatment options for MDR-TB patients and better integration of MDR-TB services into HIV care in high TB burden settings.

Introduction

Tuberculosis (TB) is the leading cause of death among people living with HIV (PLHIV) (1). In 2019, HIV-positive individuals accounted for about 8% of incident TB cases and 15% of TB mortality globally (1). Compared to HIV-negative individuals, PLHIV have an increased risk of developing active TB disease, even when on antiretroviral therapy (ART) (2, 3). There is also evidence of an association between HIV and drug-resistant TB; outbreaks involving PLHIV have been well-documented in high HIV burden countries (4, 5).

Multidrug-resistant tuberculosis (MDR-TB) challenges global TB control and is associated with high mortality (4). Strategies for controlling MDR-TB include drug susceptibility testing (DST) to guide treatment and the completion of an adequate treatment regimen. However, managing MDR-TB is difficult, particularly in low-and middle-income countries (LMICs), where there is limited access to both DST and newer, more effective MDR-TB drugs (6, 7).

For PLHIV co-infected with MDR-TB, effective coordination among services is particularly important given the complexity of second-line anti-TB treatment and the need for a careful monitoring of side effects. The integration of HIV and TB services has been identified as a global priority (8, 9), especially in regions where both diseases are widespread, which include sub-Saharan Africa, Asia and Latin America. In a multi-regional study conducted in 2012 among 47 ART clinics, we showed that only 26% offered integrated HIV-TB services, with large regional disparities (10). However, it is unclear to what extent integrated services included the management of drug-resistant TB, especially MDR-TB, among PLHIV. Therefore, we conducted a multi-regional survey assessing the capacity and routine practices of ART clinics related to the diagnosis and treatment of MDR-TB among PLHIV.

Methods

Study design

This cross-sectional survey was conducted within the International Epidemiology Databases to Evaluate AIDS (IeDEA, www.iedea.org) network, a large consortium of ART clinics predominantly located in LMICs (11, 12). ART clinics within IeDEA are mostly public facilities but are often supported by non-governmental organisations or academic institutions. ART clinics from the Asia-Pacific, Africa, and South America IeDEA regions located in countries defined by the World Health Organisation (WHO) as high MDR-TB, high TB, or high TB/HIV burden were eligible to participate (13). In West Africa, none of the countries participating in IeDEA were belonging to the aforementioned WHO definitions. Therefore, we selected a random sample of countries to ensure the representation of that region. In this analysis, we focused on the management of adults living with HIV and co-infected with MDR-TB.

Data collection

IeDEA representatives from each region and an advisory group of TB experts were involved in developing the survey tool. The survey was available in English and French and pilot-tested in both languages by the advisory group of TB experts and selected clinics. Survey data were electronically collected and managed using REDCap (Research Electronic Data Capture) (14, 15). All survey respondents were health care workers (HCWs) involved in managing TB patients as clinicians (medical doctors, clinical officers or nurses). The survey consisted of three components: (A) basic information on the ART clinic, including level of care, size, adult/paediatric services, cost of services, and infection control measures; (B) information about the management of adult TB, including case

definitions, and availability of diagnostic tools; (C) hypothetical clinical scenarios presenting cases of adult patients co-infected with TB/HIV to assess the clinical practices of treating HCWs. Components A and B were completed by one respondent per clinic. Component C was completed by two to three different respondents per clinic who were asked to respond based on routine practices. The respondent was required to be a HCW at the ART facility who provided care to TB patients (e.g. medical doctor, clinical officer, nurse, etc.) and could consult with others to answer questions. Data collection took place between September 2019 and March 2020.

Definitions

We defined MDR-TB as resistance to both isoniazid and rifampicin, and extensively drug-resistant TB (XDR-TB) as MDR plus resistance to any fluoroquinolone and at least one second-line injectable drug (16). We defined an ART clinic with integrated HIV-TB basic services as a clinic where: 1) HIV-positive people are actively screened for TB at enrollment using symptom screening; 2) TB and HIV clinical services are located in the same facility, under the same roof, or available with same-day appointments; and 3) facilities have a specialised clinic/ward on-site with dedicated staff for patients with TB (10). We categorised the degrees of service integration for MDR-TB as follows: i) full integration if both DST and MDR-TB treatment were available on-site, ii) partial integration, if either DST or MDR-TB treatment was available on-site, and iii) off-site services if both DST and MDR-TB treatment were available off-site. Clinical officers were defined as trained non-physician clinicians and medical doctors as physicians with a university degree.

Any of the following were considered initial TB diagnostic tools: smear microscopy; chest x-ray; any GeneXpert including Xpert MTB/RIF, Ultra, Omni, or XDR (Cepheid, USA); any line probe assay (LPA) including Genotype® MTBDRplus or Genotype® MTBDRsl (Hain Lifescience GmbH, Germany); mycobacterial liquid or solid culture; or urine lipoarabinomannan (LAM). DST was categorised as either molecular (any GeneXpert or any LPA) or phenotypic (mycobacterial culture). The "Bangladesh regimen" is a standardised short course MDR-TB treatment regimen of 9 to 12 months. It consists of an initial 4-6 months of kanamycin, moxifloxacin, ethionamide/prothionamide, clofazimine, pyrazinamide, high-dose isoniazid, and ethambutol, followed by 5 months of moxifloxacin, clofazimine, pyrazinamide, and ethambutol (17, 18).

Analyses

We described ART clinics by the degree of service integration and by region and explored the availability of on-site versus off-site diagnostic tools and treatment for MDR-TB. We analysed differences among ART clinics using chi-square or Fisher's exact. Using descriptive statistics, we assessed routine practices related to MDR-TB management captured by hypothetical clinical scenarios. All analyses were done in Stata version 15.1 (Stata Corporation, College Station, TX, USA).

Ethics statement

The Cantonal Ethics Committee Bern (Switzerland), Vanderbilt University Medical Center IRB (Tennessee, USA), and Johns Hopkins University School of Medicine IRB (Maryland, USA) approved this project. All participating sites obtained approvals from their local institutional review boards or ethics committees to participate in IeDEA research. All participants provided informed consent before participating.

Results

Description of the ART clinics

We collected data from 29 adult ART clinics across 19 high TB burden countries (Figure 1). Of 29 ART clinics, 20 (69.0%) were in Africa, 6 (20.7%) were in Asia-Pacific, and 3 (10.3%) were in South America. Clinic-level survey components were completed by medical doctors (23/29, 79.3%), clinical officers (4/29, 13.8%), and nurses (2/29, 6.9%). The majority of respondents (24, 88.9%) had worked at the participating ART clinic for more than five years at the time of survey completion. Overall, 25/29 (86.2%) participating clinics were in urban and 4/29 (13.8%) were in rural settings. Most participating sites (19, 65.5%) were tertiary care clinics (Table 1).

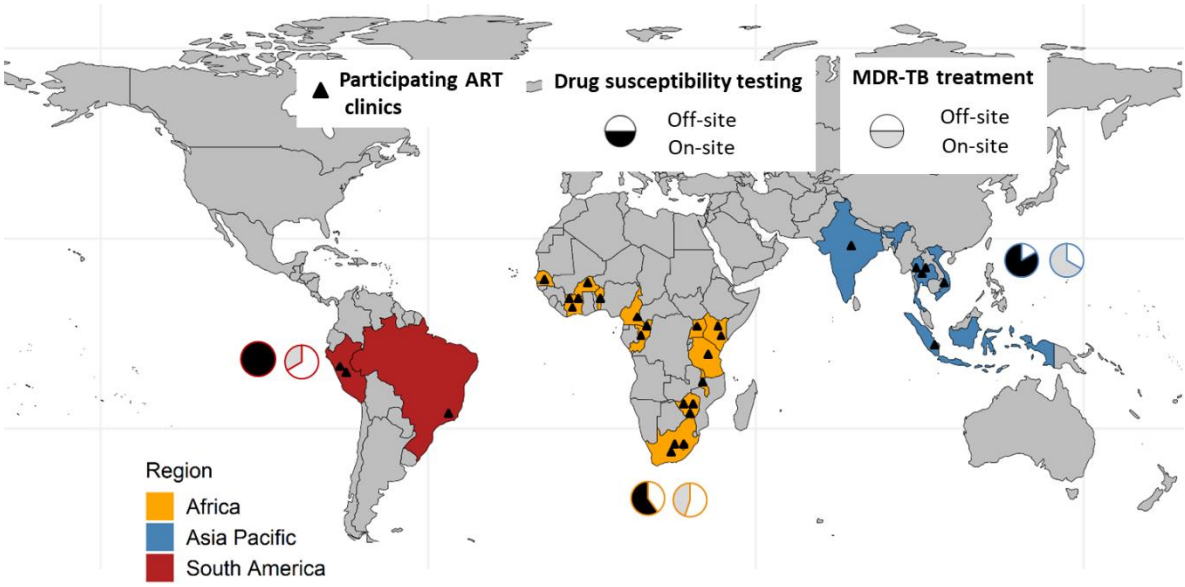

Figure 1. Map showing the participating ART clinics by regions. Pie charts indicate the proportion of clinics with drug-susceptibility testing (DST) (black) and MDR-TB treatment (grey) available on-site by region. Types of DST available included Xpert MTB/RIF, line probe assay, and culture. MDR, multidrug resistance; TB, Tuberculosis

Almost all ART clinics (27, 93.1%) reported following their national TB guidelines to screen, diagnose and treat TB patients. For infection control, environmental measures, such as regular natural ventilation, were reported to be in place at 26 (89.8%) of the ART clinics,19 clinics (65.5%) reported that staff regularly wore masks when in close contact with TB patients or TB suspects (Table S1), and 18 (62.1%) conducted TB screening among clinic staff who were in contact with TB patients or TB suspects.

Integration of HIV and MDR-TB services

The majority of participating clinics (25, 86.2%) reported offering integrated HIV-TB basic services, with 25 clinics (86.2%) offering initial TB diagnosis and 20 clinics (69.0%) first-line TB treatment on-site (Table 1).

Fourteen clinics (48.3%) reported full integration of HIV and MDR-TB services and nine (31.0%) reported partial integration, of which eight offered on-site DST only and one offered on-site MDR-TB treatment only. Six clinics (20.7%) had access to off-site MDR-TB services only for both diagnosis and treatment (Figure 2, Table 2).

We describe care pathway for patients coinfected with TB/HIV at participating clinics in Figure 3, from the identification of presumptive TB to drug resistance testing and treatment. Initial TB diagnosis was conducted off-site in four clinics (13.8%), of which half referred patients while the other half sent samples for off-site testing. Three out of four of these clinics received initial TB diagnostic result from the off-site clinic. For any type of DST, seven clinics (24.1%) relied on off-site services, of which three referred patients and four sent samples for off-site testing. Five out of seven of these clinics (71.4%) received DST results from the referral clinic. Treatment of pan-susceptible TB was prescribed off-site in nine clinics (31.0%), of which five (55.6%) received treatment outcome reports from the off-site clinic. For MDR-TB treatment, fourteen clinics (48.3%) referred patients off-site, of which nine (64.3%) received treatment outcome reports for from the referral clinic (Figure 3).

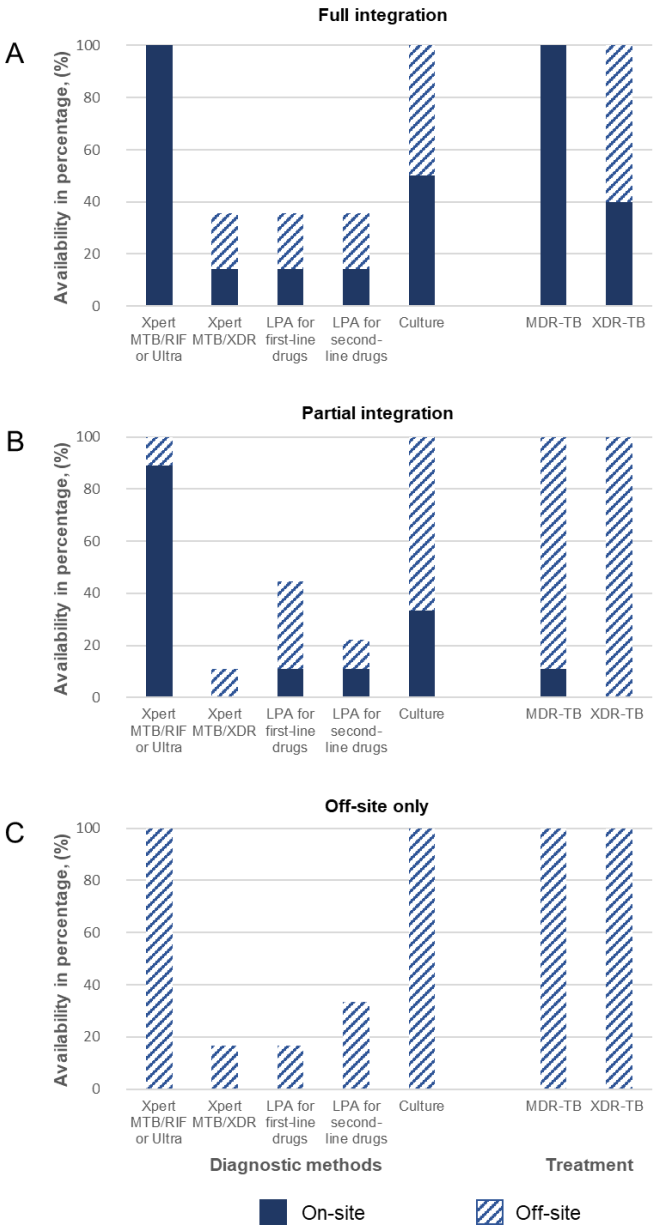

Figure 2. Models of integration of HIV and MDR-TB care. Models of integration of HIV and MDR-TB care. Panel A: Fourteen clinics with full integration of DST and MDR-TB treatment on-site; **Panel B**: Partial integration of eight clinics with DST on-site and MDR-TB treatment off-site and one clinic with the opposite; and **Panel C**: Both DST and MDR-TB treatment available off-site only at six clinics. The solid bars represent the percentage of the different molecular or phenotypic DST or MDR/XDR-TB treatment available on-site. The dashed bars represent the percentage of DST or MDR/XDR-TB treatment

Drug susceptibility testing

DST, in some form, was available on-site at 22/29 (75.9%) ART clinics. Ten ART clinics reported having molecular and phenotypic DST available on-site, whereas the remaining 12 ART clinics reported having molecular DST only (Table S2). Molecular DST was most frequently available on-site in South America (3/3, 100%) followed by Asia-Pacific (5/6, 83.3%) and Africa (14/20, 70.0%). In contrast, phenotypic DST was most frequently available on-site in Asia-Pacific (5/6, 83.3%), followed by South America (2/3, 66.7%) and Africa (3/20, 15.0%) (Table 1). Two ART clinics (6.9%) reported access to sequencing technologies.

Table 1. General clinic-level information, availability of TB services and cost model of care in the 29 ART clinics participating in the study, by region.

	Total (n=29) n (%)	Asia-Pacific (n=6) n (%)	South America (n=3) n (%)	Africa (n=20) n (%)
Clinic-level information				
Setting				
Urban	25 (86.2)	6 (100)	3 (100)	16 (80.0)
Rural	4 (13.8)	0	0	4 (20.0)
Highest level of care				
Primary	8 (27.6)	0	0	8 (40.0)
Secondary	2 (6.9)	0	0	2 (10.0)
Tertiary	19 (65.5)	6 (100)	3 (100)	10 (50.0)
Adults and/or children care				
Adults and children	17 (58.6)	1 (33.3)	2 (66.6)	14 (70.0)
Adults only	12 (41.4)	5 (66.7)	1 (33.3)	6 (30.0)
Integrated HIV-TB basic services				
Yes	25 (86.2)	6 (100)	3 (100)	16 (80.0)
No	4 (13.8)	0	0	4 (20.0)
Availability of TB services				
Initial TB diagnosis				
On-site	25 (86.2)	6 (100)	3 (100)	16 (80.0)
Off-site	4 (13.8)	0	0	4 (20.0)
Molecular DST				
On-site	22 (75.9)	5 (83.3)	3 (100)	14 (70.0)
Off-site	7 (21.1)	1 (16.7)	0	6 (30.0)
Phenotypic DST				
On-site	10 (34.5)	5 (83.3)	2 (66.7)	3 (15.0)
Off-site	19 (65.5)	1 (16.7)	1 (33.3)	17 (85.0)
Treatment pan-susceptible TB				
On-site	20 (69.0)	5 (83.3)	1 (33.3)	14 (70.0)
Off-site	9 (31.0)	1 (16.7)	2 (66.7)	6 (30.0)
Treatment MDR-TB				
On-site	15 (51.7)	5 (83.3)	1 (33.3)	9 (45.0)
Off-site	14 (48.3)	1 (16.7)	2 (66.7)	11 (55.0)
Drug-resistant TB Integration Models				
Full integration	14 (48.3)	5 (83.3)	1 (33.3)	8 (40.0)
Partial integration	9 (31.0)	0	2 (66.7)	7 (35.0)
Off-site only)	6 (20.7)	1 (16.7)	0	5 (25.0)
Cost Model				
Initial TB diagnosis				
Full payment by the patient	1 (3.4)	1 (16.7)	0	0
Cost sharing (partial payment by the patient)	6 (20.7)	2 (33.3)	0	4 (20.0)
Available at no cost for the patient	22 (75.9)	3 (50.0)	3 (100)	16 (80.0)
Pan-susceptible TB treatment				
Full payment by the patient	1 (3.4)	1 (16.7)	0	0
Cost sharing (partial payment by the patient)	1 (3.4)	1 (16.7)	0	0
Available at no cost for the patient	27 (93.1)	4 (66.7)	3 (100)	20 (100.0)
MDR-TB treatment				
Cost sharing (partial payment by the patient)	1 (3.4)	1 (16.7)	0	0
Available at no cost for the patient	28 (96.6)	5 (83.3)	3 (100)	20 (100)

ART, antiretroviral therapy; DST, drug susceptibility testing; MDR, multidrug resistance; TB, Tuberculosis; XDR, extensive drug resistance

Mycobacterial culture was available on-site at 10/29 (34.5%) clinics for identifying resistance to first-line drugs. All clinics with some form of DST on-site had a rapid molecular DST for first-line drugs available (22/29, 72.4%). Specifically, Xpert MTB/RIF was available on-site at 21/29 (72.4%), Xpert Ultra at 7/29 (24.1%), and first-line LPA (MTBDRplus) at 3/29 (10.3%, Table 2 and S2). Five (17.2%) ART clinics reported Xpert cartridge stock-outs in the preceding 12 months. In the hypothetical clinical scenario of a patient failing TB treatment, 57/72 (79.2%) HCWs would request a rapid molecular DST to identify resistance to first-line drugs (Table S3, Scenario 1). The proportion of HCWs who selected this response was higher among ART clinics with full integration of MDR-TB services (31/35, 88.6%) and lower among ART clinics with partial integration and only off-site MDR-TB services (17/24, 70.8%, and 9/13, 69.2%, respectively).

In contrast to first-line DST, DST for second-line drugs was available on-site at 9/29 (31.0%) clinics. Mycobacterial culture for second-line drugs was available at 8/29 (27.6%) clinics; rapid molecular DST for second-line drugs was rarely available. Specifically, Xpert MTB/XDR was available on-site at 2/29 (6.9%) clinics and second-line LPA (MTBDRsl) at 3/29 (10.3%) clinics (table 2 and S2).

Treatment of MDR-TB

MDR-TB treatment was reported to be offered on-site at 15/29 (51.7%) ART clinics - 83.3% of clinics in Asia-Pacific, 45.0% of clinics in Africa, and 33.3% of clinics in South America (Table 1). When treated on-site, MDR-TB treatment was most frequently prescribed by medical doctors (10/15, 66.7%), followed by clinical officers (3/15, 20.0%), and nurses (2/15, 13.3%).

Table 2. Availability of TB services by the degree of integration of HIV and MDR-TB services at 29 ART clinics

	Total	Full integration	Partial integration	Off-Site only
	(n=29)	(n=14) n (%)	(n=9) n %)	(n=6) n (%)
Availability of molecular DST for first-line drugs				
Any on-site	22 (75.9)	14 (100)	8 (88.9)	0
Xpert MTB/RIF or Xpert MTB/Ultra	*21 (72.4)*	*14 (100)*	*7 (77.8)*	*0*
Genotype MTBDRplus	*3 (10.3)*	*2 (14.3)*	*1 (11.1)*	*0*
Any off-site	12 (42.4)	3 (21.4)	3 (33.3)	6 (100)
Xpert MTB/RIF or Xpert MTB/Ultra	*7 (24.1)*	*0*	*1 (11.1)*	*6 (100)*
Genotype MTBDRplus	*7 (24.1)*	*3 (21.4)*	*3 (33.3)*	*1 (16.7)*
Availability of molecular DST for second-line drugs				
Any on-site	4 (13.8)	3 (21.4)	1 (11.1)	0
Xpert MTB/XDR	*2 (6.9)*	*2 (14.3)*	*0*	*0*
Genotype MTBDRsl	*3 (10.3)*	*2 (14.3)*	*1 (11.1)*	*0*
Any off-site	11 (37.9)	6 (42.9)	2 (22.2)	3 (50.0)
Xpert MTB/XDR	*5 (17.2)*	*3 (21.4)*	*1 (11.1)*	*1 (16.7)*
Genotype MTBDRsl	*6 (20.7)*	*3 (21.4)*	*1 (11.1)*	*2 (33.3)*
Availability of phenotypic DST				
On-site	10 (34.5)	7 (50.0)	3 (33.3)	0
First-line drugs	*10 (34.5)*	*7 (50.0)*	*3 (33.3)*	*0*
Second-line drugs	*8 (27.6)*	*6 (42.9)*	*2 (22.2)*	*0*
Off-site	19 (65.5)	7 (50.0)	6 (66.7)	6 (100)
First-line drugs	*19 (65.5)*	*7 (50.0)*	*6 (66.7)*	*6 (100)*
Second-line drugs	*8 (27.6)*	*3 (21.4)*	*3 (33.3)*	*2 (33.3)*
DR-TB treatment				
MDR-TB treatment				
On-site	15 (51.7)	14 (100)	1 (11.1)	0
Off-site	14 (48.3)	0	8 (88.9)	6 (100)
MDR-TB regimens				
Individualised according to the resistance profile	13 (44.8)	10 (71.4)	2 (22.2)	1 (16.7)
Standardised	11 (37.9)	2 (14.3)	4 (44.4)	5 (83.3)
Both (individualised and standardised)	5 (17.2)	2 (14.3)	3 (33.3)	0
XDR-TB treatment				
On-site	7 (24.1)	6 (42.9)	1 (11.1)	0
Off-site	22 (75.9)	8 (57.1)	8 (88.9)	6 (100)

DST, drug susceptibility testing; DR, drug-resistant; MDR, multidrug resistance; TB, Tuberculosis; XDR, extensive drug resistance

The remaining 14 ART clinics (48.3%) referred their MDR-TB patients off-site for MDR-TB treatment. When treated off-site, MDR-TB patients were referred to specific TB clinics (9/14, 64.3%): 6/14 (42.9%) to tertiary care facilities and 3/14 (21.4%) to secondary care facilities. Most referral clinics were located more than 20 km away from the ART clinic (12/14, 85.7%). However, more than half of ART clinics received reports from the referral clinic about their patients' treatment outcomes (9/14, 64.3%) and side effects (8/14, 57.1, Figure 3).

