



Complementing TB control strategies with end-user innovations in Sub-Saharan Africa

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Dr Emmanuel André
Promoter: Prof. Michel Delmée

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Abstract

Tuberculosis (TB) is responsible for nearly two million deaths per year around the world, and mainly affects the poorest communities. How can this happen when we know that TB disease can be prevented and treated since over 50 years?

When “top-down” guidelines fail to generate impact, end-user innovations are required for adapting these recommendations to specific contexts.

The innovations proposed in this work tend to cover a wide range of operational and scientific challenges related to TB control. These innovations include (1) tackling under-detection with active screening interventions led by TB-affected populations, (2) the development and implementation of connected laboratory networks, and (3) the development of innovative surveillance systems and technologies that allowed the detection of previously unrecognized outbreaks of multi-drug resistant TB in South-Africa.

This work took place in the Democratic Republic of Congo, in Belgium, in Rwanda and in South-Africa.

Résumé

La tuberculose (TB) est responsable de la mort de près de deux millions de personnes chaque année dans le monde. Elle touche principalement les communautés les plus pauvres. Comment en est-on arrivés là, alors que la TB peut être traitée depuis plus de 50 ans ?

Quand les recommandations ne suffisent pas à générer l'impact attendu, des innovations doivent émerger du terrain pour adapter ces recommandations aux contextes spécifiques.

Les innovations proposées dans ce travail couvrent un large éventail de défis opérationnels et scientifiques liés au contrôle de la TB. Ces innovations comprennent (1) des nouvelles approches pour lutter contre le sous-dépistage grâce à l'implication d'anciens malades, (2) le développement et l'implémentation de réseaux interconnectés de laboratoires et (3) le développement de systèmes et technologies ayant permis de mettre en évidence des foyers de TB multi-résistante qui échappaient aux stratégies actuellement recommandées.

Ce travail a été réalisé en République Démocratique du Congo, en Belgique, au Rwanda et en Afrique du Sud.

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List of abbreviations

ACF	Active case-finding	NTM	Non-tuberculous mycobacteria
ALTB	Ambassadeurs de lutte contre la tuberculose	NTP	National Tuberculosis Program
Ct	Cycle threshold	PCR	Polymerase chain reaction
DOT	Directly observed treatment	PedTB	Paediatric tuberculosis
DRC	Democratic Republic of Congo	Pre-XDR-TB	Pre-extensively drug resistant TB
DST	Drug susceptibility testing	PZA	Pyrazinamide
EMB	Ethambutol	RIF	Rifampicin
EPTB	Extra-pulmonary tuberculosis	RR-TB	Rifampicin-resistant tuberculosis
GDP	Gross domestic product	SNP	Single nucleotide polymorphism
IGRA	Interferon-gamma release assay	SS+PTB	Smear positive pulmonary tuberculosis
INH	Isoniazid	SS-PTB	Smear negative pulmonary tuberculosis
LED-FM	LED fluorescence microscopy	TB	Tuberculosis
LPA	Line probe assay	TST	Tuberculin skin test
LTBI	Latent tuberculosis infection	WGS	Whole genome sequencing
MDR-TB	Multidrug-resistant tuberculosis	WHO	World Health Organization
MTB	<i>Mycobacterium tuberculosis</i>	XRD-TB	Extensively drug-resistant TB
MTBC	<i>Mycobacterium tuberculosis</i> complex	ZN	Ziehl-Neelsen
NGS	Next generation sequencing		

Introduction

0.1. Tuberculosis

Robert Koch discovered *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB) in 1882. The clinical presentation of TB and the epidemiology of this disease are associated with its airborne route of transmission.

Following infection, only a small proportion of people infected with TB – estimated between 5 and 15% - will progress to active TB disease over the next months and years (1). The remaining people do not develop the disease in this timeframe but remain nevertheless at higher risk of developing active TB throughout their lifetime (2), particularly when they face medical or social conditions which alter their immunity such as HIV (3) and poverty (4).

The state of latent TB infection (LTBI) affects one third of the world population. Although these people do not present symptoms associated with TB disease, their infection generates a specific immunological response highlighted by a positive tuberculin skin test (TST) or a TB-oriented Interferon-Gamma Release Assay (IGRA). In LTBI, dormant mycobacteria remain under the control of the immune system. Nevertheless, the frontier between LTBI and active TB is subtle, and subclinical TB infection can occasionally be detected by positive bacteriological cultures and cause transmission (5, 6).

Active TB disease affects over 10 million people per year. The disease generally affects the lungs, but can also disseminate to all the organs. Symptoms include fever, night sweats and weight loss. In the case of pulmonary disease, a cough generally develops and persists despite usual treatments. These symptoms are non-specific and can be confused with many infectious and non-infectious diseases such as pneumonia or cancer. Therefore, for most people infected with TB, the diagnosis of TB relies not only on the clinical skills of the doctor, but also on laboratory and radiology examinations.