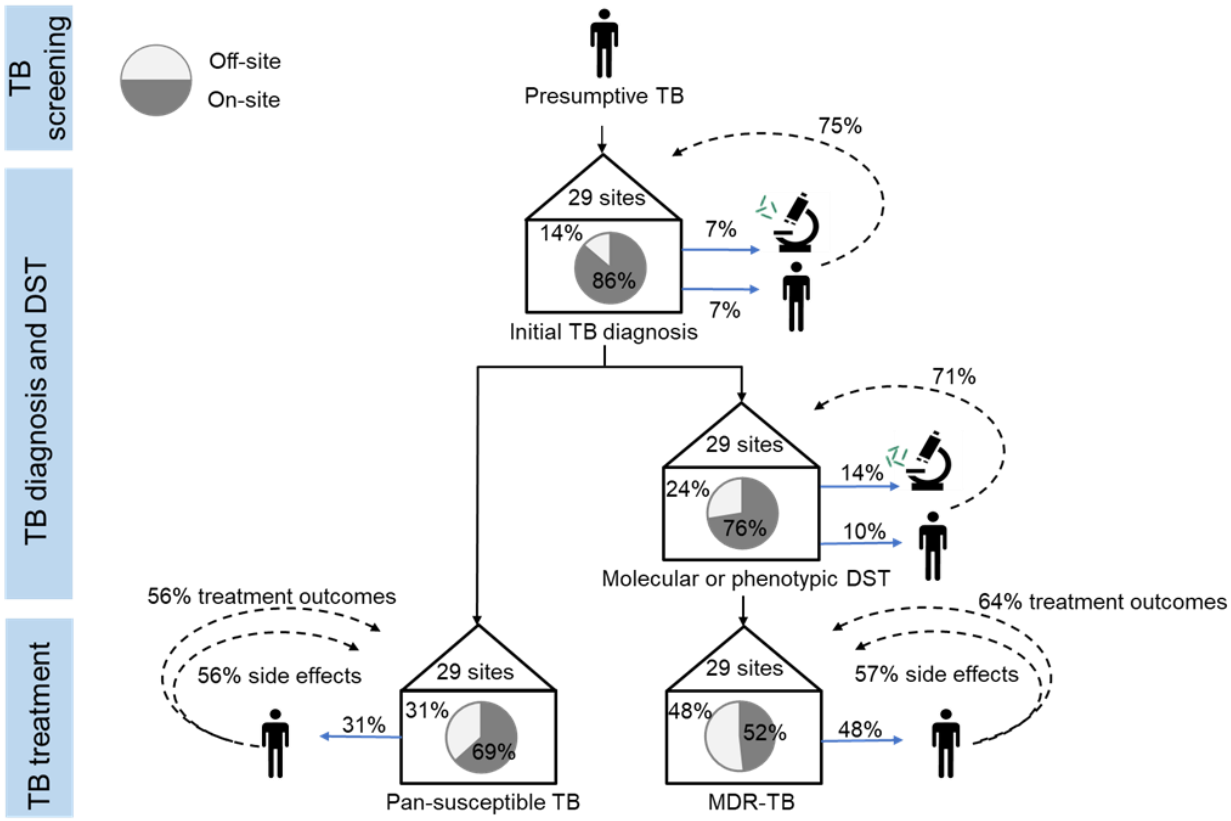

Figure 3. Care pathway for patients with HIV-TB coinfection at 29 ART clinics. Solid blue lines indicate patients or samples sent off-site for TB diagnosis, drug susceptibility testing (DST), or treatment. Dotted lines indicate feedback loops when diagnostic results, DST results, side effect or treatments outcomes are directly communicated back to the ART clinic by phone, written correspondence, or shared database entry. DST, drug susceptibility testing; MDR, multidrug resistance; TB, Tuberculosis; XDR, extensive drug resistance

Overall, 44.8% (13/29) of clinics reported that MDR-TB treatment was individualised, whereas 37.9% (11/29) reported only following standardised and pre-determined MDR-TB treatment protocols, and the remaining reported both individualised and standardised MDR-TB treatment (Table 2 and 3). In ART clinics with full integration of MDR-TB services, MDR-TB treatment was most frequently individualised (10/14, 71.4%), whereas in ART clinics with off-site services only, MDR-TB treatment was generally standardised (5/6, 83.3%, Table 2). Similarly, overall on-site MDR-TB treatment was more frequently individualised than standardised (Table 3). However, all national TB guidelines for participating clinics mentioned a standardised short MDR-TB regimen and most of them a standardised long MDR-TB regimen. Specifically, the standardised short "Bangladesh regimen" was mentioned in 13/19 (68.4%) national TB guidelines and reported to be in use at 18/29 (62.1%) ART clinics. Additionally, in the hypothetical clinical scenario of a newly diagnosed patient with HIV and rifampicin-resistant TB, 58/72 (80.6%) HCWs would start the patient on a standardised MDR-TB regimen as soon as possible (Table S3, Scenario 2). All HCWs from ART clinics with full integration selected this response (35/35, 100%), while 17/24 (70.8%) HCWs from partially integrated and 6/13 (46.2%) HCWs from ART clinics with only off-site MDR-TB services selected this option.

DOT was recommended during the entire MDR-TB treatment course at 24/29 (82.8%) ART clinics, whereas 5/29 (17.2%) clinics recommended it only during the intensive phase. Table 3 presents the different types of DOT strategies. XDR-TB treatment was available on-site at 7/29 (24.1%) ART clinics. Among the seven ART clinics that reported treating XDR-TB patients, six used individualised regimens (85.7%). Bedaquiline was routinely available at 5/29 (17.2%) clinics and upon request at another seven (24.1%); delamanid was routinely available at 3/29 (10.3%) clinics and upon request at another seven clinics (24.1%, Table 3).

Table 3. Management and treatment of MDR-TB at 29 ART clinics.

	Total (n=29, %)	On-site (n=15, %)	Off-site (n=14, %)	p-value
Treatment in line with National TB Program				0.37
Strictly in line with National TB Program	27 (93.1)	13 (86.7)	14 (100)	
Somewhat modified	1 (3.4)	1 (6.7)	0	
Individualised MDR-TB treatment	1 (3.4)	1 (6.7)	0	
Directly observed treatment				
During initiation phase only	5 (17.2)	0	5 (35.7)	0.017
During the whole duration of treatment	24 (82.8)	15 (100)	9 (64.3)	
Clinical visits intensive phase				0.75
Daily	8 (27.6)	5 (33.3)	3 (21.4)	
Weekly	9 (31.0)	4 (26.7)	5 (35.7)	
Monthly	12 (41.4)	6 (40.0)	6 (42.9)	
Clinical visits continuation phase				0.81
Daily	2 (6.9)	1 (6.7)	1 (7.1)	
Weekly	2 (6.9)	2 (13.3)	0	
Monthly	22 (75.9)	11 (73.3)	11 (78.6)	
Every third month	3 (10.3)	1 (6.7)	2 (14.3)	
Type of directly observed treatment				0.79
Self-administered	5 (17.2)	4 (26.7)	1 (7.1)	
Health facility based	11 37.9)	5 (33.3)	6 (42.9)	
Health and self-administered	11 (37.9)	6 (40.0)	5 (35.7)	
Unknown	2 (6.9)	0	2 (14.3)	
MDR-TB regimens				
Individualised according to the resistance profile	13 (44.8)	10 (66.7)	3 (21.4)	0.062
Standardised	11 (37.9)	3 (30.0)	8 (57.1)	
Standardised or according to the resistance profile	5 (17.2)	2 (13.3)	3 (21.4)	
Use of Bangladesh MDR-TB regimen				1.0
Yes	18 (62.1)	9 (60.0)	9 (64.3)	
No	9 (31.0)	5 (33.3)	4 (28.6)	
Unknown	2 (6.9)	1 (6.7)	1 (7.1)	
Availability of new drugs				
Bedaquiline				0.02
Yes, always	5 (17.2)	4 (26.7)	1 (7.1)	
Upon request	7 (24.1)	6 (40.0)	1 (7.1)	
No	17 (58.6)	5 (33.3)	12 (85.7)	
Delamanid				0.08
Yes, always	3 (10.3)	3 (20.0)	0	
Upon request	7 (24.1)	5 (33.3)	2 (14.3)	
No	19 (65.5)	7 (46.7)	12 (85.7)	

MDR, multidrug resistance; TB, Tuberculosis

Discussion

The integration of TB services into HIV care is key to TB control, especially in regions where the burden of both diseases is high. Yet, little is known about the management of MDR-TB at ART clinics in LMICs. We surveyed 29 ART clinics across three continents about the integration of HIV and MDR-TB services. About half of them offered full MDR-TB services on-site, and about three-quarters had at least access to rapid molecular testing for MDR-TB. A fifth of the clinics entirely relied on off-site services to which suspected MDR-TB cases were referred. We observed substantial regional differences in the management of MDR-TB at ART clinics.

To improve quality of care, ART clinics must strengthen the integration of HIV-TB services (9). There is evidence that full HIV-TB integration improves HIV and TB care (19). One study found a higher rate of treatment success among integrated clinics than non-integrated clinics (20). Inadequate referral mechanisms and poor communication can hamper integrated HIV-TB services in LMICs in settings with partial integration, whereas limited human resources, training, and infrastructure affect settings with full integration (21). Our findings highlight the challenge of coordinating care when MDR-TB services are partially or entirely performed off-site: fewer than two-thirds of ART clinics received reports of treatment outcomes when their patients with MDR-TB were treated off-site.

WHO recommends that Xpert MTB/RIF is used as an initial diagnostic test in individuals with suspected MDR-TB or HIV-associated TB (22). This study showed that Xpert MTB/RIF was available in about three-quarters of participating clinics. In 2012, we reported that Xpert MTB/RIF was only available on-site at 28% of ART clinics surveyed in the IeDEA consortium (23). The increase that we observed reflects efforts to roll-out Xpert MTB/RIF over the last decade, including in LMICs. In contrast, we found that culture-based phenotypic DST was only available on-site among 35% of clinics, a marginal increase from the 26% reported in 2012 (23). Culture is currently considered the gold standard DST method. Still, the technical challenges inherent to this method, including biosafety concerns, costly infrastructures, and slow turnover, limit its routine use at smaller ART clinics. In this study, we found that culture-based phenotypic DST was generally available at larger facilities outside of ART clinics, to which suspected MDR-TB cases were referred to or samples were sent.

Fifty-nine per cent of clinics could test for resistance to the first-line drugs rifampicin and isoniazid and 52% to fluoroquinolones - irrespective of whether DST testing was performed on-site or off-site. The lack of rapid tests for detecting second-line resistance can lead to inadequate treatment and increased mortality during TB treatment (7). Recent developments in sequencing technologies are promising, allowing the point-of-care identification of resistance to first- and second-line drugs simultaneously and directly on unprocessed sputum samples (24). Hopefully, this will allow for rapid diagnosis and effective, individualised treatment. However, next-generation sequencing methods were only available at two participating clinics.

There have been major changes in MDR-TB treatment guidelines in the last five years, including WHO's endorsement of the short-course "Bangladesh regimen" in 2016 (17, 18). Several studies have shown high treatment success with the "Bangladesh regimen" with minor modifications (80.2% to 95.5% success rates) (18, 25-27). However, one review found that patients with fluoroquinolone-resistant MDR-TB were much more likely to have an unfavourable outcome (≥50%) than those with a susceptible strain (<20%) unsing the "Bangladesh regimen" with our without minor modifications (28). We found that 18/29 (62%) participating clinics employed the "Bangladesh regimen", and the vast majority of HCWs surveyed would use a standardised MDR-TB regimen in routine care. This was particularly evident among sites with fully integrated MDR-TB services, suggesting that access to diagnostic tools may improve guideline adherence. Overall, the clinical scenarios demonstrated reliance on a DST-guided treatment strategies. However, only a few sites had access to rapid second-line DST. Without this important decision-making tool, sites in high MDR-TB burden settings may be unable to prescribe adequate and individualised treatment regimens and struggle to achieve widespread treatment success using a standardised MDR-TB regimen (29).

A recent meta-analysis of individual patient data in MDR-TB treatment demonstrated that new or reassigned oral drugs (bedaquiline, linezolid, clofazimine, later generation fluoroquinolones and the carbapenems) were associated with increased treatment success and reduced mortality (30). This led to new WHO guidelines, recommending the treatment of MDR-TB with only oral drugs (31). However, we observed that access to bedaquiline and delamanid remains limited among ART clinics in LMICs. In addition to ramping up access to new drugs, effective implementation of the new MDR-TB guidelines will require strong DOT and patient support, especially among PLHIV. Adherence to MDR-TB regimens remains an issue due to higher pill burden, adverse drug reactions and drug-drug interactions, especially among PLHIV on ART (28). It is promising that in our study, DOT was recommended during the entire MDR-TB treatment course in over 80% of clinics in our analysis.

Our study is limited in that it relies on clinicians' reports of available services and drugs at each site rather than patient level data. Furthermore, most participating ART clinics were in urban settings and offered tertiary level care, which could have led to an overestimation of HIV-TB service integration overall. However, a notable strength of our study is the global coverage of ART clinics in LMICs with

high TB burden and the detailed information collected on the on-site and off-site service availability. We have previously shown that the availability of diagnostic methods or second-line drugs do not necessarily imply that they were regularly used in routine practise (23). We explored this gap by assessing clinical practice with the presentation of clinical scenarios.

Conclusions

Although most surveyed ART clinics in LMICs reported integrated HIV-TB services, less than half reported full integration of MDR-TB services and access to DST for second-line anti-TB drugs was rare. There is a continued need for increased availability of diagnostic and treatment options for MDR-TB patients and better integration of advanced MDR-TB services into HIV care in TB high-burden settings. Furthermore, there is a need for novel technologies for DST and increased availability of newer, more effective MDR-TB drugs. Repeating site-level surveys is a practical method that allows for ongoing evaluation of progressive access to such testing and treatment.

Author's contribution

Conception and design: KZ, MB, LF, SC. KZ, MB, SC, LF, and JAT drafted the questionnaire. KZ, MB, SD, incorporated the questionnaire into REDCap. KZ analysed the data. KZ, MB, SC, and LF wrote the first draft. KZ, MB, SC, LF, and JAT completed the final draft of the manuscript. All authors provided input into the study design, on the questionnaire, analyses, and drafting of the paper. All authors reviewed and approved the final version of the manuscript.

Acknowledgments

We would like to thank all the participating clinics and all those who completed the questionnaire. Further, we would like to thank the advisory panel and the TB & Lung Health IeDEA working group for their inputs and feedbacks.

Source of support

The International Epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the US National Institutes of Health, National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart, Lung, and Blood Institute, the National Institute on Alcohol Abuse and Alcoholism, the National Institute of Diabetes and Digestive and Kidney Diseases, the Fogarty International Center, and the National Library of Medicine: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919. Informatics resources are supported by the Harmonist project, R24AI124872. ME was supported by special project funding (grant 189498) from the Swiss National Science Foundation. JAT was supported by NIH/NIAID (K23AI135102). The contents of this publication are solely the responsibility of the authors and do not represent the official views of any of the institutions mentioned above.

Competing interests

All authors have no conflicts of interest.

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Supplentary material

Supplementary Table 1. Infection control measures in place at the 29 ART clinics offering TB services on- and off-site.

	Total (n=29, %)	**Asia-Pacific** (n=6, %)	**South America** (n=3, %)	**Africa** (n=20, %)
Separate waiting rooms for MDR-TB patients				
Yes	15 (51.7)	3 (50.0)	1 (33.3)	11 (55.5)
No	14 (48.3)	3 (50.0)	2 (66.7)	9 (45.5)
Separate visiting hours for MDR-TB patients				
Yes	9 (31.0))	2 (33.3)	1 (33.3)	6 (30.0)
No	20 (69.0)	4 (66.7)	2 (66.7)	14 (70.0)
Natural air exchange through windows				
Yes	26 (89.7)	5 (83.3)	3 (100)	18 (90.0)
Optimized natural ventilation (airflow optimized by size of windows)	*14 (48.3)*	*4 (66.7)*	*1 (33.3)*	*9 (45.0)*
Natural ventilation, but not optimized	*12 (41.4)*	*1 (16.7)*	*2 (66.7)*	*9 (45.0)*
No natural ventilation	2 (6.9)	1 (16.7)	0	1 (5.0)
Unknown	1 (3.4)	0	0	1 (5.0)
Any protection of staff working with TB patients and presumptive TB patients				
Regular TB symptom screening (coughing, sweats, fever)	13 (44.8)	1 (16.7)	2 (66.7)	10 (50.0)
Regular screening by chest X-ray	13 (44.8)	4 (66.7)	2 (66.7)	7 (35.0)
Regular TB screening by sputum smear regardless of symptoms	2 (6.9)	0	0	2 (10.0)
Regular TB screening by culture regardless of symptoms	0	0	0	0
Regular TB screening by molecular tests regardless of symptoms	1 (3.4)	0	0	1 (5.0)
Wearing masks if in close contact to any TB patients	19 (65.5)	5 (83.3)	2 (66.7)	12 (60.0)
No specific protection measures offered to staff	4 (13.8)	1 (16.7)	1 (33.3)	2 (10.0)
Unknown	1 (3.4)	0	1 (33.3)	0

ART, antiretroviral therapy; MDR, multidrug resistance; TB, Tuberculosis

Supplementary Table 2: On- and off-site availability of diagnostic tests for initial TB diagnosis and diagnosis of drug-resistant TB.

	Total	On-site n (%)	Off-site n (%)	Not available n (%)
Initial TB Diagnosis				
Chest X-ray	29	18 (62.1)	10 (34.5)	1 (3.4)
Smear microscopy	29	22 (75.9)	7 (24.1)	-
Urine LAM	29	7 (24.1)	2 (6.9)	20 (69.0)
Molecular DST				
Any Xpert	29	22 (75.9)	7 (24.1)	-
Xpert MTB/RIF	29	21 (72.4)	7 (24.1)	1 (3.4)
Xpert MTB/RIF Ultra	29	7 (24.1)	3 (10.3)	19 (65.5)
Xpert MTB/XDR	29	2 (6.9)	5 (17.2)	22 (75.9)
Any line probe assay	29	3 (10.3)	9 (31.0)	17 (58.6)
Genotype® MTBDRplus	29	3 (10.3)	7 (24.1)	19 (65.5)
Genotype® MTBDRsl	29	3 (10.3)	6 (20.7)	20 (69.0)
Phenotypic DST				
Mycobacterial culture	29	10 (34.5)	19 (65.5)	-
First-line drugs	29	10 (34.5)	19 (65.5)	-
Second-line drugs	29	8 (27.6)	8 (27.6)	13* (44.8)

* Unknown availability

DST, drug susceptibility testing; LAM, urine lipoarabinomannan

Supplementary Table 3: Hypothetical Clinical scenarios assessing clinical practice related to the testing and treatment of MDR-TB at ART-clinics (n=72).

	Total	**Full integration**	**Partial integration**	**Off-Site only**
	(n=72)	(n=35) n (%)	(n=24) n %)	(n=13) n (%)
Scenario 1: A 23-year-old man living with HIV who has been on ART for 2 years was diagnosed with TB 3 months ago. He was started on 2HRZE, 4HR and his symptoms improved within a few days. However, his smear microscopy at month 1 was positive and his smear microscopy at month 2 remained positive as well. What would you do?				
Request a rapid molecular DST to determine the need to start second-line treatment	57 (79.2)	31 (88.6)	17 (70.8)	9 (69.2)
Extend the intensive phase of first-line treatment and re-evaluate after one month	6 (8.3)	2 (5.8)	4 (16.7)	0
Consider the patient to be non-adherent and do not make any treatment changes. Begin the patient on the continuation phase of first-line treatment and counsel him adherence.	4 (5.6)	2 (5.8)	0	2 (15.4)
Refer the patient to a TB clinic	5 (6.9)	0	3 (12.5)	2 (15.4)
Scenario 2: A 28-year-old female was recently been diagnosed with HIV. During her evaluation to start ART, she was diagnosed with MDR-TB using Xpert MTB RIF. Her sputum is being cultured on DST solid culture media and the result will be available in two months. What would you do?				
Start the patient on a standardised MDR-TB regimen as soon as possible and then start the patient on ART.	58 (80.6)	35 (100)	17 (70.8)	6 (46.2)
Wait until the TB facility starts the patient on MDR-TB treatment and then start the patient on ART.	12 (16.7)	0	6 (25.0)	6 (46.2)
Immediately start the patient on ART and refer her to another facility for MDR-TB treatment because	2 (2.8)	0	1 (4.2)	1 (7.7)
MDR-TB drugs are not prescribed at your clinic				

ART, antiretroviral therapy; DST, drug susceptibility testing; MDR, multidrug resistance; TB, Tuberculosis; H, isoniazid; R, rifiampicin, Z, pyrazinamide; E, ethambutol

7. Paper 5 – Phenotypic drug susceptibility testing and mortality

Drug susceptibility testing and mortality in patients treated for tuberculosis in high-burden countries: a multicentre cohort study

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The article has been published in the Lancet Infect Disease online on the February 7, 2019.
DOI: http://dx.doi.org/10.1016/S1473-3099(18)30673-X

Contribution: I did the data cleaning for the clinical and DST dataset. I performed the statistical analysis and made all tables and figures. Together with M.B I wrote the first draft of the manuscript and addressed all comments from co-authors and reviewers until the final published version.

Abstract

Background: Drug resistance is a challenge for the global control of tuberculosis. We examined mortality in patients with tuberculosis from high-burden countries, according to concordance or discordance of results from drug susceptibility testing done locally and in a reference laboratory.

Methods: This multicentre cohort study was done in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, South Africa, Peru, and Thailand. We collected *Mycobacterium tuberculosis* isolates and clinical data from adult patients aged 16 years or older. Patients were stratified by HIV status and tuberculosis drug resistance. Molecular or phenotypic drug susceptibility testing was done locally and at the Swiss National Center for Mycobacteria, Zurich, Switzerland. We examined mortality during treatment according to drug susceptibility test results and treatment adequacy in multivariable logistic regression models adjusting for sex, age, sputum microscopy, and HIV status.

Results: We obtained *M tuberculosis* isolates from 871 patients diagnosed between 2013 and 2016. After exclusion of 237 patients, 634 patients with tuberculosis were included in this analysis; the median age was 33·2 years (IQR 26·9–42·5), 239 (38%) were women, 272 (43%) were HIV-positive, and 69 (11%) patients died. Based on the reference laboratory drug susceptibility test, 394 (62%) strains were pan-susceptible, 45 (7%) monoresistant, 163 (26%) multidrug-resistant (MDR), and 30 (5%) had pre-extensively or extensively drug resistant (pre-XDR or XDR) tuberculosis. Results of reference and local laboratories were concordant for 513 (81%) of 634 patients and discordant for 121 (19%) of 634. Overall, sensitivity to detect any resistance was 90·8% (95% CI 86·5–94·2) and specificity 84·3% (80·3–87·7). Mortality ranged from 6% (20 of 336) in patients with pan-susceptible tuberculosis treated according to WHO guidelines to 57% (eight of 14) in patients with resistant strains who were under-treated. In logistic regression models, compared with concordant drug susceptibility test results, the adjusted odds ratio of death was 7·33 (95% CI 2·70–19·95) for patients with discordant results potentially leading to under-treatment.

Conclusions: Inaccurate drug susceptibility testing by comparison with a reference standard leads to under-treatment of drug-resistant tuberculosis and increased mortality. Rapid molecular drug susceptibility test of first-line and second-line drugs at diagnosis is required to improve outcomes in patients with MDR tuberculosis and pre-XDR or XDR tuberculosis.

Funding: National Institutes of Allergy and Infectious Diseases, Swiss National Science Foundation, Swiss National Center for Mycobacteria.

Research in context

Evidence before this study

Multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis are serious threats to WHO's End-TB strategy, because of restricted access to both laboratory tests for rapid identification of drug resistance and appropriate treatment in many countries with a high tuberculosis burden. We searched PubMed for systematic reviews and original research articles published in any language up to March 31, 2018. We combined terms for "tuberculosis", "drug resistance testing", and "mortality".

Several individual studies and systematic reviews have documented poor outcomes of MDR tuberculosis and pre-XDR/XDR tuberculosis in high-burden countries. Two Cochrane reviews investigated the accuracy of molecular tests detecting specific mutations associated with resistance, such as the Xpert MTB/RIF, which is recommended by WHO to detect rifampicin resistance directly from sputum.

Added value of this study

To our knowledge, this is the first multicentre cohort study assessing the accuracy of drug susceptibility testing in routine settings in high-burden countries by comparing local drug susceptibility test results with those from a tuberculosis reference laboratory and assessing the impact on mortality. The study shows that the accuracy of local drug susceptibility testing to detect any resistance in high-burden countries was moderate (sensitivity 90·8%, specificity 84·3%). Results from the reference and local laboratories were discordant in about 20% of patients. Mortality during treatment was increased almost two-fold in patients with discordant drug susceptibility test results compared to patients with concordant results. Mortality ranged from 6% in adequately treated patients with pan-susceptible strains to 57% in inadequately treated patients with drug-resistant strains. In multivariable analyses, associations with mortality changed little after adjustment for sex, age, sputum microscopy result, and HIV status. Notably, HIV infection was not associated with mortality during tuberculosis treatment.

Implications of all the available evidence

Drug-resistant tuberculosis is difficult to diagnose and treat, particularly in high-burden settings, where resources are scarce. In these settings, inaccurate drug susceptibility testing leading to inappropriate treatment contributes to the high mortality associated with drug-resistant tuberculosis. Local access to accurate and rapid drug susceptibility testing for first-line and second-line drugs is required to improve outcomes in patients with MDR tuberculosis and pre-XDR or XDR tuberculosis. Whole-genome sequencing is the most promising approach to reach this goal, but much work remains to be done to make this approach feasible and affordable in high-burden countries.

Introduction

Tuberculosis is a global public health concern. In 2017, an estimated 10·0 million individuals developed active tuberculosis worldwide, of whom an estimated 0·9 million (920 000; 9%) were HIV positive.[1] Scale-up of combination antiretroviral therapy (ART) has substantially improved the prognosis of HIV-positive patients[2,3] and reduced the incidence of tuberculosis in this population.[4,5] However, the risk of tuberculosis among HIV-positive patients on ART remains four times higher than among HIV-negative patients.[6]

The emergence of multidrug-resistant (MDR) tuberculosis and extensively drug-resistant (XDR) tuberculosis is another threat to the control of this disease.[7–9] In 2017, it was estimated that 3·5% of new patients and 18% (>50% in eastern Europe) of previously treated patients had MDR tuberculosis.[1] Treatment of MDR tuberculosis and XDR tuberculosis is challenging because of the longer treatment duration, adverse effects, and lower efficacy of second-line drugs.[10,11] Strategies to prevent drug-resistant tuberculosis include monitoring of the prevalence of MDR tuberculosis, widespread drug susceptibility testing, and ensuring rapid initiation and completion of full courses of effective treatment regimens.[12,13] Culture-based phenotypic drug susceptibility testing is considered the gold standard, but is time and resource intensive, and too slow to influence decisions about starting treatment.[14] Molecular-based resistance testing offers an alternative to culture-based drug susceptibility testing.[15] Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) detects resistance to rifampicin directly from sputum and provides results within 1·5 h,[16] whereas line probe assays (LPAs) from sputum detect resistance to isoniazid, rifampicin, ethambutol, fluoroquinolones, or second-line injectable drugs (amikacin, capreomycin, or kanamycin) and provide results within 1–2 days.[15]

Laboratories in high-burden settings use different tests and strategies to diagnose MDR tuberculosis, but the accuracy of drug susceptibility testing in routine settings in high-burden countries is unknown. We compared results of resistance testing done locally in ART and tuberculosis programmes in countries with a high tuberculosis burden with results from gold standard phenotypic drug susceptibility testing done in a Swiss reference laboratory, and examined mortality in HIV-positive and HIV-negative patients with tuberculosis who had concordant and discordant test results.

Methods

Study design

This multicentre cohort study is part of a larger research project about the development of drug-resistant *Mycobacterium tuberculosis* in the context of HIV co-infection within the International epidemiology Databases to Evaluate AIDS (IeDEA), a global consortium of ART programmes.[17,18] Isolates and clinical data were collected from patients with tuberculosis in seven high-burden countries in sub-Saharan Africa, Asia, and Latin America. The sample size was calculated so that the study had adequate power to detect differences in the prevalence of drug resistance between HIV-positive and HIV-negative patients.

Local institutional review boards or ethics committees approved the study at all participating sites. The study was also approved by the Cantonal Ethics Committee in Bern, Switzerland. Written informed consent was obtained at all sites, except in Nigeria and South Africa, where no informed consent was required for archived samples.

Data collection

Prospective recruitment of participants took place between January, 2013, and December, 2016. We included adult patients aged 16 years or older who were treated for active pulmonary tuberculosis in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, South Africa, Peru, and Thailand. All seven countries are defined by WHO as countries with a high tuberculosis burden; Democratic Republic of the Congo, Kenya, Nigeria, South Africa, and Thailand also have a high MDR tuberculosis burden and high HIV/tuberculosis burden.[19]

HIV-positive patients with tuberculosis were recruited prospectively from ART clinics participating in IeDEA and HIV-negative patients were recruited prospectively from tuberculosis clinics serving the same population. In South Africa, recruited patients came from strain collections held at the University of Cape Town. Sites were asked to contribute pulmonary pre-treatment *M tuberculosis* isolates from 25 or more patients within each of the four strata defined by HIV status (positive or negative) and drug resistance (MDR or pan-susceptible), for a total of 100 patients per site. The appendix summarises the characteristics of participating sites. Patient characteristics were entered online in French or English at baseline, with the Research Electronic Data Capture (REDCap) tool,[20] including site, type of tuberculosis patient as defined by WHO, age, sex, HIV status, CD4 cell count at start of tuberculosis treatment (if HIV positive), sputum smear microscopy result, and risk factors for tuberculosis. Treatment regimens were updated and outcomes entered during follow-up visits within routine care.

Outcomes

Treatment outcomes were defined according to WHO as follows: cured, treatment completed, treatment failure, death, lost to follow-up, transferred to other clinics, ongoing treatment at the time of evaluation, or unknown treatment outcome.[21] Treatment success included cured patients and patients who completed treatment.[21] The main outcome for this study was mortality during tuberculosis treatment. Outcome data received up to March 31, 2018, were included in analyses.

Drug susceptibility testing

Drug susceptibility testing was done locally with liquid or solid cultures or molecular methods: Xpert MTB/RIF or LPAs, such as Genotype MTBDRplus or MTBDRsl tests (Hain Lifesciences, Nehren, Germany). Drug susceptibility testing at participating clinics was dictated by local guidelines and the availability of tests. The reference laboratory of the Swiss National Center for Mycobacteria, Zurich, Switzerland, did drug susceptibility testing with the Mycobacteria Growth Indicator Tube liquid medium system (MGIT, Becton Dickinson, Franklin Lakes, NJ, USA), with the following drug concentrations: 0·1 mg/L for isoniazid, 1·0 mg/L for rifampicin, 100·0 mg/L for pyrazinamide, 5·0 mg/L for ethambutol, 1·0 mg/L for amikacin, and 0·25 mg/L for moxifloxacin, in line with the critical concentrations published by WHO.[22]

WHO defines monoresistance as resistance to one first-line tuberculosis drug (isoniazid, rifampicin, pyrazinamide, or ethambutol); MDR as resistance to isoniazid and rifampicin; pre-XDR as MDR with additional resistance to any fluoroquinolone or one of the second-line injectable drugs (amikacin, capreomycin, or kanamycin); and XDR as MDR with additional resistance to any fluoroquinolone and at least one of the second-line injectable drugs.[21] The "other drug resistance" category included any other combination. We defined pan-susceptible tuberculosis as no resistance against the six drugs tested at the reference laboratory and any resistance as resistance against at least one of the tested

drugs. First-line regimens (standard treatment) included first-line tuberculosis drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) and second-line regimens included a combination of first-line and second-line drugs.[21,23]

Exposure definition and data analysis

We calculated test accuracy statistics for diagnosis of any drug resistance. We further classified comparisons between the phenotypic and molecular drug susceptibility test results obtained in the local laboratories and the reference laboratory as follows: concordant results, discordance potentially leading to under-treatment, discordance potentially leading to over-treatment, and other discordant results. We defined drug regimens received by patients as compatible with WHO guidelines in place during the study period, as under-treatment, or as over-treatment, based on the reference drug susceptibility test results. First-line regimens for pan-susceptible tuberculosis, first-line or second-line regimens prescribed to isoniazid monoresistant patients, and second-line regimens prescribed to rifampicin monoresistant patients, patients with MDR tuberculosis, and patients with pre-XDR or XDR tuberculosis were classified as being in accordance with WHO guidelines. Under-treatment included first-line regimens given to rifampicin mono-resistant patients, patients with MDR tuberculosis, and those with pre-XDR or XDR tuberculosis; and over-treatment included second-line regimens given to patients with pan-susceptible tuberculosis. The appendix shows the classification of regimens.

We used descriptive statistics to describe patient characteristics by levels of drug resistance based on drug susceptibility testing done at the reference laboratory and by HIV status. We examined determinants of mortality in multivariable logistic regression models. Patients with unknown or missing treatment outcome, ongoing treatment, missing treatment regimen, missing sputum microscopy, and other drug-resistant tuberculosis were excluded from logistic regression analyses. Logistic regression models were adjusted for age, sex, sputum microscopy result, and HIV status. We stratified models by study site by including an indicator variable for all sites except for South Africa (the reference group). We calculated the population attributable fraction of mortality due to discordant drug susceptibility test results based on the adjusted model as described by Greenland and Drescher.[24] Other variables, such as smoking history, diabetes, substance abuse, and contact with other patients with tuberculosis worsened the fit of the model. For HIV-positive individuals, models were additionally adjusted for CD4 cell count at the start of tuberculosis treatment. All analyses were done with Stata, version 15.

Role of the funding source

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

Results

We obtained *M tuberculosis* isolates from 871 patients diagnosed between 2013 and 2016. We excluded 237 patients from analyses of the accuracy of drug susceptibility testing, mainly because isolates were contaminated or not viable, and excluded a further 61 patients from analyses of mortality, mainly because treatment was ongoing or outcomes were unknown at the time of closing the database (appendix). Excluded patients were similar in terms of age, sex, HIV status, and site of tuberculosis, but had lower CD4 counts and were more likely to have recurrent tuberculosis and to be on treatment after failure or default (appendix).

634 patients with tuberculosis were included in the analysis; the median age was 33·2 years (IQR 26·9–42·5) and 239 (38%) patients were women (table 1). The reference laboratory identified 394 (62%) pan-susceptible *M tuberculosis* strains, 45 (7%) monoresistant strains, 163 (26%) MDR strains, 30 (5%) pre-XDR or XDR strains, and two (<1%) strains with other drug resistance profiles (appendix).

Table 1. Patient characteristics by phenotypic drug resistance profiles obtained at the Swiss National Center for Mycobacteria

	Pan-susceptible	Any resistance	p value	Monoresistance			Polyresistance		
	(n=394)	(n=240)		INH (n=29)	RIF (n=14)	PZA (n=2)	MDR (n=163)	Pre-XDR or XDR (n=30)	Other (n=2)
Sex									
Women	150 (38%)	89 (37%)	0·80	6 (21%)	3 (21%)	0	65 (40%)	14 (47%)	1 (50%)
Men	244 (62%)	151 (63%)	··	23 (79%)	11 (79%)	2 (100%)	98 (60%)	16 (53%)	1 (50%)
Age (years)	34·6 (27·8–44·6)	31·5 (25·3–40·2)	0·003	34·3 (26·5–43·2)	27·1 (24·9–35·5)	26·1 (23·3–28·9)	31·5 (25·4–41·4)	30·3 (24·2–37·5)	27·3 (24·4–30·2)
HIV status									
Negative	200 (51%)	162 (68%)	<0·0001	20 (69%)	8 (57%)	1 (50%)	114 (70%)	18 (60%)	1 (50%)
Positive	194 (49%)	78 (32%)	··	9 (31%)	6 (43%)	1 (50%)	49 (30%)	12 (40%)	1 (50%)
CD4 count at baseline, cells per µL	215 (85–369)	161 (61–369)	0·79	92·5 (55–161)	63·5 (43–81)	43	259 (151–528)	32 (5–105)	213
Number of observations (%)	155 (39%)	45 (19%)	··	6 (21%)	6 (42·9%)	1 (50%)	24 (15%)	7 (23%)	1 (50%)
Treatment regimen									
First line	369 (94%)	46 (19%)	<0·0001	27 (93%)	0	2 (5%)	14 (9%)	2 (7%)	1 (50%)
Second line	25 (6%)	188 (78%)	··	2 (7%)	14 (100%)	0	143 (85%)	28 (93%)	1 (50%)
Unknown	0	6 (3%)	··	0	0	0	6 (6%)	0	0
Treatment outcomes									
Success	287 (73%)	124 (52%)	<0·0001	15 (52%)	7 (50%)	0	88 (54%)	13 (43%)	1 (50%)
Mortality	24 (6%)	45 (19%)	··	7 (24%)	2 (14%)	1 (50%)	24 (15%)	10 (33%)	1 (50%)
Treatment failure	12 (3%)	10 (4%)	··	0	0	1 (50%)	5 (3%)	4 (13%)	0
Lost to follow-up	29 (7%)	30 (13%)	··	1 (3%)	3 (21%)	0	26 (16%)	0	0
Transfer	15 (4%)	14 (6%)	··	0	2 (14%)	0	9 (6%)	3 (10%)	0
Ongoing treatment/ unknown	27 (7%)	17 (7%)	··	6 (21%)	0	0	11 (7%)	0	0
Country									
Côte d'Ivoire	48 (12%)	51 (21%)	<0·0001	3 (10%)	0	0	44 (27%)	4 (13%)	0
DRC Congo	33 (8%)	29 (12%)	··	0	1 (7%)	0	19 (12%)	9 (30%)	0
Kenya	24 (6%)	11 (5%)	··	2 (7%)	1 (7%)	0	8 (5%)	0	0
Nigeria	20 (5%)	36 (15%)	··	1 (3%)	5 (36%)	0	26 (16%)	4 (13%)	0
Peru	66 (17%)	38 (16%)	··	8 (28%)	0	0	27 (17%)	3 (10%)	0
South Africa	130 (33%)	57 (24%)	··	6 (21%)	7 (50%)	1 (50%)	32 (20%)	10 (33%)	1 (50%)
Thailand	73 (19%)	18 (8%)	··	9 (31%)	0	1 (50%)	7 (4%)	0	1 (50%)

Data are n (%) or median (IQR). Analysis based on 634 patients (see appendix). INH=isoniazid. RIF=rifampicin. PZA=pyrazinamide. MDR=multidrug resistant. XDR=extensively drug resistant

Among the 163 patients with MDR tuberculosis, 85 (52%) had resistance to rifampicin and isoniazid only, whereas the remaining patients were also resistant to pyrazinamide or ethambutol, or both. Among the 24 patients with pre-XDR tuberculosis, resistance to moxifloxacin (n=15) was more frequent than resistance to amikacin (n=9; appendix). Patients with resistant strains were more likely to receive second-line tuberculosis treatment and to experience unfavourable treatment outcomes than were patients with pan-susceptible strains (table 1).

Table 2. Concordance and discordance of drug susceptibility results obtained from reference and local laboratories (n=634)

	Drug susceptibility test results by laboratory		Test used at local laboratories			
	Reference laboratory (phenotypic)	Local laboratories	Xpert MTB/RIF*	Culture	LPA	Combination of tests
Concordance						
513 (100%)	Total	..	216 (100%)	154 (100%)	11 (100%)	73 (100%)
332 (65%)	Pan-susceptible	Pan-susceptible	167 (77%)	101 (66%)	1 (9%)	5 (7%)
8 (2%)	RIF monoresistance	RIF monoresistance	0	0	0	7 (10%)
8 (2%)	INH monoresistance	INH monoresistance	0	8 (5%)	0	0
153 (30%)	MDR	MDR	49 (23%)	44 (29%)	8 (73%)	52 (71%)
12 (2%)	Pre-XDR or XDR	Pre-XDR or XDR	0	1 (1%)	2 (18%)	9 (12%)
Discordance potentially leading to under-treatment						
23 (100%)	Total	..	8 (100%)	9 (100%)	0	6 (100%)
5 (22%)	MDR	Pan-susceptible	2 (25%)	2 (22%)	0	1 (17%)
18 (78%)	Pre-XDR or XDR	MDR	6 (75%)	7 (78%)	0	5 (83%)
Discordance potentially leading to over-treatment						
67 (100%)	Total	..	5 (100%)	44 (100%)	3 (100%)	14 (100%)
14 (21%)	Pan-susceptible	RIF monoresistance	0	0	3 (100%)	10 (71%)
14 (21%)	Pan-susceptible	MDR	3 (60%)	8 (18%)	0	3 (21%)
33 (49%)	Pan-susceptible	Other monoresistance†	2 (40%)	31 (71%)	0	0
5 (8%)	Other monoresistance‡	MDR	0	5 (11%)	0	0
1 (2%)	MDR	Pre-XDR or XDR	0	0	0	1 (7%)
Other discordance						
31 (100%)	Total	..	16 (100%)	6 (100%)	1 (100%)	7 (100%)
1 (3%)	Pan-susceptible	EMB, SM	0	1 (17%)	0	0
7 (23%)	RIF monoresistance	MDR	2 (13%)	0	0	5 (29%)
17 (55%)	Other monoresistance§	Pan-susceptible	13 (81%)	3 (50%)	0	0
1 (3%)	INH, MOX	INH monoresistance	0	1 (17%)	0	0
1 (3%)	INH, PZA	MDR	0	1 (17%)	0	0
3 (10%)	MDR	RIF monoresistance	0	0	1 (100%)	2 (71%)
1 (3%)	MDR	EMB, SM	1 (6%)	0	0	0

Data are n (%). Analysis based on 634 patients (see appendix). MDR=multidrug resistance. XDR=extensively drug resistant. RIF=rifampicin. LPA=line probe assay. INH=isoniazid. XDR=extensively drug resistant. EMB=ethambutol. SM=streptomycin. MOX=moxifloxacin. PZA=pyrazinamide. In some patients the test used to diagnose drug-resistant infection at the local laboratories was unknown. Therefore, numbers do not always add up to the row totals. *RIF resistance diagnosed with Xpert MTB/RIF was classified as MDR. †21 strains were resistant to EMB, ten to SM, and two to INH. ‡Five strains were resistant to INH. §15 strains were resistant to INH and two to PZA.

Comparing local results with reference laboratory results for any resistance, there were 218 true and 62 false positives and 332 true and 22 false negatives, for an overall sensitivity of 90·8% (95% CI 86·5–94·2) and specificity of 84·3% (80·3–87·7). For Xpert MTB/RIF, sensitivity was 79·5% (95% CI 68·4–88·0) and specificity 97·1% (93·3–99·0); for culture, sensitivity was 93·1% (84·5–97·7) and specificity 71·6% (63·4–78·9); for LPA, sensitivity was 100% (71·5–100·0) and specificity 25·0% (0·63–80·6); and for combinations of tests, sensitivity was 98·8% (93·4–100·0) and specificity 27·8% (9·7–53·5). For all four categories of drug resistance considered together (rifampicin mono-resistance, isoniazid monoresistance, MDR, and pre-XDR or XDR), results from the reference laboratory and local laboratories were concordant for 513 (81%) of 634 patients and discordant for 121 (19%). Results were concordant in 216 (88%) of 245 patients for Xpert MTB/RIF, in 154 (72%) of 213 for culture, in 11 (73%) of 15 for LPA, and in 73 (73%) of 100 for a combination of tests (p<0·0001).

23 (4%) of 634 patients had discrepancies potentially leading to under-treatment, 67 (11%) had discordant results potentially leading to over-treatment, and 31 (5%) had other discordances (table 2; appendix). Treatments received were compatible with WHO guidelines in 491 (97%) of 507 patients with concordant drug susceptibility test results compared with 94 (78%) of 121 patients with discordant results (p<0·0001).

After excluding 61 (10%) of 634 patients with unknown treatment outcomes, missing data, or other drug resistance (appendix), mortality ranged from 6% (17 of 302) among patients with pan-susceptible strains and concordant drug susceptibility test results to 44% (eight of 18) among patients with pre-XDR or XDR tuberculosis and discordant drug susceptibility test results (table 3).

Table 3. Mortality by phenotypic drug resistance profiles obtained at the Swiss National Centre for Mycobacteria and by concordance with local results

	Concordant results	Discordant results	Total
Pan-susceptible	17/302 (6%)	6/57 (11%)	23/359 (6%)
Any resistance	29/164 (18%)	15/50 (30%)	44/214 (21%)
Monoresistance			
INH	5/8 (63%)	2/15 (13%)	7/23 (30%)
RIF	0/7 (0%)	2/7(29%)	2/14 (14%)
PZA	..	1/2 (50%)	1/2 (50%)
Polyresistance			
MDR	22/138 (16%)	2/8 (25%)	24/146 (16%)
Pre-XDR or XDR	2/11 (18%)	8/18 (44%)	10/29 (34%)
Total	46/466 (10%)	21/107 (20%)	67/573 (12%)

Analysis based on 573 patients with complete data (see appendix). INH=isoniazid. RIF=rifampicin. PZA=pyrazinamide. MDR=multidrug resistance. XDR=extensively drug resistant.

In patients with discordant results potentially leading to over-treatment, mortality was 10% (six of 61) whereas in patients with discordant results potentially leading to under-treatment it was 41% (nine of 22; figure, table 4). Mortality ranged from 6% (23 of 359) in patients with pan-susceptible strains to 35% (ten of 29) in patients with pre-XDR or XDR tuberculosis (table 4). Mortality was higher in patients with isoniazid monoresistant strains (seven [30%] of 23) than in patients with rifampicin monoresistant strains (two [14%] of 14) but the difference was not significant (p=0·38, table 3) and the two categories were combined in further analyses. Finally, mortality ranged from 6% (20 of 336) in patients with pan-susceptible tuberculosis treated according to WHO guidelines to 57% (eight of 14) in patients with resistant strains who were under-treated (figure, table 4).

In multivariable logistic regression models adjusted for sex, age, sputum microscopy result, and HIV status, discordant drug susceptibility test results continued to be associated with increased mortality compared with concordant drug susceptibility test results (table 4). Compared with concordant results, the adjusted odds ratio (aOR) of death was 7·33 (95% CI 2·70–19·95) for patients with discordant results potentially leading to under-treatment. The population attributable fraction for mortality associated with any type of discordance obtained from the logistic model was 15·15% (95% CI 2·08–26·47).

Drug resistance of any type was associated with higher mortality than pan-susceptible tuberculosis (aOR 5·18; 95% Cl 2·78–9·66) and mortality was highest for pre-XDR or XDR tuberculosis (15·19; 5·45–42·36; table 4). Finally, when compared with patients treated according to WHO guidelines with pan-susceptible strains, adequately treated patients with resistant strains had higher mortality (aOR 4·66; 95% CI 2·38–9·14), as did patients with resistant strains receiving inadequate regimens (19·32; 5·59–66·73; table 4). Patients with pan-susceptible tuberculosis who were over-treated also had an increased risk of death compared with patients who had pan-susceptible tuberculosis treated according to WHO guidelines, although the difference was not significant (aOR 3·31; 95% CI 0·82–13·45; p=0·10). Sex, positive sputum smear microscopy, and HIV status were not associated with the odds of death (table 4). Results from univariable models were similar to those from multivariable models (appendix). When restricting the analysis to HIV-positive patients, mortality was higher among patients with CD4 cell counts less than 50 cells per µL than in patients with higher CD4 counts at the start of tuberculosis treatment (aOR 6·89; 95% CI 1·57–30·26).

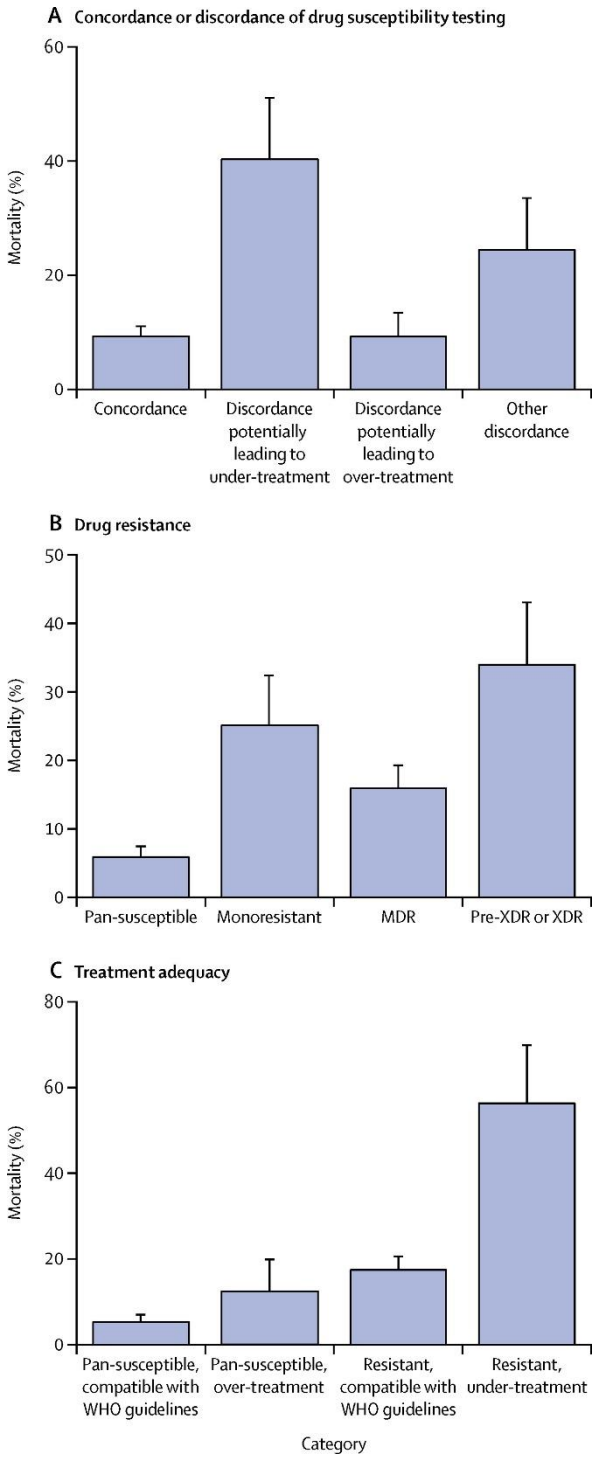

Figure Mortality according to drug resistance, concordance or discordance of drug susceptibility test results, and treatment adequacy
Error bars are standard errors. All p values are less than 0·001 for difference in mortality across categories. Analysis based on 573 patients with complete data.

Table 4. Results from logistic regression models showing the probability of death during tuberculosis treatment. Models based on 573 patients with complete data for all variables shown (see appendix).

Model 1 was adjusted for concordance or discordance of drug susceptibility test results, sex, age, sputum microscopy, and

	Number of patients	Number of deaths	Model 1, aOR (95% CI)	Model 2, aOR (95% CI)	Model 3, aOR (95% CI)
Concordance or discordance of drug susceptibility test results					
Concordance	466	46 (10%)	1	..	..
Discordance potentially leading to under-treatment	22	9 (41%)	7·33 (2·70–19·95)	..	..
Discordance potentially leading to over-treatment	61	6 (10%)	0·81 (0·31–2·11)	..	..
Other discordance	24	6 (25%)	4·92 (1·69–14·33)	..	..
Drug resistance*					
Pan-susceptible	359	23 (6%)	..	1	..
Monoresistance	39	10 (26%)	..	6·05 (2·36–15·56)	..
MDR	146	24 (16%)	..	3·83 (1·88–7·81)	..
Pre-XDR or XDR	29	10 (35%)	..	15·19 (5·45–42·36)	..
Treatment adequacy by drug resistance					
Pan-susceptible, compatible with WHO guidelines	336	20 (6%)	..	..	1
Pan-susceptible, over-treatment	23	3 (13%)	..	..	3·31 (0·82–13·45)
Any resistance, compatible with WHO guidelines	200	36 (18%)	..	..	4·66 (2·38–9·14)
Any resistance, under-treatment	14	8 (57%)	..	..	19·32 (5·59–66·73)
Sex					
Women	219	20 (10%)	1	1	1
Men	354	47 (13%)	1·47 (0·81–2·67)	1·42 (0·78–2·60)	1·46 (0·80–2·70)
Age (per 1-year increase)	573	67 (12%)	1·04 (1·01–1·06)	1·04 (1·01–1·06)	1·04 (1·01–1·06)
Sputum microscopy					
Negative	111	10 (9%)	1	1	1
Positive	462	57 (12%)	1·14 (0·51–2·56)	1·03 (0·45 -2·37)	0·90 (0·40–2·07)
HIV status					
Negative	337	43 (13%)	1	1	1
Positive	236	24 (10%)	0·90 (0·50–1·61)	1·19 (0·65–2·20)	1·19 (0·65–2·20)

HIV status; model 2 was adjusted for drug resistance, sex, age, sputum microscopy, and HIV status; model 3 was adjusted for treatment adequacy, sex, age, sputum microscopy, and HIV status. aOR=adjusted odds ratio. MDR=multidrug resistant. XDR=extensively drug-resistant. *Results from the Swiss National Reference Center for Mycobacteria.

Discussion

The results of this multicentre cohort study of patients treated for drug-resistant or drug-susceptible tuberculosis in seven high tuberculosis burden countries show that the accuracy of drug susceptibility testing in routine care was moderate, with discordant results from local drug susceptibility testing compared with phenotypic drug susceptibility testing in a reference laboratory in about 20% of patients. Discordant results led to inadequate treatment and contributed to the excess mortality associated with drug-resistant tuberculosis. As expected, mortality was highest in patients with pre-XDR or XDR tuberculosis and higher in patients who were under-treated than in those who were adequately treated. Patients with pan-susceptible tuberculosis who were over-treated also had higher mortality than did those who were adequately treated, although the difference was not significant. It is possible that over-treated patients had worse adherence and were at higher risk of adverse drug effects than were adequately treated patients. To our knowledge, this is the first study to assess the accuracy of drug susceptibility testing in real world, routine settings and to examine the impact of inaccurate results on mortality. Our findings support the recent call for a precision medicine approach to the treatment of drug-resistant tuberculosis, guided by detailed molecular drug susceptibility testing done locally, to replace the standardised, empirical combination regimens used in many low-income and middle-income countries with a high tuberculosis burden.[25]

At present, WHO recommends that "Xpert MTB/RIF be used as an initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB",[26] based on a Cochrane review of test accuracy studies in adults with suspected rifampicin resistance or MDR tuberculosis.[27] In line with this recommendation, Xpert MTB/RIF was the most commonly used test in our study sites. The Cochrane review reported a pooled sensitivity of 95%, based on 17 studies and 555 patients with rifampicin-resistant strains.[27] The pooled specificity was 98%. We examined the accuracy of drug susceptibility testing strategies at the level of the local laboratories in high-burden countries, in routine care settings, rather than by examining a single test. Our estimates of sensitivity and specificity, for detection of any drug resistance, were lower overall (90·8% and 84·3%), and lower for Xpert MTB/RIF (79·5% and 97·1%) and for culture (93·1% and 71·6%), indicating that drug susceptibility testing is less accurate in routine settings than in test accuracy studies.[27]

There are concerns about both false-negative and false-positive Xpert MTB/RIF test results, and a policy of confirmatory testing has been introduced in South Africa and Brazil.[28,29] The discordant drug susceptibility test results that potentially led to under-treatment of drug-resistant tuberculosis (false negative for resistance) were mainly based on locally done cultures, Xpert MTB/RIF tests, or a combination of the two. Notably, the recently developed Xpert MTB/RIF Ultra assay has been shown to improve detection of rifampicin resistance.[30] Culture-based tests dominated discordance that potentially led to over-treatment, whereas Xpert MTB/RIF dominated in the category of discordance with unclear clinical significance. Some discordance could be explained by mixed infections, heteroresistance, or minority resistant populations.[31,32]

LPAs were rarely used in our study, possibly because they have been widely replaced by Xpert MTB/RIF, which is easier to use and provides results in a shorter time. Additionally, LPAs have suboptimal accuracy for isoniazid resistance, and WHO recommends that culture-based drug susceptibility testing for isoniazid should still be used, particularly in patients with suspected MDR tuberculosis in whom the LPA result does not detect isoniazid resistance.[33] In one case, the local laboratory detected resistance to ethambutol but this could not be confirmed in the reference laboratory: drug susceptibility testing is challenging for ethambutol and less reproducible.[34]

Data about treatment outcomes in drug-resistant tuberculosis are scarce, particularly for sub-Saharan Africa. A systematic review of treatment outcomes in patients with MDR tuberculosis included data on mortality among adults from seven studies done in sub-Saharan Africa, six in South Africa and one in Lesotho.[35] In these studies, mortality during tuberculosis treatment ranged from 12·4% in patients with MDR tuberculosis treated in a referral hospital in the Western Cape, South Africa,[36] to 45·8% in a study of patients with XDR tuberculosis from three South African provinces.[37] Our results extend these data to other countries in the region and also provide data for Peru and Thailand.

Our study confirms the poor outcome in patients with isoniazid monoresistant tuberculosis who are treated with first-line regimens (as recommended by WHO during the study period[38]), in line with a study from Durban, South Africa,[39] and a systemic review and meta-analysis.[40] Mortality in patients with monoresistant tuberculosis, especially isoniazid-resistant tuberculosis, was higher than in patients with MDR tuberculosis. This might be due to the treatment of almost all patients with isoniazid monoresistant tuberculosis with first-line regimens, whereas most patients with MDR tuberculosis received second-line treatment. WHO has updated its guidelines recommending the inclusion of fluoroquinolones in the treatment of isoniazid monoresistant tuberculosis.[41] Chance is another explanation: few patients had monoresistant tuberculosis and in the analysis of mortality the confidence intervals of the odds ratios for monoresistant and MDR tuberculosis overlapped widely. In patients with HIV co-infection, treatment of drug-resistant tuberculosis is challenging for several

reasons, including poorer absorption of drugs,[42] the risk of immune reconstitution inflammatory syndrome,[43] and interactions between antiretroviral and second-line tuberculosis drugs.[44–46] In contrast to previous studies from South Africa, which reported higher mortality at the end of treatment in HIV-positive patients with MDR tuberculosis compared with HIV-negative patients with MDR tuberculosis,[36,47] we found no association with HIV infection, although the confidence intervals were wide. The median CD4 cell count of HIV-positive patients was considerably higher in our study (192 cells per µL) than in the South African studies,[36,47] which might explain the discrepant results. A study from Lesotho[48] also found little evidence for a difference in mortality between HIV-positive patients (median CD4 cell count 185 cells per µL) and HIV-negative patients. Finally, for patients with XDR tuberculosis, treatment outcomes have been uniformly poor in previous studies, irrespective of HIV status.[37]

Our study has several limitations. We sampled eligible patients within strata defined by drug resistance and HIV infection, and therefore could not estimate the incidence or prevalence of drug-resistant tuberculosis in HIV-positive or HIV-negative patients. In previous studies, HIV infection has not been consistently associated with drug resistance,[28] but it is clear that in regions with a high burden of HIV, the majority of patients with MDR tuberculosis will have HIV co-infection.[28] Although we initially exceeded the planned sample size, about a quarter of patients had to be excluded from analyses of drug susceptibility, mainly because of contamination or insufficient growth of cultures, and about a third were excluded from the analysis of mortality outcomes, mainly because vital status was unknown at database closure. The reference laboratory tested resistance against six drugs, and we would have missed resistance against other drugs used, such as kanamycin, ethionamide, or levofloxacin. Furthermore, the presence of different subpopulations of *M tuberculosis* in isolates tested at the local sites versus the reference laboratory might have introduced variability in phenotypic or molecular drug susceptibility testing.[49]

Conclusions

In conclusion, our study shows that the accuracy of drug susceptibility testing in routine care in high-burden countries was inadequate and that inaccurate results led to inadequate treatment and contributed to the excess mortality associated with drug-resistant tuberculosis. Our results support the notion that access to rapid molecular drug susceptibility testing of first-line and second-line drugs at treatment initiation is required to improve outcomes in patients with MDR tuberculosis and pre-XDR/XDR tuberculosis.[28] Whole-genome sequencing is the most promising approach to reach this goal, but much work remains to be done to make this approach feasible and affordable in low-income and middle-income countries.[28] In particular, direct testing of sputum samples should become routine to circumvent lengthy mycobacterial cultures.[40] A standardised approach for the interpretation of mutations conferring drug resistance has been developed.[50] In the meantime, the capacity for the phenotypic and molecular drug susceptibility testing recommended by WHO should be increased to ensure the most adequate treatment of drug-resistant tuberculosis in these settings.

Supplementary webappendix

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

Supplement to: Zürcher K, Ballif M, Fenner L, et al, on behalf of the International epidemiology Databases to Evaluate AIDS (IeDEA) consortium. Drug susceptibility testing and mortality in patients treated for tuberculosis in high-burden countries: a multicentre cohort study. Lancet Infect Dis 2019; published onlined Feb 7. http://dx.doi.org/10.1016/S1473-3099(18)30673-X.

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KZ, MB, and ME wrote the first draft of the paper, which was reviewed by all authors and revised on the basis of the comments received by co-authors. MB coordinated data and strain collection across study sites. ECB and PMK supervised drug susceptibility testing at the Swiss National Center for Mycobacteria, which were done by RHö. HC, JG, OM, MY, LD, EJC, NR, RJW, NE, AGA, JC, AA, and KK supervised drug susceptibility testing at the local laboratory and the collection of clinical data. ME and KZ did statistical analyses. All authors approved the final version of the manuscript.

Declaration of interests

AA has received honoraria fees from Jensen-Cilag, Gilead, and Bristol-Myers Squibb. All other authors declare no competing interests.

Acknowledgments

We thank all sites that participated in this survey and the patients whose data were used in this study. We are grateful to the Tuberculosis Working Group of IeDEA for helpful discussions, and to the reviewers for their thoughtful comments. We also thank all regional data centres that contributed to the coordination of the study. This research was supported by the Swiss National Science Foundation (grant numbers 153442, 310030_166687 and 174281), the National Institutes of Allergy and Infectious Diseases (NIAID) under award numbers U01 AI096299, U01 AI069919, U01 AI069924, U01 AI069911, U01 AI069907, U01 AI096186, and U01 AI069923, and the Swiss National Center for Mycobacteria, University of Zurich, Switzerland. RJW is supported by the Francis Crick Institute (grant number 10218), which is funded by the Wellcome Trust, Cancer Research UK, and Research Councils UK. RJW also receives support from the Wellcome Trust (104803 and 203135). HC is supported by a Wellcome Trust fellowship and reports grants from UK Medical Research Council and the National Research Foundation of South Africa. SG is supported by the Swiss National Science Foundation (310030_166687, IZRJZ3_164171, IZLSZ3_170834, and CRSII5_177163), the European Research Council (309540-EVODRTB) and SystemsX.ch. KK was supported by the National Research University Program, Office of the Higher Education Commission, Bangkok, Thailand.

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8. Paper 6 – Whole-genome sequencing and mortality

Mortality from drug-resistant tuberculosis in high-burden countries comparing routine drug susceptibility testing with whole-genome sequencing: a multicentre cohort study

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The article has been published in the Lancet microbe online on the April 29, 2021.
DOI: http://dx.doi.org/10.1016/S1473-3099(18)30673-X

Contribution: I did the data cleaning for the clinical dataset. I helped with the statistical analysis. Together with M.R I wrote the first draft of the manuscript and addressed all comments from co-authors and reviewers until the final published version.

Abstract

Background: Drug resistance threatens global tuberculosis control. We aimed to examine mortality in patients with tuberculosis from high-burden countries, according to concordance or discordance of results from drug susceptibility testing done locally and whole-genome sequencing (WGS).

Methods: In this multicentre cohort study, we collected pulmonary *Mycobacterium tuberculosis* isolates and clinical data from individuals with tuberculosis from antiretroviral therapy programmes and tuberculosis clinics in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, Peru, South Africa, and Thailand, stratified by HIV status and drug resistance. Sites tested drug susceptibility using routinely available methods. WGS was done on Illumina HiSeq 2500 in the USA and Switzerland, and TBprofiler was used to analyse the genomes. We included individuals aged 16 years or older with pulmonary tuberculosis (bacteriologically confirmed or clinically diagnosed). We analysed mortality in multivariable logistic regression models adjusted for sex, age, HIV status, history of tuberculosis, and sputum positivity.

Findings: Between Sept 1, 2014, and July 4, 2016, of 634 patients included in our previous analysis, we included 582 patients with tuberculosis (median age 33 years [IQR 27–43], 225 [39%] women, and 247 [42%] HIV-positive). Based on WGS, 339 (58%) isolates were pan-susceptible, 35 (6%) monoresistant, 146 (25%) multidrug-resistant, and 24 (4%) pre-extensively drug-resistant (pre-XDR) or XDR. The analysis of mortality was based on 530 patients; 63 (12%) died and 77 (15%) patients received inappropriate treatment. Mortality ranged from 6% (18 of 310) in patients with pan-susceptible tuberculosis to 39% (nine of 23) in patients with pre-XDR or XDR tuberculosis. The adjusted odds ratio for mortality was 4·92 (95% CI 2·47–9·78) among undertreated patients, compared with appropriately treated patients.

Interpretation: In seven countries with a high burden of tuberculosis, we observed discrepancies between drug resistance patterns obtained locally and WGS. The underdiagnosis of drug resistance resulted in inappropriate treatment and higher mortality. WGS can provide accurate and detailed drug resistance information required to improve the outcomes of drug-resistant tuberculosis in high-burden settings. Our results support WHO's call for point-of-care tests based on WGS.

Funding: National Institutes of Allergy and Infectious Diseases, Swiss National Science Foundation, and Swiss National Center for Mycobacteria.

Research in context

Evidence before this study

Drug-resistant tuberculosis, in particular multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis, is threatening the control of tuberculosis worldwide. WHO has highlighted the need to improve drug susceptibility testing and treatment of drug-resistant tuberculosis, particularly in countries with a high burden of tuberculosis. Whole-genome sequencing (WGS) has the potential to provide resistance profiles for all first-line and second-line anti-tuberculosis drugs and is increasingly replacing other drug resistance testing methods. Yet, the potential of WGS in routine clinical care has not been shown in low-income and middle-income countries, where the burden of drug-resistant tuberculosis is high. We searched PubMed for systematic reviews and original research articles published in any language up to June 29, 2020. We combined terms for "tuberculosis", "whole-genome sequencing", and "mortality". Several validation studies showed that WGS could accurately predict drug resistance; however, we could not find any study showing the potential benefit of WGS-based drug resistance testing on survival.

Added value of this study

In this study, we compared drug resistance profiles from WGS with routine drug susceptibility test results in seven countries across three continents with a high tuberculosis burden and assessed the effect of undiagnosed drug resistance on mortality. Results from WGS and routine drug susceptibility testing were discordant in 22% of patients. Resistance to isoniazid and rifampicin was accurately identified at local clinics, whereas resistance to ethambutol, pyrazinamide, and second-line drugs was rarely tested locally. Mortality ranged from 6% in patients with pan-susceptible tuberculosis who were appropriately treated to 32% in patients with drug-resistant tuberculosis who were undertreated.

Implications of all the available evidence

Routine drug susceptibility testing in resource-limited settings with a high tuberculosis burden is often insufficient to inform the prescription of the most effective treatment regimen, which in turn contributes to higher mortality. Our results support the implementation of point-of-care protocols for WGS, ideally directly from sputum to obtain comprehensive drug resistance profiles and facilitate the initiation of personalised and effective treatment regimens.

Introduction

Tuberculosis is caused by bacteria of the *Mycobacterium tuberculosis* complex and is the leading cause of death by a single infectious agent worldwide.[1] In 2019, ten million people were estimated to have developed active tuberculosis, of whom 8% also had HIV. In the same year, around 1·2 million people died from tuberculosis, including 208 000 people with HIV.[1] Tuberculosis accounts for approximately 40% of HIV and AIDS-related adult deaths, and half of these remain undiagnosed.[2] The emergence of drug-resistant *M tuberculosis* strains threatens tuberculosis control. In 2019, 3% of new tuberculosis cases worldwide were estimated to be multidrug-resistant (MDR) tuberculosis, and 18% of individuals who had been previously treated had MDR tuberculosis.[1] People with HIV are at greater risk of acquiring MDR tuberculosis than people who are HIV-negative.[3] Also, treatment outcomes in people with HIV and MDR tuberculosis are worse than among HIV-negative patients with MDR tuberculosis.[3] Pre-extensively drug-resistant (pre-XDR) or XDR tuberculosis poses additional challenges for treatment and control of the disease.[4] Strategies to control and prevent drug-resistant tuberculosis include surveillance, rapid drug susceptibility testing, and ensuring the completion of an appropriate treatment regimen. The limited access to detailed drug susceptibility testing and effective second-line anti-tuberculosis drugs, insufficient adherence and drug dosages, and comorbidities challenge the management of drug-resistant tuberculosis in low-income and middle-income countries.[2,5–7]

The present study is part of a research programme investigating drug-resistant tuberculosis of the International epidemiology Databases to Evaluate AIDS.[8] In a previous analysis, we compared the results of drug susceptibility testing from high-burden countries in Africa, Asia, and Latin America with phenotypic drug susceptibility testing results from the Swiss National Center for Mycobacteria.[9] We found that the accuracy of testing done at participating sites was moderate, and that discordant results and inappropriate treatment were associated with increased mortality. The Swiss reference laboratory tested drug resistance to six drugs only: isoniazid, rifampicin, pyrazinamide, ethambutol, amikacin, and moxifloxacin. Therefore, other resistances could have been missed, including resistance to streptomycin, kanamycin, ethionamide, levofloxacin, or newer drugs.

Whole-genome sequencing (WGS) can simultaneously provide information on resistance to first-line and second-line drugs, for which drug-resistance-conferring mutations are known. WGS has the potential to overcome many of the limitations of conventional drug susceptibility testing with higher throughput.[10] We and others showed that drug susceptibility predicted from *M tuberculosis* genomes correlates with phenotypic drug susceptibility testing.[11,12] WHO recommends WGS for drug resistance surveillance and is evaluating sequencing technologies for routine drug susceptibility testing.[1,13] Here, we aimed to compare the drug resistance patterns routinely obtained in seven countries with a high tuberculosis burden with the results from WGS, and examined the mortality associated with discordant resistance profiles using WGS as the reference.

Methods

Study design and participants

We did a multicentre cohort study. As described in detail elsewhere,[9] we recruited patients from antiretroviral therapy programmes and tuberculosis clinics in their corresponding catchment areas in Côte d'Ivoire, Democratic Republic of the Congo, Kenya, Nigeria, Peru, South Africa, and Thailand. In South Africa, we used strain collections held at the University of Cape Town (Cape Town, South Africa). All patients had bacteriologically confirmed, or clinically diagnosed tuberculosis. We included

individuals aged 16 years or older with pulmonary tuberculosis. We excluded patients for whom no viable isolate was available, patients with extrapulmonary tuberculosis only, patients with missing data that were necessary for the analyses, and patients for whom the *M tuberculosis* genome could not be sequenced (appendix p 2). Recruitment was stratified by HIV status and drug resistance as defined at local clinics. We collected demographic and clinical characteristics of participants using a standardised questionnaire. *M tuberculosis* isolates were subcultured at the recruitment sites.

The Cantonal Ethics Committee in Bern, Switzerland, and local institutional review boards approved the study. Written informed consent was obtained at all sites, except in South Africa, where consent was not required for the use of archived samples.

Procedures

The local laboratories tested molecular or phenotypic drug susceptibility according to routine procedures. DNA was extracted from isolates using standard protocols.[14] Libraries were prepared using the Illumina Nextera XT kit (Illumina, San Diego, CA, USA) and sequenced on Illumina HiSeq 2500 at the Department of Biosystems Science and Engineering of the Swiss Federal Institute of Technology in Basel, Switzerland and the Broad Institute in Cambridge, MA, USA. Sequences had 101, 138, or 151 bp paired-end reads. After Illumina adaptors were clipped and low-quality reads trimmed with Trimmomatic, version 0.38, reads shorter than 36 bp were excluded. The minimum read depth at each position was 10 × in 99% of the genome (IQR 99–99, range 77–100; seven genomes were less than 90%). BCFtools, version 1.11 mpileup was used to map the reads to the H37Rv reference genome. We included reads with a minimum mapping quality of eight. We screened one isolate per patient for anti-tuberculosis drug resistance mutations using the TBprofiler, version 2.8.2 pipeline.[10,15] The pipeline aligns reads to the reference genome using BWA, version 0.7.17 and calls variants with SAMtools, version 1.9.10,[16–18] The variants were then compared to a drug-resistance database. Single-nucleotide polymorphisms, insertions, and deletions responsible for resistance to 19 anti-tuberculosis drugs were identified:[10,15,19] streptomycin, para-aminosalicylic acid, isoniazid, pyrazinamide, cycloserine, kanamycin, ethionamide, ethambutol, amikacin, rifampicin, capreomycin, ofloxacin, ciprofloxacin, moxafloxacin, levofloxacin, linezolid, bedaquiline, clofazimine, and delamanid. A coverage of ten reads was needed to call a polymorphism. We considered all drug resistance alleles with a variant frequency equal to or higher than 90%.

WHO defines monoresistance as resistance to one of the first-line drugs (ie, isoniazid, pyrazinamide, ethambutol, and rifampicin).[1,13] MDR tuberculosis is defined as resistance to both isoniazid and rifampicin. Pre-XDR tuberculosis is defined as resistance to isoniazid and rifampicin plus fluoroquinolones or one of the three second-line injectable drugs (ie, amikacin, ciprofloxacin, or kanamycin). XDR tuberculosis is defined as drug resistance against isoniazid, rifampicin, fluoroquinolones, and at least one of the three second-line injectable drugs.

We compared the drug resistance profiles obtained at sites using routine drug susceptibility testing to drug resistance patterns obtained from whole-genome sequences. We considered any drug resistance obtained from the tests that a patient underwent locally. Drug resistance profiles were defined as concordant or discordant according to the resistance categories defined by WHO.[1] Discordant results were further categorised into discordant results potentially leading to undertreatment, or potentially leading to overtreatment (appendix p 6).[1,13] Discordances with no clear implications for treatment were defined as other discordances. We assessed the appropriateness of prescribed anti-tuberculosis treatment according to WHO guidelines (appendix p 7).[1,13] Effective drugs were defined as drugs to which no drug-resistance-conferring mutations were

observed in WGS (appendix p 8). The prescription of less than three effective drugs was defined as undertreatment, except for patients with isoniazid-resistant or rifampicin-resistant isolates. In these patients, a regimen comprising fewer than four effective drugs was considered as undertreatment, according to WHO guidelines. Overtreatment included second-line drugs given to patients for whom first-line regimens would have been appropriate. The classification of regimens is shown in the appendix (p 11).

Statistical analysis

We used descriptive statistics for patient characteristics by levels of drug resistance based on WGS. We compared the following drug resistance categories: pan-susceptible tuberculosis, monoresistant tuberculosis (any monoresistance), MDR tuberculosis, pre-XDR or XDR tuberculosis, any isoniazid-resistant tuberculosis (including isoniazid-monoresistant, MDR, and pre-XDR or XDR tuberculosis), any rifampicin-resistant tuberculosis (including rifampicin-monoresistant, MDR, and pre-XDR or XDR tuberculosis). Patients with missing data for treatment regimen, treatment outcome, ongoing treatment, or sputum microscopy were excluded from the analysis of mortality.

Four logistic regression models were calculated to assess the effects of: any drug resistance; drug resistance categories; discordant diagnoses; and treatment appropriateness on mortality. Logistic regression models were adjusted for sex, age, HIV status, history of tuberculosis, and sputum positivity. The country of origin was included as a random effect on the intercept.20 We did three sensitivity analyses. First, we repeated all logistic regression analyses after restricting the data to drug resistances that could be diagnosed with the locally available tests. We thus excluded drug resistances that were missed due to unavailable testing methods. Second, we repeated the logistic regression for mortality by treatment appropriateness, excluding patients with pre-XDR or XDR tuberculosis. Third, we examined the effect of different variant frequency cutoffs on each logistic regression (≥0% and 100%). All analyses were done in R, version 3.6.1, or Python, version 3.7.6.[21,22]

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Sept 1, 2014, and July 4, 2016**,** of the 634 patients included in our previous analysis,9 we were unable to sequence 52 (8%) isolates due to poor bacterial growth, DNA quality, or failures in the library preparation (appendix p 2). We therefore included 582 patients with tuberculosis, 406 (70%) from Africa, 93 (16%) from Latin America, and 83 (14%) from Asia. 172 (30%) patients came from South Africa, 94 (16%) from Côte d'Ivoire, 93 (16%) from Peru, 83 (14%) from Thailand, 59 (10%) from Democratic Republic of the Congo, 53 (9%) from Nigeria, and 28 (5%) from Kenya (table 1).

Table 1. Patient characteristics by resistance profiles obtained by whole-genome sequencing

	Pan-susceptible	Any resistance	p value	Monoresistance					Polyresistance			
				All	Isoniazid	Pyrazinamide	Ethambutol	Rifampicin	All	MDR	Pre-XDR/ XDR	Other
Total	339	243	..	35	8	2	1	24	208	146	24	38
Sex	..	..	0·99	..	..	..	..	..	..	..	..	..
Women	131 (39%)	94 (39%)	..	10 (29%)	3 (38%)	0 (0%)	1 (100%)	6 (25%)	84 (40%)	56 (38%)	13 (54%)	15 (39%)
Men	208 (61%)	149 (61%)	..	25 (71%)	5 (63%)	2 (100%)	0	18 (75%)	124 (60%)	90 (62%)	11 (46%)	23 (61%)
Age, years at diagnosis	35 (28–45)	32 (25–40)	0·0067	32 (25–40)	40 (31–49)	26 (25–28)	36 (36–36)	29 (25–39)	32 (26–40)	31 (25–39)	30 (25–34)	36 (29–44)
HIV status	..	..	<0·0001	..	..	..	..	..	..	..	..	..
HIV-negative	169 (50%)	166 (68%)	..	23 (66%)	7 (88%)	1 (50%)	0	15 (63%)	143 (69%)	103 (71%)	14 (58%)	26 (68%)
HIV-positive	170 (50%)	77 (32%)	..	12 (34%)	1 (13%)	1 (50%)	1 (100%)	9 (38%)	65 (31%)	43 (29%)	10 (42%)	12 (32%)
***Mycobacterium tuberculosis* lineage**			0·039	..	..	..	..	..	..	..	..	..
L1	18 (5%)	6 (2%)	..	2 (6%)	0	0	0	2 (8%)	4 (2%)	1 (1%)	0	3 (8%)
L2	79 (23%)	56 (23%)	..	7 (20%)	3 (38%)	1 (50%)	0	3 (13%)	49 (24%)	23 (16%)	8 (33%)	18 (47%)
L3	15 (4%)	3 (1%)	..	0	0	0	0	0	3 (1%)	2 (1%)	1 (4%)	0
L4	225 (66%)	178 (73%)	..	26 (74%)	5 (63%)	1 (50%)	1 (100%)	19 (79%)	152 (73%)	120 (82%)	15 (63%)	17 (45%)
L5	1 (<1%)	0	..	0	0	0	0	0	0	0	0	0
L6	1 (<1%)	0	..	0	0	0	0	0	0	0	0	0
Country	..	..	<0·0003	..	..	..	..	..	..	..	..	..
Côte d'Ivoire	46 (14%)	48 (20%)	..	5 (14%)	2 (25%)	0	1 (100%)	2 (8%)	43 (21%)	39 (27%)	3 (13%)	1 (3%)
Democratic Republic of the Congo	29 (9%)	30 (12%)	..	1 (3%)	0	0	0	1 (4%)	29 (14%)	19 (13%)	8 (33%)	2 (5%)
Kenya	21 (6%)	7 (3%)	..	1 (3%)	1 (13%)	0	0	0	6 (3%)	5 (3%)	0	1 (3%)
Nigeria	19 (6%)	34 (14%)	..	6 (17%)	0	0	0	6 (25%)	28 (13%)	20 (14%)	4 (17%)	4 (11%)
Peru	57 (17%)	36 (15%)	..	2 (6%)	2 (25%)	0	0	0	34 (16%)	28 (19%)	2 (8%)	4 (11%)
South Africa	111 (33%)	61 (25%)	..	15 (43%)	0	1 (50%)	0	14 (58%)	46 (22%)	28 (19%)	7 (29%)	11 (29%)
Thailand	56 (17%)	27 (11%)	..	5 (14%)	3 (38%)	1 (50%)	0	1 (4%)	22 (11%)	7 (5%)	0	15 (39%)
History of TB	..	..	<0·0001	..	..	..	..	..	..	..	..	..
No	269 (79%)	104 (43%)	..	13 (37%)	7 (88%)	1 (50%)	1 (100%)	4 (17%)	91 (44%)	56 (38%)	5 (21%)	30 (79%)
Yes	70 (21%)	139 (57%)	..	22 (63%)	1 (13%)	1 (50%)	0	20 (83%)	117 (56%)	90 (62%)	19 (79%)	8 (21%)
Treatment outcomes	..	..	<0·0001	..	..	..	..	..	..	..	..	..
Success	248 (73%)	129 (53%)	..	16 (46%)	5 (63%)	0	1 (100%)	10 (42%)	113 (54%)	76 (52%)	11 (46%)	26 (68%)
Mortality	19 (6%)	45 (19%)	..	6 (17%)	1 (13%)	1 (50%)	0	4 (17%)	39 (19%)	24 (16%)	9 (38%)	6 (16%)
Treatment failure	11 (3%)	10 (4%)	..	3 (9%)	0	1 (50%)	0	2 (8%)	7 (3%)	5 (3%)	2 (8%)	0
Lost to follow-up	26 (8%)	29 (12%)	..	5 (14%)	0	0	0	5 (21%)	24 (12%)	22 (15%)	0	2 (5%)
Transfer	13 (4%)	15 (6%)	2 (6%)	0	0	0	2 (8%)	13 (6%)	10 (7%)	2 (8%)	1 (3%)	0
Ongoing, unknown	22 (6%)	15 (6%)	..	3 (9%)	2 (25%)	0	0	1 (4%)	12 (6%)	9 (6%)	0	3 (8%)
Sputum	..	..	0·089	..	..	..	..	..	..	..	..	..
Positive	264 (78%)	205 (84%)	..	25 (71%)	7 (88%)	1 (50%)	1 (100%)	16 (67%)	180 (87%)	129 (88%)	17 (71%)	34 (89%)
Negative	68 (20%)	36 (15%)	..	10 (29%)	1 (13%)	1 (50%)	0	8 (33%)	26 (13%)	17 (12%)	6 (25%)	3 (8%)

Data are n (%) or median (IQR). p values show the difference between pan-susceptible and any resistance, obtained with the χ^2 test (L5 and L6 were excluded and for age the t test was used). The category other included the following drug resistances: cycloserine (n=1); ethionamide (n=5); streptomycin (n=9); ethambutol and rifampicin (n=1); ethambutol and streptomycin (n=1); isoniazid and ethionamide (n=14); isoniazid and pyrazinamide (n=1); isoniazid and streptomycin (n=1); ethambutol, isoniazid, and streptomycin (n=1); isoniazid, ethionamide, and streptomycin (n=1); rifampicin, pyrazinamide, streptomycin, and ethionamide (n=1); isoniazid, levofloxacin, moxifloxacin, ofloxacin, para-aminosalicylic acid, and ciprofloxacin (n=1); ethambutol, rifampicin, levofloxacin, moxifloxacin, ofloxacin, ciprofloxacin, and streptomycin (n=1). XDR=extensively drug-resistant. Due to rounding, some group percentage totals are more than 100%. *For nine patients with tuberculosis, no sputum was available.

The median age was 33 years (IQR 27–43), 225 (39%) were women, and 247 (42%) were HIV-positive. Six *M tuberculosis* lineages were represented: 24 (4%) cases of L1, 135 (23%) L2, 18 (3%) L3, 403 (69%) L4, one (<1%) L5, and one (<1%) L6. Based on WGS, 339 (58%) isolates were pan-susceptible and 35 (6%) were monoresistant: 24 rifampicin, eight isoniazid, two pyrazinamide, and one ethambutol monoresistant isolates. There were 208 (36%) polyresistant isolates, including 146 (25%) MDR, 24 (4%) pre-XDR or XDR isolates, and 38 (7%) other types of polyresistances (table 1; figure 1). Among the 24 patients with pre-XDR or XDR, nine had resistance to fluoroquinolones, six to injectable drugs, and nine to both.

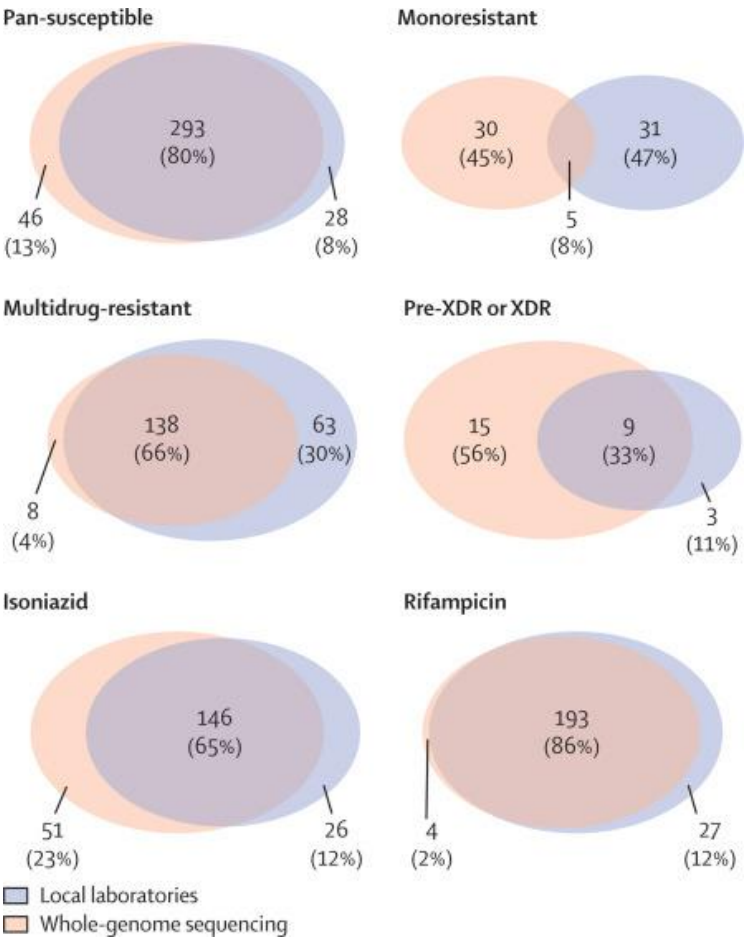

Figure 1. Distribution of diagnosed drug resistance between whole-genome sequencing and local drug susceptibility testing. The categories include pan-susceptible, monoresistant (any monoresistance), multidrug-resistant, pre-XDR or XDR, any isoniazid-resistant, or any rifampicin-resistant tuberculosis. Due to rounding, some group percentage totals are more than 100%. XDR=extensively drug-resistant.

Local drug susceptibility testing results were based on the molecular Xpert MTB/RIF test system, line probe assays, and culture-based phenotypic tests, or a combination of these methods (table 2). Among the 582 isolates, 130 (22%) of 582 had discordant drug resistance results when comparing local drug susceptibility testing with WGS. 65 (11%) discordant drug resistance results potentially led to inappropriate treatment of patients with tuberculosis (table 2). We then looked at the regimens prescribed to patients. For six patients, we had no treatment information. Of 576 patients with known treatment, we observed that overall 86 (15%) of 576 patients received inappropriate treatment according to WGS results and WHO treatment guidelines: 67 (12%) of 576 patients were undertreated, and 19 (3%) were overtreated. Consequently, 490 (85%) patients were appropriately treated.

The agreement between local drug susceptibility testing and WGS was 80% for pan-susceptible, 8% for monoresistant, 66% for MDR, and 33% for pre-XDR or XDR tuberculosis (figure 1). Agreement of local drug susceptibility testing and WGS for rifampicin resistance was 86% and it was 65% for isoniazid resistance. Rifampicin resistance was, in contrast to other drug resistance, more frequently diagnosed with local drug susceptibility testing than with WGS (figure 1). Only three sites tested for drugs other than rifampicin and isoniazid. Two sites tested for streptomycin, two for fluoroquinolones, and two for injectable drugs. One site tested for pyrazinamide and one site for ethambutol. Resistance to pyrazinamide, cycloserine, ethambutol, linezolid, bedaquiline, clofazimine, and delamanid was not tested at any site. WGS did not identify any resistance to bedaquiline, clofazimine, or delamanid (appendix p 8).

Table 2. Drug resistance results from whole-genome sequencing and local testing by diagnosis concordance and potential consequences for treatment

Drug resistance		n (%)	Local drug susceptibility test diagnosis method			
Based on whole-genome sequencing	Based on local tests		Xpert MTB/RIF*	Culture	Line probe assay	Combination of tests
Concordance between resistance patterns						
Total	..	452 (100%)	242/452 (54%)	195/452 (43%)	60/452 (13%)	102/452 (23%)
Pan-susceptible	Pan-susceptible	293 (65%)	196	139	49	53
Monoresistant (3 isoniazid, 2 rifampicin)	3 isoniazid, 2 rifampicin	5 (1%)	0	4	1	0
MDR	MDR	138 (31%)	45	44	8	44
Pre-XDR or XDR	Pre-XDR or XDR	9 (2%)	1	1	2	5
Other (7 streptomycin)	7 streptomycin	7 (2%)	0	7	0	0
Discordance between resistance patterns						
Total	..	130 (100%)	35/130 (27%)	55/130 (42%)	9/130 (7%)	46/130 (35%)
Potentially leading to undertreatment	..	34 (26%)	17/130 (13%)	12/130 (9%)	1/130 (1%)	1/130 (1%)
Pan-susceptible	..	0	0	0	0	0
Monoresistant (3 isoniazid)	3 pan-susceptible	3 (2%)	2	1	0	0
MDR	3 pan-susceptible, 1 streptomycin-ethambutol	4 (3%)	2	1	0	1
Pre-XDR or XDR	15 MDR	15 (12%)	5	6	0	4
Other (10 isoniazid-ethionamide, 1 isoniazid-streptomycin, 1 isoniazid-ethionamide-streptomycin)	12 pan-susceptible	12 (9%)	8	4	0	0
Potentially leading to overtreatment	..	31 (24%)	3/130 (2%)	12/130 (9%)	2/130 (2%)	14/130 (11%)
Pan-susceptible	1 isoniazid, 18 MDR, 4 rifampicin	23 (18%)	3	9	2	9
Monoresistant (2 isoniazid)	2 MDR	2 (2%)	0	2	0	0
MDR	3 Pre-XDR or XDR	3 (2%)	0	0	0	3
Pre-XDR or XDR	..	0	0	0	0	0
Other (1 isoniazid-ethionamide, 1 isoniazid-pyrazinamide, 1 isoniazid-ethambutol-streptomycin)	3 MDR	3 (2%)	0	1	0	2
Other discordance	..	65 (50%)	15/130 (12%)	31/130 (9%)	6/130 (1%)	27/130 (4%)
Pan-susceptible	20 ethambutol, 1 monoresistant†, 2 streptomycin	23 (18%)	2	20	0	1
Monoresistant (1 ethambutol, 2 pyrazinamide, 22 rifampicin)	1 pan-susceptible, 2 pan-susceptible, 22 MDR	25 (19%)	6	1	1	20
MDR	1 rifampicin	1 (1%)	0	0	1	0
Pre-XDR or XDR	..	0	0	0	0	0
Other‡						
1 cycloserine,	1 pan-susceptible					
5 ethionamide	5 pan-susceptible					
2 streptomycin	1 pan-susceptible,					
3 isoniazid-ethionamide	1 streptomycin-ethambutol					
1 isoniazid-levofloxacin-moxifloxacin-ofloxacin-para-aminosalicylic acid-ciprofloxacin	3 isoniazid					
	1 isoniazid					
1 ethambutol-rifampicin-levofloxacin-moxifloxacin-ofloxacin-ciprofloxacin-streptomycin	1 streptomycin					
1 ethambutol-rifampicin	1 MDR					
1 ethambutol-streptomycin	1 MDR					
1 rifampicin-pyrazinamide-streptomycin-ethionamide	1 MDR					

MDR=multidrug-resistant. XDR=extensively drug-resistant. *Rifampicin resistance diagnosed with Xpert MTB/RIF was classified as MDR.
†Exact monoresistance is not known

We excluded 52 (9%) of 582 patients from the mortality analyses due to missing data (appendix p 2). Based on WGS, the isolates of 310 (58%) of 530 patients were pan-susceptible, 32 (6%) monoresistant, 131 (25%) MDR, 23 (4%) pre-XDR or XDR, and 34 (6%) other polyresistances. Among the 530 patients, 121 (23%) had discordant drug susceptibility testing results. For 29 (66%) of 44 patients, underdiagnosis of drug resistance potentially led to undertreatment, and for 28 (36%) of 77, overdiagnosis potentially led to overtreatment. During treatment, 63 (12%) of 530 patients died (table 3). Mortality was 6% (18 of 310) in patients with pan-susceptible tuberculosis, 19% (six of 32) in patients with monoresistant tuberculosis, and 18% (24 of 131) in patients with MDR tuberculosis. Patients with pre-XDR or XDR tuberculosis had a mortality of 39% (nine of 23; figure 2A). Overall, mortality ranged from 6% (16 of 267) among patients with pan-susceptible strains and concordant diagnosis to 47% (seven of 15) among patients with pre-XDR or XDR tuberculosis and a discordant diagnosis potentially leading to undertreatment (table 3). In patients with a discordant diagnosis potentially leading to undertreatment, mortality was 28% (eight of 29), and in patients with a discordant diagnosis potentially leading to overtreatment, it was 4% (one of 28; figure 2B). Mortality ranged from 6% (17 of 293) in patients with pan-susceptible tuberculosis treated according to WHO guidelines to 32% (19 of 60) in undertreated patients and 6% (one of 17) in patients who were overtreated (figure 2C).

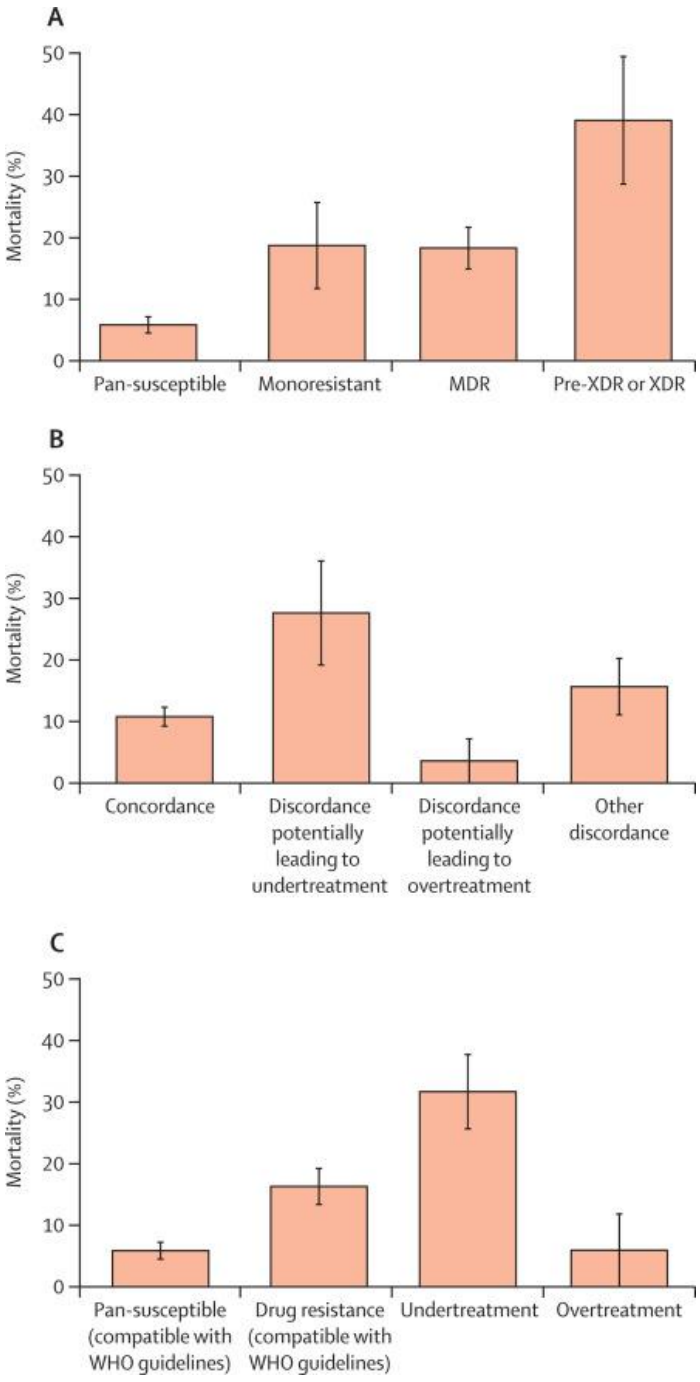

Figure 2. Mortality according to drug resistance, concordance of diagnosis, and treatment appropriateness. Mortality data are shown based on drug resistance (A), concordance of diagnosis (B), and treatment appropriateness (C). Appropriateness was considered according to WHO guidelines (appendix pp 6–7). Error bars are SEs. Analysis based on 530 patients with complete data. Mortality was calculated by dividing deaths by the number of patients in the respective category. MDR=multidrug-resistant. XDR=extensively drug-resistant.

Table 3. Mortality by concordance of local diagnosis and whole-genome sequencing

	Total	Concordant with diagnosis at sites	Discordant with diagnosis at sites			
			Any discordance	Potentially leading to undertreatment	Potentially leading to overtreatment	Other discordance
Resistance based on whole-genome sequencing	63/530 (12%)	44/409 (11%)	19/121 (16%)	8/30 (27%)	1/28 (4%)	10/63 (16%)
Pan-susceptible	18/310 (6%)	16/267 (6%)	2/43 (5%)	0/0	0/20	2/23 (9%)
Any resistance	45/220 (20%)	28/142 (20%)	17/78 (22%)	8/30 (27%)	1/8 (13%)	8/40 (20%)
Monoresistance	6/32 (19%)	2/4 (50%)	4/28 (14%)	0/1	0/2	4/25 (16%)
Isoniazid	1/6 (17%)	1/3 (33%)	0/3	0/1	0/2	0/0
Pyrazinamide	1/2 (50%)	0/0	1/2 (50%)	0/0	0/0	1/2 (50%)
Ethambutol	0/1	0/0	0/1	0/0	0/0	0/1
Rifampicin	4/23 (17%)	1/1 (100%)	3/22 (14%)	0/0	0/0	3/22 (14%)
Polyresistance	39/188 (21%)	26/138 (19%)	13/50 (26%)	8/29 (28%)	1/6 (17%)	4/15 (27%)
Multidrug resistance	24/131 (18%)	23/123 (19%)	1/8 (13%)	1/4 (25%)	0/3	0/1
Pre-XDR or XDR	9/23 (39%)	2/8 (25%)	7/15 (47%)	7/15 (47%)	0/0	0/0
Other	6/34 (18%)	1/7 (14%)	5/27 (19%)	0/9	1/3 (33%)	4/15 (27%)

Analysis based on 530 patients with complete data. The category other discordance includes the following drug resistances: cycloserine (n=1); ethionamide (n=5); streptomycin (n=9); ethambutol and rifampicin (n=1); isoniazid and ethionamide (n=12); isoniazid and pyrazinamide (n=1); ethambutol, isoniazid, and streptomycin (n=1); ethambutol, isoniazid, and streptomycin (n=1); rifampicin, pyrazinamide, streptomycin, and ethionamide (n=1); isoniazid, levofloxacin, moxifloxacin, ofloxacin, para-aminosalicylic acid, and ciprofloxacin (n=1); and ethambutol, rifampicin, levofloxacin, moxifloxacin, ofloxacin, ciprofloxacin, and streptomycin (n=1). XDR=extensively drug-resistant.

In the multivariable logistic regression, resistance to any of the anti-tuberculosis drugs was associated with higher mortality (figure 3). The adjusted odds ratio (OR) was 5·58 (95% CI 2·86–10·90). The association with mortality became stronger with a higher degree of drug resistance. Compared with pan-susceptible tuberculosis, the adjusted OR for monoresistant was 5·88 (95% CI 1·92–17·98), for MDR was 5·55 (2·53–12·20), and for pre-XDR or XDR tuberculosis was 23·03 (7·16–74·05; figure 3). The adjusted OR for mortality during tuberculosis treatment was 4·07 (95% CI 1·58–10·47) in patients with a diagnosis potentially leading to undertreatment, and 0·29 (0·04–2·19) in the case of a diagnosis potentially leading to overtreatment, compared with patients with appropriate treatment (figure 3). Overall, 77 (15%) of 530 patients received inappropriate treatment based on WGS drug resistance results and WHO guidelines (appendix p 7). 60 (11%) of 530 patients were undertreated, and 17 (3%) of 530 were overtreated. The OR for mortality for undertreatment was 4·92 (95% CI 2·47–9·78), and for overtreatment was 0·52 (0·07–4·20), compared with patients receiving appropriate treatment (figure 3). In a sensitivity analysis, we showed that mortality among undertreated patients remained higher than among appropriately treated patients after excluding patients with pre-XDR or XDR tuberculosis (adjusted OR 5·97 [95% CI 2·58–13·80]). The unadjusted covariate ORs for mortality during tuberculosis treatment are shown in the appendix (p 13). The sensitivity analysis of the logistic regression models using different variant frequency cutoffs (≥0% and 100%) produced similar results (appendix pp 3–4). When restricting the analysis to drug resistances that could be diagnosed at sites, again similar results were obtained (appendix p 5).

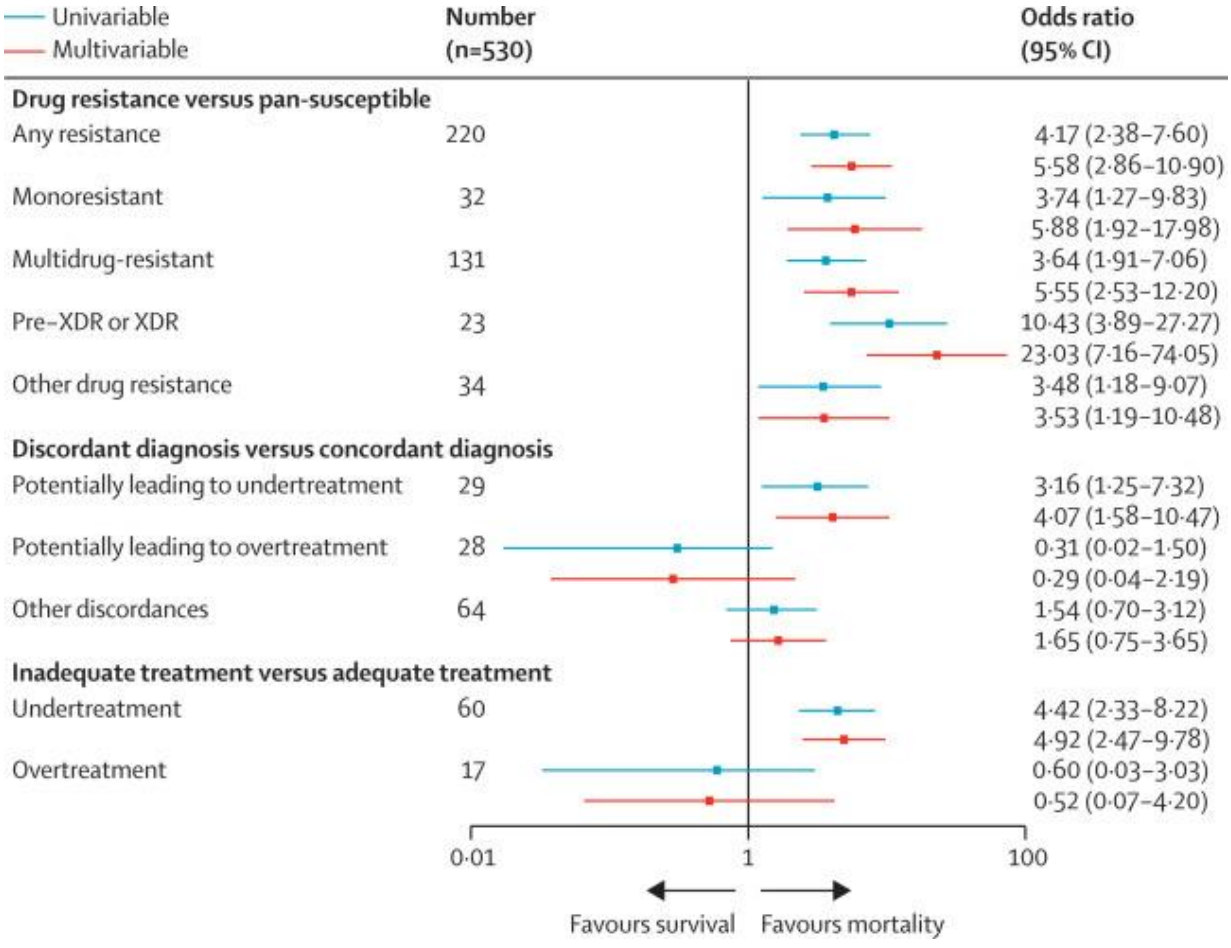

Figure 3. Logistic regression models to assess the effect of any drug resistance, drug resistance categories, diagnosis discordance, and treatment appropriateness on mortality The models were adjusted for sex, age, HIV status, history of tuberculosis, and sputum microscopy, and country of participating site was included as random effect on the intercept. Appropriateness was considered according to WHO guidelines (appendix pp 6–7). XDR=extensively drug-resistant.

Discussion

In this multicentre cohort study, we compared drug resistance predicted by WGS with the results from local drug susceptibility testing in seven countries with a high burden of tuberculosis. We examined mortality by drug resistance predicted by WGS, and by concordance or discordance with local diagnosis and the appropriateness of treatment. We found that the diagnosis was discordant between local drug resistance results and WGS in about one in five patients. The agreement between local and centralised WGS was the highest for rifampicin and isoniazid, but low for other drugs. Of note, resistance to streptomycin, para-aminosalicylic acid, pyrazinamide, cycloserine, ethionamide, ethambutol, fluoroquinolones, and injectable drugs was rarely investigated locally. Mortality during treatment ranged from 6% among patients with pan-susceptible strains and concordant results between WGS and local drug resistance testing to 47% among patients with pre-XDR or XDR tuberculosis and discordant results.

To our knowledge, this is the first study to compare the results from drug susceptibility testing in real-world settings in high-burden countries with WGS and to examine the effect of discordant resistance results on mortality. In a previous analysis of this cohort, we compared the results from local drug susceptibility testing with those obtained at the Swiss National Center for Mycobacteria

for six drugs.[9] In the present study, we used a well established bioinformatics pipeline and its corresponding database to analyse the WGS data.[10] The analysis covered 19 anti-tuberculosis drugs, including streptomycin, kanamycin, pyrazinamide, ethionamide, ethambutol, and levofloxacin, as well as newer drugs. Specifically, we were able to detect more single-drug resistance with WGS than with phenotypic drug susceptibility testing.

Rapid and accurate diagnosis, prompt and appropriate treatment, and the control of airborne infection are key strategies to prevent drug-resistant tuberculosis.[23] Routine testing at sites focused mainly on the identification of rifampicin and isoniazid resistance used to diagnose MDR tuberculosis and did not address the efficacy of other drugs. Also, isoniazid monoresistance would typically be missed if drug susceptibility testing relies on the Xpert MTB/RIF system, which could lead to the undertreatment of some patients. Furthermore, culture-based drug susceptibility testing is challenging for several drugs—eg, pyrazinamide, ethionamide, and ethambutol— due to poor drug solubility.[11,24] Yet, pyrazinamide is essential for shortening tuberculosis therapy, and resistance to pyrazinamide is associated with worse outcomes.[23] However, pyrazinamide resistance testing is often unavailable. Only one site could test pyrazinamide resistance in our study.

WGS has the potential to predict resistance profiles for most anti-tuberculosis drugs without the need for time-consuming phenotypic drug susceptibility testing.[10–12,19] WGS provides simultaneous and comprehensive information on relevant mutations conferring resistance to first-line and second-line drugs, anywhere in the genome. By contrast, targeted sequencing only identifies mutations in a priori defined regions covered by the amplifications. WGS allows effective individualised treatment, and thus reduces the risk of propagating drug resistance. Ineffective treatment could lead to the acquisition of additional drug resistance and increases the risk of transmitting drug-resistant strains.[23] These considerations support the use of WGS to replace the current drug susceptibility testing methods, which cover only a limited number of drugs.

The broader range of drug resistance captured by WGS explains some of the discordant results found in this study; however, restricting the analysis of discordances between drug resistance diagnosed locally and by WGS to the most clinically relevant WHO categories of drug resistance will have minimised this effect.[13] Thus, discordant results potentially leading to inappropriate treatment were mainly due to important drug resistance not captured with the available local tests at sites, rather than to a wider range of drug resistances captured by WGS. The detection of drug resistance is also influenced by the type of sample collected, and the methods used for culturing, DNA extraction and sequencing, and the pipeline used to analyse the sequences.[25] The pipeline used to analyse the sequences was determined by a 90% or greater variant frequency cutoff, the robustness of the TBprofiler pipeline, and its coverage of all relevant resistance-conferring mutations. Our sensitivity analysis showed that the cutoff for variant frequency had little effect on results.

For new drugs, most resistance-conferring mutations are unknown at the time of introduction, and relevant drug resistance mechanisms become apparent only when the mutation becomes established in the population. The TBprofiler database is continuously updated with newly identified resistance-conferring mutations, such as bedaquiline in 2013 and dalamanid in 2014. Yet, the accuracy of the prediction of phenotypic resistance by molecular markers varies by drugs, depending on the molecular mechanisms involved and the evidence generated so far. We showed that the identification of drug-resistance-conferring mutations predicted phenotypic resistance to rifampicin better than to ethambutol.[11] Discrepancies in results between local drug susceptibility testing and WGS might also be explained by mixed infections, heteroresistance, minority resistant populations, or methodological differences,[25–27] which can lead to uncertainties in treatment decisions.[28] Of note, overtreatment did not increase mortality, but the analysis was based on few patients (n=28) and

should be interpreted with caution. Anti-tuberculosis drugs, especially second-line drugs, can cause serious side-effects, which can lead to treatment interruption, and failure, or acquired drug resistance, and should therefore only be used when needed.[29]

Our study has several limitations. We sampled eligible patients within strata defined by drug resistance and HIV infection, and therefore, could not estimate the incidence or prevalence of drug-resistant tuberculosis in patients who were HIV-coinfected or HIV-negative. Also, we could not evaluate differences in drug resistance between *M tuberculosis* lineages because the sample size was small for several lineages. Our analysis is mainly based on L2 and L4 strains, as expected from the geographical distribution of these lineages.[30] Further, we sequenced strains before treatment and thus could not diagnose potentially acquired drug resistance, which might influence treatment outcomes. Finally, this study reflects the years 2013–16. Since then, the availability of drug resistance tests has increased (appendix p 14). For example, the MTBDRsl assay (Hain Lifescience, Nehren, Germany), a line probe assay for the detection of pre-XDR or XDR, is now available at four sites. However, three of the seven sites still have no access to rapid molecular tests to diagnose resistance to second-line drugs. In general, there were only a few changes in the drug resistances that are tested routinely between the study period and 2020 (appendix p 14).

Treatment guidelines also changed over the study period. In 2013, WHO published an interim policy guideline on bedaquiline, and in 2014 on delamanid in the treatment of MDR tuberculosis.[31,32] In our study, patients were rarely given newer drugs such as bedaquiline or delamanid. In 2020, only South Africa included bedaquiline in their short and long MDR tuberculosis regimens. By contrast, the other sites are still using the so-called Bangladesh regimen (ie, a standardised short course MDR tuberculosis treatment regimen of 9–12 months), although guidelines will probably change in the near future. Identifying the emergence of resistance to recently introduced drugs will be crucial alongside the roll-out of new regimens.[33]

Our study shows that treatment strategies guided by comprehensive drug resistance data are likely to save lives. Our results thus support WHO's call for an accurate point-of-care test based on WGS that can be done directly from sputum samples.[34] Such tests would allow rapid diagnosis and efficient, individual-based treatment of drug-resistant tuberculosis.[35] Test systems performing WGS on sputum samples, using new laboratory and bioinformatics pipelines are in development. High-burden countries should consider building central, high-throughput sequencing capacities.[36] The establishment of a trustworthy, widely accepted drug resistance database similar to the Stanford HIV drug resistance database will be essential in this context.[37] Finally, we support the call for clinical trials evaluating the safety, efficacy, and tolerability of new drugs and drug susceptibility testing strategies for drug-resistant tuberculosis.[23,29] The role of new drugs like bedaquiline, delamanid, and pretomanid in regimens with fewer, more effective, and safer drugs needs to be evaluated.[23] Future studies should also examine treatment duration and adherence.[23] The duration of the intensive and continuation phases of tuberculosis treatment and treatment adherence are crucial for efficient therapy.

In conclusion, our study shows that both the accuracy of drug susceptibility testing in routine care, and the access to testing for resistance for several essential drugs is limited in high-burden tuberculosis countries, which leads to inappropriate treatment, and contributes to higher mortality. Our results support the role of WGS to improve the management of drug-resistant tuberculosis in high-burden settings.

Supplementary appendix

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

Supplement to: Zürcher K, Reichmuth ML, Ballif M, et al. Mortality from drug-resistant tuberculosis in high-burden countries comparing routine drug susceptibility testing with whole-genome sequencing: a multicentre cohort study. Lancet Microbe 2021; published online April 29. https://doi.org/10.1016/S2666-5247(21)00044-6

Author contributors

MLR, KZ, MB, and ME wrote the first draft of the paper, which was reviewed by all authors and revised based on the comments received by coauthors. MB coordinated data and strain collection across study sites. MLR, KZ, MB, ME, SB, ECB, LF, and VS were involved in study design. CL, SB, and MR were involved in whole-genome sequencing. AA, AGA, OM, JC, EJC, RJW, HC, and MY supervised drug susceptibility testing at the local laboratory and the collection of clinical data. MLR and KZ verified the data. MLR did statistical analyses. All authors approved the final version of the manuscript.

Declaration of interests

RJW reports grants from Wellcome, European and Developing Countries Clinical Trials Partnership, UK Research and Innovation, Cancer Research UK, and National Institutes of Health, during the conduct of the study. ECB reports personal fees from AID Diagnostika and COPAN, outside the submitted work. MY reports grants from US National Institutes of Health, during the conduct of the study. All other authors declare no competing interests.

Data sharing

Whole-genome sequencing data from the strains included in this analysis have been submitted to the National Center for Biotechnology Information (PRJNA300846; appendix p 15).

Acknowledgments

We thank all sites and patients who participated in this study. We are also grateful to the Tuberculosis Working Group of International epidemiology Databases to Evaluate AIDS (IeDEA) for helpful discussions. Calculations were done on UBELIX, the HPC cluster at the University of Bern. The IeDEA is supported by the US National Institutes of Health, National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart, Lung, and Blood Institute, the National Institute on Alcohol Abuse and Alcoholism, the National Institute of Diabetes and Digestive and Kidney Diseases, the Fogarty International Center, and the National Library of Medicine: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919. ME was supported by special project

funding from the Swiss National Science Foundation (grant number 17481). KZ, MB, CL, LF and ME were supported by the Swiss National Science Foundation (grant number 320030_153442). SG, CL, SB, and MR were supported by the Swiss National Science Foundation (grant numbers 153442, 310030_188888, IZRJZ3_164171, IZLSZ3_170834 and CRSII5_177163) and by the European Research Council (grants 309540 and 883582). RJW received support from the Francis Crick Institute, which is funded by UK Research and Innovation, Cancer Research UK (grant number FC0010218), and Wellcome (grant numbers 104803, 203135). This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

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Co-author papers

9. Paper 7 – Low-fitness in drug resitant *Mtb* strains

HIV Coinfection Is Associated with Low-Fitness *rpoB* Variants in Rifampicin-Resistant *Mycobacterium tuberculosis*

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The article has been published in Antimicrobial Agents and Chemotherapy on the 21 September 2020.
DOI: https://doi.org/10.1128/AAC.00782-20.

Contribution: I contributed to the methodology. I did cleaning data of the clinical data and provided C.L the dataset. I gave inputs to the analysis. I commented on the first draft and all subsequent drafts of the manuscript.

Abstract
We analyzed 312 drug-resistant genomes of *Mycobacterium tuberculosis* isolates collected from HIV-coinfected and HIV-negative TB patients from nine countries with a high tuberculosis burden. We found that rifampicin-resistant *M. tuberculosis* strains isolated from HIV-coinfected patients carried disproportionally more resistance-conferring mutations in *rpoB* that are associated with a low fitness in the absence of the drug, suggesting these low-fitness *rpoB* variants can thrive in the context of reduced host immunity.

Tuberculosis (TB), caused by members of the *Mycobacterium tuberculosis* complex, is a leading cause of death worldwide, killing more people than any other infectious disease. Among the many factors driving the global TB epidemics, two factors stand out as particularly important: antibiotic resistance and HIV coinfection (1). Although the impact of both of these factors individually is well recognized, the interaction between them is less clear and likely depends on the particular epidemiologic setting (2). HIV coinfection and drug-resistant TB often coexist in severe epidemics, which indicates spread of drug-resistant *M. tuberculosis* strains from immunocompromised patients (3–5). The propensity of drug-resistant *M. tuberculosis* strains to spread is influenced by the fitness cost associated with drug resistance determinants (6). Specifically, bacterial strains that have acquired drug resistance-conferring mutations may be less transmissible than their susceptible counterparts, although this fitness cost can be ameliorated by compensatory mutations (7–10). Moreover, the effect of different resistance-conferring mutations on fitness can be heterogeneous (11). In the clinical setting, there is a selection for high-fitness and/or compensated drug-resistant *M. tuberculosis* strains in TB patients (12). However, in immunocompromised hosts, such as HIV-coinfected patients, even strains with low-fitness resistance mutations might propagate efficiently (13–15), which could partially explain why drug-resistant TB has been associated with HIV coinfection (16, 17). However, to date, no evidence directly supports the notion that the immunological environment created by HIV coinfection modifies the fitness of drug-resistant *M. tuberculosis* (5, 18, 19).

In this study, we tested the hypothesis that resistance-conferring mutations with low fitness in *M. tuberculosis* are overrepresented among HIV-coinfected TB patients. We focused our analysis on isoniazid and rifampicin, the two most important first-line anti-TB drugs, for which resistance-conferring mutations have been shown to differ in their fitness effects when measured in the laboratory (11). In addition, the frequency of the resistance alleles found in a clinical setting correlates well with the in vitro fitness of strains (12, 20). To explore the association between HIV coinfection and the fitness effect of different drug resistance-conferring mutations in *M. tuberculosis*, we compiled a collection of drug-resistant strains using the global International Epidemiology Databases to Evaluate AIDS (IeDEA, http://www.iedea.org) consortium (21, 22) as a platform. For this study, 312 strains were collected from HIV-coinfected and HIV uninfected TB patients originating from nine countries on three continents: Peru, Thailand, South Africa, Kenya, Côte d'Ivoire, Botswana, Democratic Republic of the Congo, Nigeria, and Tanzania (Fig. 1; see also Table S1 in the supplemental material). The association between the fitness of isoniazid resistance-conferring mutations and HIV coinfection was tested in a univariate analysis (Fig. S1).

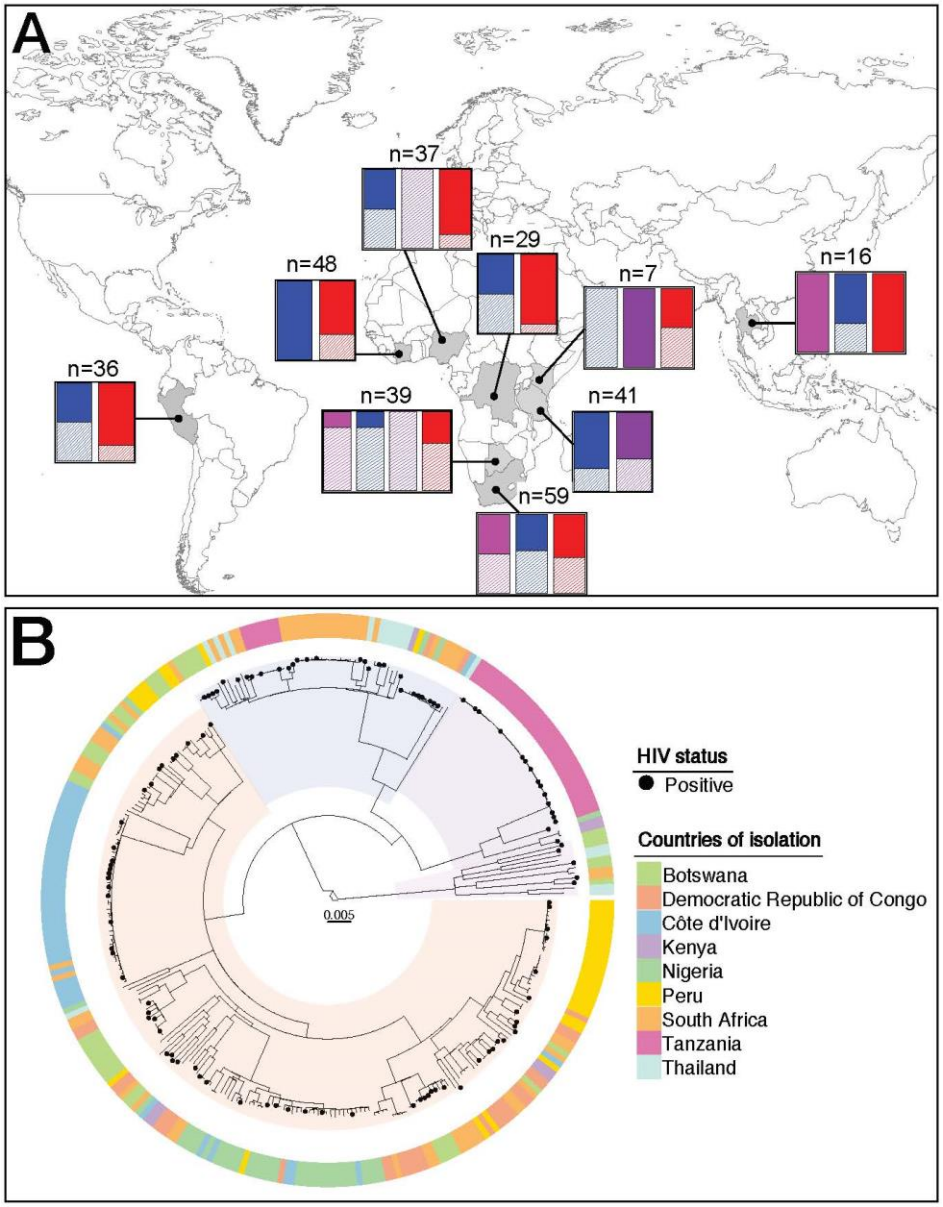

Figure 1. (A) Frequency of *M. tuberculosis* lineages by HIV status for countries sampled. Countries colored in gray were sampled. The bar plots indicate the proportion of each lineage represented in this study. Magenta corresponds to *M. tuberculosis* lineage 1, blue corresponds to *M. tuberculosis* lineage 2, purple corresponds to *M. tuberculosis* lineage 3, and red corresponds to *M. tuberculosis* lineage 4. Solid color corresponds to HIV-negative patients, and hatches correspond to HIV-coinfected TB patients. The number of genomes sampled in each country is indicated on top of the bar plots. (B) Phylogenetic tree of the data set used in the study. Maximum likelihood phylogeny of 312 whole-genome sequences based on 18,531 variable positions. The scale bar indicates the number of substitutions per polymorphic site. The phylogeny was rooted on *Mycobacterium canettii*. *M. tuberculosis* strains isolated from HIV-coinfected patients are indicated by black dots. The peripheral ring depicts the country of isolation of the strains sequenced.

Isoniazid resistance-conferring mutations were divided into three groups, as previously described (23): *katG* S315T mutation, *katG* mutations other than S315T, and *inhA* promoter mutations only. The S315T substitution in *katG* causes high-level isoniazid resistance while retaining some catalase/peroxidase functions (24). Conversely, the *inhA* promoter mutation does not affect *KatG* activity. Other substitutions/deletions in *katG* have been associated with a lower fitness in the laboratory and are observed only rarely among clinical isolates (23, 25, 26). In the case of rifampicin, the association between the fitness of *rpoB* variants and HIV coinfection was tested in both a univariate and multivariate analysis (Table 1). Resistance-conferring variants in *rpoB* were classified into two groups based on their fitness effects documented previously (11, 20, 27). The mutation *rpoB*

S450L was considered high fitness, since this mutation was previously shown to confer a low fitness cost in the laboratory (11) and is generally the most common in clinical strains (28). Any other resistance-conferring variant affecting *rpoB* was considered low fitness (11). The multivariable logistic regression model with outcome of low-fitness *rpoB* variants was adjusted for host-related factors (history of TB, country of isolation, sex, and age) (29) and bacterial factors (*M. tuberculosis* lineage, presence of an *rpoA-C* compensatory mutation, clustering of the genome inferred by genetic relatedness). Seventy-six patients from Tanzania and Botswana were excluded from the model due to missing or unknown clinical data (see the supplemental methods file).

Out of 312 patients, 113 (36.2%) were HIV coinfected, 120 (38.5%) were women, 115 (36.9%) were newly diagnosed TB cases (therefore, treatment naive), 276 (88.5%) harbored isoniazid resistance-conferring mutations, with or without additional resistance, and 282 (90.4%) harbored rifampicin resistance-conferring mutations, with or without additional resistance. In total, 78.8% (n = 246) of the strains were classified as being at least multidrug resistant, defined as resistance to isoniazid and rifampicin with or without additional resistance to second-line drugs. Among the 113 HIV-coinfected individuals, 34 (30%) were on antiretroviral therapy (ART), 26 (23%) were not, and 53 (47%) had an unknown ART start date. Four of the eight known *M. tuberculosis* lineages were represented in the following proportions: 11 L1 (3.5%), 57 L2 (18.3%), 38 L3 (12.2%), and 206 L4 (66.0%). After dividing a total of 276 isoniazid-resistant strains into the three groups of isoniazid resistance-conferring mutations defined above, we found similar proportions in HIV-coinfected and HIV-uninfected patients (chi-square test, P = 0.54; Fig. S1), and, as expected, the *katG* S315T mutation was the most frequent mutation in both categories (overall, found in 80% of isoniazid-resistant strains). In the case of rifampicin resistance, a univariate and multivariate analysis of 203 strains with complete clinical records indicated that HIV-coinfected TB patients carried a higher proportion of low-fitness *rpoB* resistance variants than HIV-negative patients (72.3% versus 51.4%). The univariate analysis showed higher odds of having a low-fitness *rpoB* variant in HIV-coinfected patients (odds ratio, 2.46 [95% confidence interval, 1.30 to 4.66], P = 0.006) (Table 1). Our multivariable regression analysis confirmed these results and showed an association between low-fitness *rpoB* variants and HIV coinfection while controlling for other factors (odds ratio, 4.58 [95% confidence interval, 1.69, 12.44], P = 0.003) (Table 1). This association can be explained in at least two ways. First, HIV-coinfected patients are thought to have fewer lung cavities on average and lower sputum bacillary load (30, 31). The resulting smaller *M. tuberculosis* population size would lead to fewer replication events, possibly reducing the number of mutations available for selection to act upon. In other words, low-fitness variants and high-fitness variants would co-occur less often in an HIV-coinfected patient, such that competition between them would be less likely. This scenario would be relevant for de novo acquisition of low-fitness drug-resistant variants within an HIV-coinfected patient. Second, following the transmission of a drug-resistant strain with low fitness to a host with reduced immunity, weaker immune pressure acting on this strain might lead to better bacterial survival. The association between low-fitness *rpoB* variants and HIV coinfection remained significant even after adjusting for the different epidemiologic settings (i.e., countries) and the strain genetic background (i.e., *M. tuberculosis* lineages). We also observed that strains carrying the *rpoB* S450L resistance-conferring mutation were more likely to also carry a compensatory mutation in *rpoA-C* (97.4% versus 2.6%) (Table 1). Even though this phenomenon seems counterintuitive, it has been described multiple times (7, 9, 32–34) and, thus, might point to different mechanisms of compensation in strains carrying resistance mutations other than *rpoB* S450L.

Table 1. Results of the univariate and multivariate analysis showing host and bacterial factors associated with low fitness *rpoB* variants in 203 TB patients.

		Low-fitness	High-fitness	univariable		multivariable	
Parameters for fitness of *rpoB* variants				**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
HIV status	HIV-	71 (51.4)	67 (48.6)	reference		reference	
	HIV+	47 (72.3)	18 (27.7)	2.46 (1.30-4.66)	0.006	4.58 (1.69-12.44)	0.003
Presence of a compensatory	No	117 (71.3)	47 (28.7)	reference		reference	
mutation in *rpoA/C*	Yes	1 (2.6)	38 (97.4)	0.01 (0.00-0.08)	< 0.0001	0.01 (0.00-0.06)	< 0.0001
***Mtb* lineage**	Lineage 2	16 (44.4)	20 (55.6)	reference		reference	
	Lineage 4	99 (61.5)	62 (38.5)	2.00 (0.96-4.14)	0.06	3.10 (0.94-10.21)	0.06
	Other (L1 or L3)	3 (50.0)	3 (50.0)	1.25 (0.22-7.05)	0.80	0.97 (0.11-8.31)	0.98
Clustering of the genome	No	109 (59.6)	74 (40.4)	reference		reference	
	Yes	9 (45.0)	11 (55.0)	0.56 (0.22-1.41)	0.21	1.05 (0.28-3.90)	0.94
Country of isolation	South Africa	29 (55.8)	23 (44.2)	reference		reference	
	Democratic Republic of Congo	11 (37.9)	18 (62.1)	0.48 (0.19-1.23)	0.13	0.39 (0.12-1.34)	0.14
	Côte d'Ivoire	35 (79.5)	9 (20.5)	3.08 (1.24-7.70)	0.02	2.04 (0.58-7.23)	0.27
	Kenya	4 (66.7)	2 (33.3)	1.59 (0.27-9.44)	0.61	0.94 (0.10-8.42)	0.96
	Nigeria	20 (58.8)	14 (41.2)	1.13 (0.47-2.72)	0.78	1.00 (0.29-3.40)	0.99
	Peru	16 (53.3)	14 (46.7)	0.91 (0.37-2.23)	0.83	1.49 (0.33-6.70)	0.60
	Thailand	3 (37.5)	5 (62.5)	0.48 (0.10-2.20)	0.34	0.42 (0.07-2.65)	0.36
Age	Mean (SD)	32.5 (10.4)	34.3 (12.3)	0.99 (0.96-1.01)	0.25	0.97 (0.94-1.01)	0.10
Sex	Female	47 (59.5)	32 (40.5)	reference			
	Male	71 (57.3)	53 (42.7)	0.91 (0.51-1.62)	0.75	0.77 (0.34-1.71)	0.52
History of TB disease	No	35 (52.2)	32 (47.8)	reference			
	Yes	83 (61.0)	53 (39.0)	1.43 (0.79-2.58)	0.23	0.96 (0.34-2.73)	0.94

Number of observations in model = 203; CI = confidence interval; The odds ratio and p-value are obtained from the regression model.

In addition, in our study, L4 strains were associated with low-fitness *rpoB* variants compared to L2 (odds ratio, 3.10 [95% confidence interval, 0.94, 10.21], P = 0.06) (Table 1), indicating that the strain genetic background plays a role in shaping the cost of resistance, as was previously shown for other bacterial species (35) and for other drugs (36). In the regression analysis, we had several categorical variables with only a few observations. Therefore, statistical power, especially for country of isolation, was low, and the results should be interpreted with care.

HIV-coinfected TB patients are generally thought to have a reduced potential for TB transmission (30, 37), because these patients have reduced formation of lung cavities, more extrapulmonary disease, and a shorter period of infectiousness due to earlier diagnosis or higher mortality, especially in the absence of antiretroviral treatment and if antibiotic resistance is already present (4). Based on the overrepresentation of low-fitness *rpoB* mutations in the context of HIV coinfection, one would expect a further reduction of the transmission potential of drug-resistant TB in this context. However, outbreaks of drug-resistant TB in HIV-coinfected patients have been reported (3). Such outbreaks might be explained by (i) a higher risk of *M. tuberculosis* infection and reinfection due to diminished host immunity, (ii) ongoing transmission of drug-resistant M. tuberculosis from a larger pool of immunocompetent TB patients to immunocompromised patients, (iii) transmission occurring in conducive environments, such as health care settings, where both HIV-coinfected individuals and drug-resistant TB patients are more likely to coexist, and (iv) *M. tuberculosis* strains carrying high-fitness drug resistance mutations.

In summary, using a global sample of drug-resistant *M. tuberculosis* clinical strains from HIV-coinfected and HIV-negative TB patients, we showed that low-fitness *rpoB* variants were overrepresented in HIV-coinfected patients, and that this association was independent from other potential confounding factors. Taken together, our results provide new insights into how HIV coinfection can impact the fitness of drug-resistant *M. tuberculosis*.

Data availability

The *M. tuberculosis* whole-genome sequences from the patients are available on NCBI under several project identifiers. The accession number for each genome is indicated in Supplemental Table S1.

Supplemental material

Supplemental material is available online only (https://journals.asm.org/doi/10.1128/AAC.00782-20). SUPPLEMENTAL FILE 1, PDF file, 0.1 MB. SUPPLEMENTAL FILE 2, XLSX file, 0.03 MB.

Acknowledgments

We thank all the sites that participated in this study and the patients whose data were used in this study. We thank Jan Hattendorf for providing statistical help and Sebastian M. Gygli for critically reading the manuscript. Calculations were performed at the sciCORE (http://scicore.unibas.ch/) scientific computing core facility at University of Basel. This research was supported by the Swiss National Science Foundation (grant numbers 153442, 310030_188888, 174281, and IZRJZ3_164171). The International Epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart, Lung,

and Blood Institute, the National Institute on Alcohol Abuse and Alcoholism, the National Institute of Diabetes and Digestive and Kidney Diseases, the Fogarty International Center, and the National Library of Medicine: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; and West Africa, U01AI069919. Informatics resources are supported by the Harmonist Project, R24AI124872. This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above. R.J.W. is supported by the Francis Crick Institute, which receives funding from Wellcome (FC0010218), CRUK (FC0010218), and UKR1 (FC0010218). He is also supported by Wellcome (104803 and 203135).

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10. Paper 8 – Delamanid resistance in drug-naïve patients

Natural Polymorphisms in *Mycobacterium tuberculosis* Conferring Resistance to Delamanid in Drug-Naïve Patients

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The article has been published in Antimicrobial Agents and Chemotherapy on the 20 October 2020.
DOI: https://doi.org/10.1128/AAC.00513-20.

Contribution: I contributed to the methodology and I provided the clinical data. I gave inputs to the analysis. I commented on the first draft and all subsequent drafts of the manuscript.

Abstract

Mutations in the genes of the F_{420} signaling pathway of *Mycobacterium tuberculosis* complex, including *dnn, fgd1, fbiA, fbiB, fbiC*, and *fbiD*, can lead to delamanid resistance. We searched for such mutations among 129 *M. tuberculosis* strains from Asia, South America, and Africa using whole-genome sequencing; 70 (54%) strains had at least one mutation in one of the genes. For 10 strains with mutations, we determined the MIC of delamanid. We found one strain from a delamanid-naïve patient carrying the natural polymorphism Tyr29del (*ddn*) that was associated with a critical delamanid MIC.

In 2014, the new anti-tuberculosis (anti-TB) drug delamanid (also known as OPC-67683, or Deltyba) was introduced (1). The World Health Organization (WHO) recommends the administration of delamanid if a standard effective drug regimen cannot be prescribed due to drug toxicity or resistance (2, 3). Thus, the European Medicines Agency (EMA) conditionally approved delamanid for the treatment of multidrug-resistant (MDR) TB (1, 3, 4). Of note, 6 years after its market launch, robust and widely accepted breakpoints that define susceptibility and resistance to delamanid still do not exist (5). The few available studies suggest a critical MIC between 0.125 mg/liter and 0.2 mg/liter, and an epidemiological cutoff value (ECOFF) of 0.04 mg/liter (6–9). This ECOFF is in line with the WHO technical report (10). Delamanid is a drug of the bicyclic nitroimidazole class with potent anti-TB activity (1, 11). It is a prodrug that is activated by the deazaflavin (F_{420})-dependent nitroreduc tase (*ddn*) through hydride transfer, forming unstable intermediates, which in turn lead to the formation of reactive nitrogen species (nitric oxide, nitrous acid) (12, 13). Activated delamanid thus has a dual bactericidal mode of action: the primary decomposition product prevents mycolic acid synthesis, while the reactive nitrogen species cause respiratory poisoning (12–15). Loss-of-function mutations in ddn or one of the genes encoding the five coenzymes (*fgd1, fbiA, fbiB, fbiC, and fbiD*) have been proposed as a mechanism of resistance to delamanid (12, 13, 16, 17). In vitro, the frequencies of delamanid resistance-conferring mutations in the *Mycobacterium tuberculosis* laboratory strain H37Rv and in *Mycobacterium bovis* range from 2.51 X 10-5 to 6.44 X 10-6 (13). Previous studies have found several resistance-conferring mutations, including Leu107Pro (*ddn*), 51–101del (*ddn*), Trp88STOP (*ddn*), Gly81Asp (*ddn*), Gly81Ser (*ddn*), Gly53Asp (*ddn*), c.146_147insC (*fgd1*), Gln88Glu (*fgd1*), Lys250STOP (*fbiA*), Arg175His (*fbiA*), and Val318Ile (*fibC*) (6–8, 18–22).

This multicenter study has been described in detail elsewhere and is part of the work of the International epidemiology Databases to Evaluate AIDS (IeDEA) (23). We identified putative delamanid resistance-conferring mutations in *M. tuberculosis* strains from TB patients living with HIV (PLWH) and delamanid-naïve, HIV-negative TB patients by whole-genome sequencing (WGS) and MIC determination. We collected information on the demographic and clinical characteristics of patients who were recruited between 2013 and 2016 in Peru, Thailand, Côte d'Ivoire, the Democratic Republic of the Congo (DRC), Kenya, and South Africa (24, 25). The Cantonal Ethics Committee in Bern, Switzerland, and local institutional review boards approved the study. Written informed consent was obtained at all locations, except in South Africa, where consent was not required for archived samples.

The sequencing pipeline has been described previously (25). In brief, *M. tuberculosis* DNA was extracted and sequenced using the Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA). For the analysis, we used the well-established pipeline TBprofiler (https://github.com/jodyphelan/TBProfiler) (26, 27). It aligns short reads to the *M. tuberculosis* reference strain H37Rv (GenBank accession no. NC_000962.3) with bowtie2 (v2.3.5), BWA (v0.7.17), or minimap2 (v2.16) and then calls variants with SAMtools (v1.9) (28–31). To identify putative

delamanid resistance-conferring mutations, we analyzed F_{420} genes (*ddn, fgd1, fbiA, fbiB, fbiC, and fbiD*) with variant frequencies of >75%. A subset of *M. tuberculosis* strains with at least one mutation in the F_{420} genes was recultured in liquid medium and subjected to delamanid MIC determination (see Fig. S1 in the supplemental material). We assumed that 0.04 mg/liter indicates a critical MIC (9).

We included 129 *M. tuberculosis* isolates, among them 51 isolates (39.5%) from Peru, 13 (10.1%) from Thailand, 49 (38%) from Côte d'Ivoire, 14 (10.9%) from the DRC, and 1 (0.8%) each from Kenya and South Africa. We identified 70 (54.3%) isolates with polymorphisms in at least one of the six F_{420} genes compared to the reference genome (Table S1). All patients infected with either of these strains were naïve to delamanid. We selected strains fulfilling the following criteria: (i) mutations in a part of the gene encoding regions of catalytic or structural importance predicted by ARIBA and then the PhyResSE pipeline (32, 33), (ii) availability of a culture of the strain, and (iii) bacterial growth amenable to microdilution (25). MICs were determined for 10 isolates with mutations in the F420 genes. Four isolates showed MICs of >0.015 mg/ liter: specifically, MICs of 0.5 (patient 1), 0.03 (patients 6 and 10), and >8 (patient 9) mg/liter (Table 1; Fig. S1). The isolate from patient 1 had a polymorphism in *fgd1* (Lys270Met) and was susceptible to the six drugs tested (isoniazid, rifampin, ethambutol, pyrazinamide, moxifloxacin, and amikacin). The patient was cured. The isolate from patient 9 had two alterations: a deletion in *ddn* (Tyr29del) and a nucleotide change in *fgd1* (T960C). The strain showed an elevated delamanid MIC and was phenotypically susceptible to six other drugs tested. The patient died. The MIC for the isolates of patients 10 and 6 was above 0.015 but below 0.04 mg/liter (Table 1). This suggests low-level resistance to delamanid (22), which could be due to the combination of various mutations: Ala416Val (*fbiC*), Trp678Gly (*fbiC*), Arg64Ser (*fgd1*), and T960C (*fgd1*).

Table 1. Observed polymorphisms in F_{420} genes and MIC values of delamanid[1]

Patient no. or reference	Lineage	Country	HIV status	Age (yr) at TB diagnosis	Gender	Mutation(s) in the F_{420} genes	Treatment outcome	MIC(mg/liter) in the microdilution
Reference	H37Rv (ATCC 27294)					Control (wt)		<0.015
1	**L4.1.2.1**	**Côte d'Ivoire**	**Negative**	**29**	**Female**	***fgd1* Lys270Met**	**Cured**	**0.5**
2	L4.6.2.2	Côte d'Ivoire	Negative	51	Male	*ddn* C168T	Died	<0.015
3	L2.2.1	Kenya	Positive	40	Male	*fgd1* T960C	Died	<0.015
4	L2.2.1	Peru	Positive	28	Male	*fgd1* T960C	Unknown	<0.015
5	L4.3.2	Peru	Negative	21	Male	*fbiC* C1161T	Cured	<0.015
6	**L4.1.2.1**	**Peru**	**Positive**	**45**	**Male**	***fgd1* Lys270Met**	**Unknown**	**0.03**
7	L4.1.2.1	Peru	Positive	36	Male	*fbiC* G-11A, *fgd1* Lys270Met	Unknown	<0.015
8	L4.1.2	South Africa	Negative	57	Female	*fbiA* Ile208Val	Cured	<0.015
9	**L2.2.1**	**Thailand**	**Unknown**	**76**	**Male**	***fgd1* T960C, *ddn* 85-87del (Tyr29del)**	**Died**	**>8**
10	**L1.1.1**	**Thailand**	**Negative**	**42**	**Male**	***fbiC* Ala416Val Trp678Gly, *fgd1* Arg64Ser T960C**	**Unknown**	**0.03**

[1]All patients were treated with 2 months of daily isoniazid, rifampin, pyrazinamide, and ethambutol, followed by 4 months of daily rifampin and isoniazid. Data forisolates for which the MIC was >0.015 are shown in boldface. wt, wild type; L, lineage.

In summary, in the subset of 10 isolates with polymorphisms in the six targeted genes, six had no elevated MIC in the microdilution, while four isolates had elevated MICs (Table 1). In line with previous studies, we found that Lys270Met in *fgd1* is a natural polymorphism characteristic of *M. tuberculosis* lineage 4.1.2.1, which may (patients 1 and 6) or may not (patient 7) lead to an increased delamanid MIC (19, 34, 35). All 16 strains of lineage 4.1.2.1 showed this lineage-specific marker (Table S1). Furthermore, T960C (*fgd1*) is a synonymous substitution and was found in three other patient isolates which, as expected, did not have a critical MIC. The increase in the delamanid MIC for the isolate of patient 9 was due to the deletion in ddn (7). Our results thus suggest that Tyr29del is a

natural polymorphism leading to an increased delamanid MIC. Our study was too small to estimate the prevalence of strains that are naturally resistant to delamanid. In 2020, Lee et al. screened 14,876 *M. tuberculosis* strains and found 2 strains with Tyr29del, for a prevalence of 0.013% (36). However, in their study, only the *ddn* gene was screened, and the prevalence of natural resistance could, therefore, be higher.

In conclusion, we confirm that mutations in F_{420} genes can confer an elevated delamanid MIC (13, 19). Whether our findings also apply to the related drug, pretomanid should be investigated in future studies. The occurrence of clinical *M. tuberculosis* isolates from previously untreated patients for which delamanid MICs are naturally elevated calls for careful drug susceptibility testing (DST) prior to delamanid treatment (5, 36). However, access to DST is limited in high-burden countries. This dilemma highlights the conflict between making new drugs available in high-burden countries and avoiding the spread of drug-resistant strains.

Data availability

WGS data from patients' *M. tuberculosis* strains shown in Table 1 have been submitted to the NCBI (BioProject accession no. PRJNA300846) (Table S1).

Supplemental material

Supplemental material is available online only (https://journals.asm.org/doi/10.1128/AAC.00513-20). SUPPLEMENTAL FILE 1, PDF file, 0.2 MB. SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

Acknowledgments

We thank those at all sites who participated, the patients whose data were used in this study, and Marie Ballif for contributing to the data collection and for critical reading of the manuscript. Calculations were performed on UBELIX (http://www.id.unibe.ch/ hpc), the HPC cluster at the University of Bern. This research was supported by the Swiss National Foundation (project grants 153442, 310030_166687, 310030_188888, IZRJZ3_164171, IZLSZ3_170834, and CRSII5_177163). The International epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Cancer Institute, National Institute of Mental Health, National Institute on Drug Abuse, National Heart, Lung, and Blood Institute, National Institute on Alcohol Abuse and Alcoholism, National Institute of Diabetes and Digestive and Kidney Diseases, Fogarty International Center, and National Library of Medicine (Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919). R.J.W. receives support from the Francis Crick Institute, which is funded by UKRI, CRUK, and the Wellcome Trust (grants FC0010218, 104803, and 203135). This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

We have no potential conflicts of interest to disclose.

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Discussion and Conclusion

11. Discussion

This thesis studied different epidemiological aspects of TB in PLHIV and HIV-negative people in low- and middle-income countries with a focus on (i) estimating the risk of *Mtb* transmission in health care clinics, (ii) the management of TB from TB diagnosis including DST to treatment and treatment outcomes, and (iii) the evolution of drug-resistant TB and clinical consequences. For each sub-aim, I will first summarize the main findings of the first author studies (chapter 3 to 8) and the co-author studies (chapter 9 and 10). Then I will discuss and interpret the findings and their implications. Finally, I will provide an outlook for future research in this field and will give an overall conclusion of this thesis. The discussion will be broader compared to the discussion of each study.

11.1. Sub-aim 1: The risk of *Mtb* transmission in a health care clinic

Summary

Paper 1 (chapter 3) is the study protocol describing a novel approach using patient and environmental data to study *Mtb* transmission at primary health care clinics in high TB burden countries. Paper 2 (chapter 4) found an increased risk of *Mtb* transmission with the presence of young adults and higher room humidity. We estimated the risk of *Mtb* transmission during a clinic visit of one hour to be 3% - 6%, and increasing to 9% - 29% for patients with regular monthly visits.

Interpretation of the findings and their implications

In paper 2 we found more copies of *Mtb* DNA in the air when young adults visited the clinic than when clinic visitors were older. When adolescents and young adults' transit from child to adult healthcare, they face specific age-related challenges accessing appropriate healthcare (215, 216). These challenges can result in delayed HIV or TB diagnosis and treatments. A global study using the WHO TB notification database showed that 17% of all newly diagnosed people with TB were among people aged 10-24 years (217). Similar a study from Cape Town, South Africa showed that TB notification was highest among young adults (218). In addition, behaviour and social contact patterns differ by age, and could play a role in the risk of *Mtb* transmission (219, 220). A study showed that young index cases (<40 years) had more close contacts in all age groups whereas older index cases had fewer contacts and mainly within their own age group (220).

We also observed that increasing relative humidity was associated with an increased number of copies of *Mtb* DNA in the air. It has been shown elsewhere that relative humidity plays an important role in the transmission of respiratory diseases (73). Consistent with our findings, a more recent study showed that *Mtb* genome copies were likely to be present in the air when the relative humidity was above 65% (99).

Our approach could be expanded to other settings such as schools, prisons, or churches where airborne transmission is possible and to other respiratory diseases such as COVID-19. In the COVID-19 pandemic, governments and primary care clinics introduced infection control measures such as increased hand hygiene, physical distancing, and wearing face masks in public spaces. From the history of TB, we know that improving living conditions, improving hygiene, and separating and sending people with active TB to Sanatoria reduced TB mortality, even though antibiotics were unavailable (6, 10-14). In addition, a systematic review showed that physical distancing, face masks, and eye protection for health care workers and the general population reduces their risk of SARS-CoV-2 infection (221). These

infectious control measures would likely reduce the risk of *Mtb* transmission and other airborne diseases. For example, during the COVID-19 pandemic in 2020, almost no influenza cases were diagnosed in the northern or southern hemisphere (222, 223).

Outlook and further research

Further research in this area looking at *Mtb* transmission in other clinics as well as in other high-risk setting would be highly valuable. We have received a grant from the Swiss National Science Foundation to continue studying *Mtb* transmission. We would like to extend this approach to other clinics, settings and countries and are planning to measure the impact of interventions on *Mtb* transmission. Such interventions include basic infectious control measures such as (i) provide any coughing attendee or all attendees with a face mask at the registration area and (ii) ventilate the waiting room every hour by opening all the windows and doors for 5-15 minutes depending on the room size. We will assess the impact of the interventions on the risk of TB transmission in a before-after study (110, 224). Importantly, this will allow us to evaluate interventions to mitigate *Mtb* transmission in high-risk locations such as primary care clinics. Further, we will expand this approach to other respiratory diseases. As a first step, we will attempt collecting SARS-CoV-2 RNA in the air ascertained from the dried filter unites.

11.2. Sub-aim 2: Management of TB at ART clinics

Summary

In paper 3 (chapter 5) we showed the challenges in obtaining bacteriological confirmation in PLHIV who have pulmonary TB (52%) or extra-pulmonary TB (42%). We found no association between mortality in PLHIV and the type of TB they developed (extra-pulmonary or pulmonary). However, bacteriological confirmation was associated with reduced mortality in PLHIV who have pulmonary TB or extra-pulmonary TB compared to PLHIV with a negative diagnostic result. Paper 4 (chapter 6) showed that 14 out of 29 (48%) ART clinics had full MDR-TB services integration. 76% of ART clinics with on-site molecular DST could identify drug resistance to first-line drugs but rarely to second-line drugs. ART clinics with full integration of MDR-TB services were more likely to prescribe individualized MDR-TB treatment compared to off-site services.

Interpretation of the findings and their implications

Paper 3, paper 4, paper 5, and paper 6 identified gaps in the management of TB in low-and middle-income countries. Paper 3 underlined the challenges of initial TB diagnosis in routine care in PLHIV. Bacteriological confirmation was obtained in 52% of the PLHIV who developed pulmonary TB. The most frequently used diagnostic test was sputum smear microscopy. The risk of a false negative sputum smear is increased in PLHIV who have pulmonary TB due to the reduced lung cavitation and a lower bacterial load in the sputum compared to HIV-negative people who have TB (57, 95, 97, 153, 225). This finding is consistent with paper 5, which showed that PLHIV who have pulmonary TB are more likely to have a negative sputum smear microscopy result compared to HIV-negative people who have TB. We also showed that bacteriological confirmation in PLHIV who developed extra-pulmonary TB is even more challenging than in PLHIV who developed pulmonary TB. Extra-pulmonary TB's pauci-bacillary nature in body fluid, tissue, or cerebrospinal fluid requires an invasive specimen collection by biopsy or fine needle aspiration for bacteriological confirmation (226, 227). For both types of TB (pulmonary

TB and extra-pulmonary TB) mycobacterial culture is the recommended diagnostic test, however rapid molecular technologies are an alternative. End of 2010, WHO recommended the routine use of the Xpert MTB/RIF as the initial diagnostic test in people presumptive of MDR-TB or HIV-associated TB, as the sensitivity of *Mtb* detection is higher compared to smear microscopy. Further, the Xpert MTB/RIF and later next generation Xpert MBT/RIF Ultra reliably diagnose extra-pulmonary TB (228, 229). Despite the enormous scale up in many low-and middle-income countries, the Xpert MTB/RIF was rarely used in our study, even when available. Reasons for this could be stock out in cartilage, power outages causing the machine not to run, staff being unsure of how to perform the test or not knowing the benefits of the Xpert MTB/RIF over the sputum smear. Therefore, when implementing new diagnostic tests, it is important that the equipment is available and that the staff are trained. Further, we need diagnostic tests which can be used decentralised and do not require biosafety 2 or 3 laboratories.

Paper 4, paper 5, and paper 6 identified gaps in the access to point of care DST, especially for second-line anti-TB drugs. In paper 4 we showed that some form of DST was available on-site at almost 80% of ART clinics. All clinics with on-site DST used rapid molecular DST to identify resistance to rifampicin or isoniazid, and DST for second-line drugs was only available in 31% of the clinics. Similarly, paper 6 showed that routinely obtained DST profiles at ART or TB clinics in low-and middle-income countries identified resistance to isoniazid and rifampicin with a relatively high agreement to WGS, while DST to second-line drugs or other first-line drugs were rarely obtained in routine care. To prevent further resistance and to ensure an appropriate treatment, a rapid and comprehensive DST is needed. A very promising technology is WGS, as WGS identifies drug-resistant-conferring mutations simultaneously to first- and second-line anti-TB drugs (230). However, the implementation of the sequencing technology is challenging in low-and middle-income countries because of the high cost of equipment costs, the technical trainings needed for staff, the need for expert interpretation of the sequencing data, and finally the lack of a simple and affordable end sequencing technology to obtain sequencing data directly from sputum samples. One possibility in the near future could be the use of targeted sequencing on unprocessed sputum samples (178). There is also a need for validated protocols and standardised workflows for laboratories.

The last gap we identified is in the treatment of drug-resistant TB (paper 4, paper 5, and paper 6). Paper 4, provided more evidence about the current MDR-TB treatment practise from 19 high TB burden countries. The treatment of drug-resistant TB has changed over the years. We found that MDR-TB treatment differed depending on the level of MDR-TB services integration at the ART clinic. For example, individualised MDR-TB treatment was reported to be offered more frequently at ART clinics with full MDR-TB service integration compared to clinics with partially integration or all off-site (paper 4). A systematic review and meta-analysis showed that people with MDR-TB who received an individualised regimen had higher treatment success rates than people who received an standardised regimen (64% vs. 52% $p<0.001$) (231). In addition, studies from high-resource settings, where individualised treatment is the standard of care, showed treatment success rates greater than 80% (232-234). Individualised treatment reduces the risk of drug toxicities, drug-drug interaction and prescribing inappropriate drugs because of undiagnosed resistances, particularly in people with TB and with co-morbidities such as HIV infection or diabetes. In 2018, there was a call for individualised treatment guided by detailed and comprehensive DST for first- and second-line anti-TB drugs to improve treatment outcomes in low-and middle-income countries. At the time, the treatment for drug-resistant TB was mainly standardised or empirical combination regimens in low-and middle-income countries (235). As mentioned in the previous paragraph we showed a lack of rapid DST to second-line drugs (paper 4, paper 5, and paper 6). Discordant DST results, due to missed resistances, led to an inappropriate treatment, which contributed to an excess mortality associated with drug-resistant TB (papers 5 and paper 6). We support the call for individualised treatment to increase

treatment success if comprehensive DST to first and second-line anti-TB drugs is performed. Finally, to achieve the END TB targets we need better access to point-of-care diagnostic tests for initial diagnosis, DST for first- and second-line drugs, as well as access to second-line anti-TB drugs in low-and middle-income countries.

Outlook and further research

A follow-up study, ideally in the same clinics, would be valuable as in the last 10 years new diagnostic technologies have emerged. For example, the Xpert is now recommended by WHO as the initial diagnostic test and it would be interesting to see if we can observe a shift from sputum smear microscopy to the Xpert MTB/RIF compared to the survey from 2012-2014. In addition, we would like to expand the previous survey and follow the study participants up to two years after TB treatment to study post-TB complications. Death is the most severe complication of TB, and can occur even after successful treatment due to lung destructions. Other non-fatal negative health consequences following TB treatment can occur, such as bronchiectasis, emphysema, obstructive and restrictive lung impairments, which are likely to be more severe in people with MDR-TB than in people with drug-susceptible TB (236-238). We plan to collect data on lung function using spirometry (239, 240), functional capacity using the 6-minutes walking test (241) and the 1-minute sit-to-stand (242). With these additional data on post-TB complications, we will be able to estimate the additional burden on the health care system caused by TB.

The research presented here focuses on TB in adults but there is also value in expanding the study to include children and adolescents. In children and adolescents, especially those living with HIV, TB is a major cause of morbidity and mortality (18). Studies have identified critical gaps in diagnosis, and treatment of TB among children and adolescents (243, 244). Childhood TB is severely under-diagnosed, as compared to older age groups, due to several factors including the limited recognition of TB manifestations in children, the inability of young children to produce sputum for evaluation, and the nature history of TB in children, which decreases the sensitivity of TB diagnostics (244, 245). Given low ascertainment of cases, estimates of childhood TB rely on mathematical modelling (246, 247). It is estimated that 1.2 million children (<15 years of age) developed TB in 2019, and that 227,000 died (18). Longitudinal observational studies looking at the TB care cascade - from onset of symptoms to post-TB complications – in children and adolescents can contribute to fill the current knowledge gap and are useful to understand where interventions are needed to reduce related child and adolescent morbidity and mortality.

11.3. Sub-aim 3: Evolution of drug-resistant TB and clinical consequences

Summary

In paper 5 and paper 6 (chapter 7 and chapter 8) we showed that (i) the degree of drug resistance, (ii) discordant DST results potentially leading to under-treatment, and (iii) discordant DST results, which resulted in under-treatment, all led to increased mortality, especially among people with drug-resistant TB. Further, we demonstrate that DST for second-line drugs was rarely available locally. Paper 7 (chapter 9) showed that mutations with low-fitness in *rpoB* variants are more likely in PLHIV who have rifampicin-resistant TB than in HIV-negative people who have rifampicin-resistant TB. Finally, paper 8 (chapter 10) demonstrated that drug resistance to delamanid occurred in people with TB who were drug naïve to delamanid.

Interpretation of the findings and their implications

In paper 5, paper 6, paper 7, and paper 8 we looked at the epidemiology of drug resistance in *Mtb* in the context of HIV co-infection. In paper 5 and paper 6, we found that mortality increased with the degree of drug resistance. Our results are in line with a systematic review of treatment outcomes in adults with MDR-TB from seven studies from sub-Saharan Africa (248). These studies reported mortality during TB treatment ranging from 12.4% in people with MDR-TB to 45.8% in people with XDR-TB (249, 250). Another systematic review and meta-analysis, which included 74 studies, showed no treatment success in 39% of people with MDR-TB (67 studies) and 74% in people with XDR-TB (7 studies)(231).

The treatment of drug-resistant TB is more challenging in people co-infected with HIV for several reasons. These are fore example poor absorption of drugs, the risk of immune reconstitution inflammatory syndrome, and interactions between antiretroviral and anti-TB drugs (203, 251, 252). In contrast to an individual patient data meta-analysis, which showed that mortality was increased in PLHIV who have MDR-TB compared to HIV-negative people who have MDR-TB (253), we found no association between mortality and HIV-status (paper 5). One reason for this discrepant result could be the relatively high median CD4 cell count of the PLHIV in our study (192 cells per µL). Similar to our study, a study from Lesotho found little evidence for a difference in mortality between PLHIV (median CD4 cell count 185 cells per µL) and HIV-negative people (254). For patients with XDR-TB, treatment outcomes have been poor in previous studies, irrespective of HIV status (250).

The association between MDR-TB and HIV infection has been widely discussed. There are several factors suggesting that HIV and MDR-TB are associated. First, PLHIV are more likely to develop active TB disease due to the immunodeficiency (42). Second, HIV-coinfection is associated with malabsorption of anti-TB drugs, which can lead to treatment failure, relapse or acquired drug resistance (255). The first systematic review found no association between MDR-TB and HIV or acquired MDR-TB and HIV (256). However, the systematic review and meta-analysis by Mesfin et al., including 24 observational studies published between 1994 and 2011, found an association between MDR-TB and HIV (257). The most recent systematic review and meta-analysis, including 54 studies published between 2010 and 2020, confirmed the finding from Mesfin et al (257, 258). Further, all three systematic reviews found an association between primary MDR-TB and HIV (256-258). From the molecular point of view an important question is, if HIV infection directly contributes to the accumulation of drug-resistant mutations. It is known that many drug-resistant mutations cause a fitness cost for the bacterium (256, 257). Therefore, drug-resistant strains might be more successful in PLHIV with immunodeficiency due to a reduced immune pressure on the bacillus (45-47). In paper 7 we found differences in the distribution of *rpoB* variants by HIV-status. Low-fitness in rifampicin-resistant *Mtb* strains were associated with disproportionally more drug-resistant-conferring mutations in *rpoB* in PLHIV compared to HIV-negative people, suggesting that low-fitness *rpoB* variants can persist in reduced host immunity.

Two new drugs bedaquiline and delamanid became available in 2013 and 2014 respectively, which are recommended by WHO in the treatment of MDR-TB or XDR-TB (259, 260). In study 4 we showed that in 29 ART clinics from 19 high TB burden countries bedaquiline or delamanid were rarely available. This is in line with the results from paper 5 and paper 6, which showed that people with TB were rarely treated with bedaquiline or delamanid. We showed that one strain from a delamanid-naïve person carried the natural polymorphism Tyr29del (*ddn*) which was associated with a critical delamanid MIC (paper 8).

In paper 5, paper 6, paper 7, and paper 8 we showed several challenges in the control of drug-resistant TB. In general, antimicrobial resistance is a challenge for the future, especially for TB, as there are very few new drugs and research is slow and under-invested. This is for example shown in paper 8, where a delamanid-naïve person had an elevated MIC for delamanid. We need an ongoing monitoring of drug-resistant mutations, especially in low-and middle-income countries to react early enough, especially when these new drugs become ineffective. We need one trustworthy and widely accepted drug resistance TB database similar to the Stanford HIV drug resistance database (261). Such a database would help to detect new drug-resistant mutations and help identify drug-resistant-conferring mutations associated with HIV infection.

Outlook and further research

Further research in the field of drug-resistant TB would be highly recommended. As presented in paper 4, paper 5, and paper 6 the access to comprehensive DST is limited. Without a rapid and comprehensive diagnosis and DST, drug-resistant TB will remain a challenge in the control of TB. We are planning an observational study in PLHIV and HIV-negative people with drug-resistant TB to validate portable and real-time Nanopore technology. With the Nanopore technology, targeted sequencing of *Mtb* can be performed directly from sputum and DST profiles can be obtained (262). Minimal laboratory work is needed. First, we will extract *Mtb* DNA from sputum using validated kits. Then we will enrich *Mtb* DNA and amplify selected regions of the *Mtb* genome covering drug-resistance conferring mutations by multiplexed PCR (263). Amplicons will then be sequenced on MinION, with a minimum of 10x base depth coverage (264). To validate the targeted sequencing by MinION, we will perform WGS from sputum using Illumina (Hiseq) technology on a random sample of isolates. With this targeted sequencing method directly from sputum using Nanopore technology, we will be able to provide results within seven to ten days and improve the treatment of drug-resistant TB. Finally, we hope to implement this Nanopore technology in routine care.

Currently several drug resistance TB database like ReSeqTB, CRyPTIC or TBprofiler exist. Beside the *Mtb* genome, clinical data, such as age, gender, HIV status or treatment outcome are rarely available. To better understand the evolution of drug-resistant TB in PLHIV and the clinical consequences, clinical data including HIV status should be included in the TB genome databases. Future studies should not only sequence *Mtb* strains but also collect clinical data.

12. Conclusion

In conclusion, my thesis identified several gaps in the control of TB, ranging from *Mtb* transmission, management of TB, to drug resistance and death. Our approach using multiple measurements to estimate *Mtb* transmission should be used to evaluate *Mtb* transmission interventions and thereby help to reduce transmission. We need to strengthen the access to and use of point-of-care diagnostic tests to diagnose TB disease and DST for first- and second-line anti-TB drugs as well as the access to second-line anti-TB drugs in low-and middle-income countries. In addition, we need to improve both the surveillance of drug-resistant TB and the treatment options to ensure completing of the treatment.

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Appendix

A. Acknowledgments

I am most grateful to everyone who has made this PhD journey such an enjoyable, fulling, formative, and memorable experience. Throughout my research work, I never felt unsupported. I was always surrounded by people who cared; people who were either willing to give me a hand when I needed help, or lend me an ear when I needed advice.

First, I would like to thank my PhD supervisors, Lukas Fenner and Matthias Egger. Six years ago, you gave me the opportunity to do an internship at the ISPM on the history of TB in Bern, although I had no experience in research. Throughout the years, you have supported and encouraged me to obtain the MSc in Epidemiology and now the PhD. You have introduced me to epidemiological thinking and taught me to write and communicate scientific results in a clear way. I am very grateful for the opportunities you gave me to travel to international conferences to present scientific results, to visit ART clinics, to give press interviews, and to be involved in many different projects apart of my thesis. Otto Schoch, thank you for being the external supervisor and Gertraud Schüpbach for being my mentor at the Graduate School for Cellular and Biomedical Sciences (GCB). I would like to thank Gunar Günther for being my external examiner for my PhD thesis.

Special thanks to Marie Ballif for her support, for all the discussions we had, and for your friendship. I have learnt so much from you, from developing questionnaires, writing concepts, and writing manuscripts. I would also like to thank Hans Rieder for being a consultant over all these years.

I would also like to thank all other people who contributed to the work presented in my thesis: Martina Reichmuth, Julien Riou, Carl Morrow, Robin Wood, Keren Middelkoop, Simon Bertschinger, Chloé Loiseau, and Samyra Cox.

I would like to thank the whole IeDEA team in Cape Town and Bern for a supportive work environment and especially Nanina Anderegg, Catrina Mugglin, Eliane Rohner, Kathayoun Taghavi, Martina Reichmuth, Veronika Whitesell, Andreas Haas, and Elisabeth Zanienski for the friendship and the encouragement. Nanina Anderegg, Catrina Mugglin, and Eliane Rohner it was always a pleasure to travel with you to conferences. We laughed a lot, went to lovely places for dinner and did nice things together. Thank you Martina Reichmuth for all the discussions we had, when we went for a coffee, a walk, a run or swimming in the Aare. Thank you Lilian Smith and Per von Groote for the administrative work. I would also like to thank the TB & lung health-working group for their critical feedback on manuscripts and the discussion about current or new projects.

There are those outside of my work environment whom I also must acknowledge. A special thank to my parents, my family, and my friends for all your support and the great times we spent together. Without you I would not have made it this far!

Finally and most importantly, I would like to thank all the study participants and the clinical staff at the clinics for supporting the research.

B. Curriculum vitae and list of publications

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Education

12/2018 – current	**PhD in Biomedical Science**, *Institute of Social and Preventive Medicine (ISPM), University of Bern* PhD Thesis: Tuberculosis among people living with and without HIV in lower-income countries: Transmission, Resistance, Mortality
09/2016 – 02/2018	**MSc in Epidemiology**, *Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel* Master thesis: Drug susceptibility testing, HIV-coinfection and treatment outcomes in patients diagnosed with tuberculosis in lower income countries
09/2013 – 07/2016	**MSc in Physiotherapy**, *Bern University of Applied Sciences BFH* Master thesis: Disability in HIV-positive South African children and young adults with stroke and improvements with physiotherapy
09/2009 – 07/2013	**BSc in Physiotherapy**, *Bern University of Applied Sciences BFH* Bachelor Thesis: Taping bei Schulterimpingement. Eine Pilotstudie an Volleyballspielerinnen

Work experience

08/2015 – current **60-100% as research assistant & PhD student,** *Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern*

- Study coordination
- Data collection, cleaning, management, and analysis
- Writing grants, abstracts for conferences and manuscripts for publication
- Present results at international conferences

06/2018 –04/2020 **40-50% as physiotherapist**, *Physio4life, Bern*

- Outpatient, mainly shoulder patients

01/2016 –01/2017 **20% as physiotherapist**, *PhysioSternen, Bern*

- Outpatient

02/2014 –04/2016 **50% as physiotherapist**, *Physio4life, Bern*

- Outpatient, mainly shoulder patients

08/2013 –01/2014 **60% as physiotherapist**, *Therapie und Trainingszentrum, Zollikofen*

- Outpatient, nursing homes

Presentations

2021	**GCB graduate school symposium**, virtually, Bern, Switzerland, oral presentation: Management of multidrug-resistant tuberculosis at HIV clinics in low-and middle-income countries
2020	**51th Union World Conference on Lung Health (UNION)**, Seville, Spain (presented by my colleague), oral presentation: Mutation in Mycobacterium tuberculosis Confer Resistance to Delamanid in Drug-naïve Patients
2020	**51th Union World Conference on Lung Health (UNION)**, Seville, Spain (presented by my colleague), oral presentation: Undiagnosed drug resistance in Mycobacterium tuberculosis is associated with higher mortality in countries with high tuberculosis burdens
2020	**Conference on Retroviruses and Opportunistic Infections (CROI)**, Boston, USA, poster presentation: Estimating TB transmission in primary care clinics in TB/HIV high burden settings
2020	**GCB graduate school symposium**, Bern, Switzerland, poster presentation: Estimating TB transmission in primary care clinics in TB/HIV high burden settings
2019	**Swiss TB Symposium**, **Swiss TB Award,** Magglingen, oral presentation: Drug susceptibility testing and mortality in patients treated for tuberculosis in high-burden countries: a multi-centre cohort study
2018	**AIDS conference**, Amsterdam, Nederland, oral presentation: Drug susceptibility testing, HIV-coinfection and outcomes in patients treated for tuberculosis in low-and middle-income setting
2018	**22th International Workshop on HIV and Hepatitis Observational Databases (IWHOD)**, Fuengirola, Spain, poster presentation: Accuracy and clinical significance of TB drug resistance testing at TB clinics attached to ART programs
2017	**21th International Workshop on HIV and Hepatitis Observational Databases (IWHOD)**, Lisbon, Portugal, poster presentation: Clinical and bacteriological diagnosis of pediatric extrapulmonary and pulmonary TB in HIV-positive children in lower income countries
2017	**48th Union World Conference on Lung Health (UNION)**, Guadalajara, Mexico, poster presentation: Treatment outcome and mortality in pulmonary TB in lower income countries: impact of drug resistance and HIV co-infection
2016	**47th Union World Conference on Lung Health (UNION)**, Liverpool, United Kingdom, oral presentation: Influenza pandemics and TB mortality in the 19th and 20th century in Switzerland
2015	**46th Union World Conference on Lung Health (UNION)**, Cape Town, South Africa, oral presentation: A historic view on understanding causes of the tuberculosis epidemic: mortality and living conditions in Bern, Switzerland, between 1890 and 1950

Languages skills

German:	first native language
Danish:	second native language
English:	fluent
French:	good knowledge

Computer skills

Applications:	MS Office: very good
	Excel: very good
	Power point: very good
Statistic program:	Stata: good
	R: basic knowledge
ECD software:	REDCap: very good

Personal awards

2019	Swiss Tuberculosis Award (10,000 CHF)
2017	Swiss Tuberculosis Award's Special Prize (1,000 CHF)

Publications

1. **Zürcher K**, Ballif M, Zwahlen M, Rieder HL, Egger M, Fenner L. Tuberculosis Mortality and Living Conditions in Bern, Switzerland, 1856-1950. *PLoS One*. 2016 Feb 16;11(2):e0149195. doi: 10.1371/journal.pone.0149195. eCollection 2016. PMID: 26881850

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C. Declaration of originality

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I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.

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