0.2. Mortality associated with TB

In the absence of treatment, approximately half of the people who develop active TB will die (7).

Because treatment is effective and free of charge for most people around the world, TB mortality should have become uncommon. Unfortunately, this is not the case. In the many countries of the African continent, one of four people with active TB dies despite the fact that effective treatment is available (Figure 0.1).

The reasons for such disastrous outcomes are probably multiple:

- First, people do not have access to care or present to the health system at a very late stage of the disease
- Second, the health staff may not suspect TB
- Third, the bacteriological tests used in these settings are often not sensitive enough to detect TB when it is present
- Fourth, TB has become resistant to commonly used drugs
- Last, these populations frequently present major comorbidities such as HIV or malnutrition which decrease the probability of treatment success

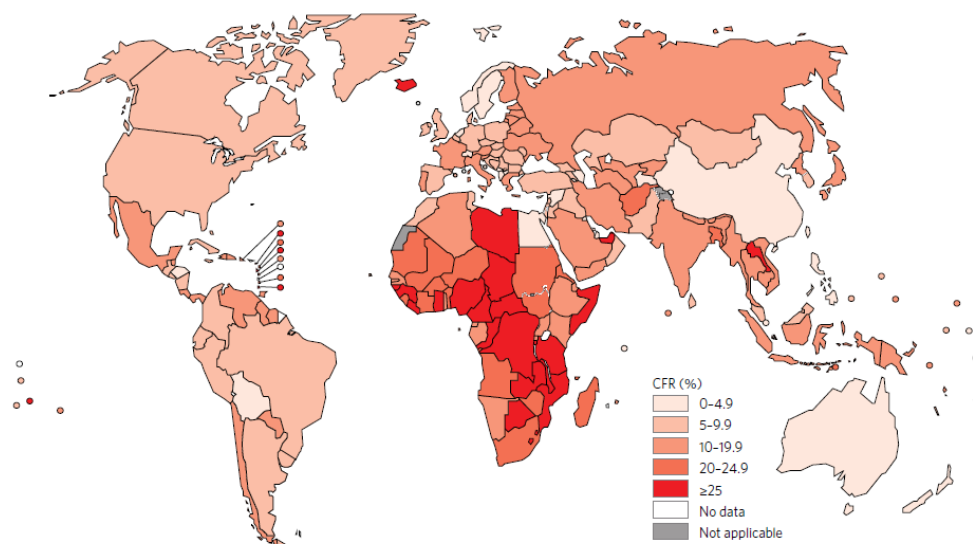


Figure 0.1: Estimates of the case fatality ratio for patients infected by TB (Source: WHO. Data 2015. In Global Tuberculosis Report 2016)

0.3. Treatment of TB

0.3.1. First line treatment regimen

Globally, 80% of all individuals diagnosed with active TB disease are infected with MTB strains that are fully susceptible to the four TB drugs composing the standard first line treatment regimen: Rifampicin (RIF), Isoniazid (INH), Ethambutol and Pyrazinamide (8).

In programmatic conditions, 85% of these people with fully susceptible TB disease have a favourable outcome following 6 months of directly observed treatment (DOTs). The other 15% of them are lost of follow-up, do not respond to treatment or die. Among people co-infected with HIV, the proportion of patients cured is 75%.

0.3.2. Emergence of drug resistance

The rationale for combining multiple active drugs to treat TB disease is based on observations made since the 1950s, which revealed the systematic emergence of drug resistance following the introduction of new drugs which were given as monotherapy (9).

The aim of combining different classes of antibiotics is to decrease the probability that a mutated strain will emerge during the course of treatment. The frequency of this natural phenomenon depends on each drug: the rate of occurrence of an INH conferring mutation is $2,5 \times 10^{-8}$, and it is $2,2 \times 10^{-10}$ for RIF (10). This frequency is to be compared to the bacterial burden in a lung cavity during active TB disease, which is estimated to be 10×10^8 (11). Emergence of drug resistant mutants during the course of treatment with only one effective drug is therefore frequent.

Outside the principle of quadruple drug therapy, there are four main principles that together can prevent the emergence of multidrug resistance (defined as the presence of at least RIF and INH resistance):

- Most cases of MDR-TB are caused by transmission from another person with MDR-TB. Notifications of MDR prevalence from most high-burden settings are consistent with most incident MDR-TB resulting from transmission rather than

new treatment-related acquisition of resistance. Preventing transmission in the community and in the clinics through infection control precautions, rapid diagnosis and appropriate treatment is therefore key for tackling the global problem of drug resistance. Still today, too many people enter hospitals without TB and acquire this infection because the health facilities do not respond appropriately to prevent nosocomial transmission (12).

- Early diagnosis and treatment of TB, prior to the development of lung cavities and preferably during the latent stage of infection, naturally decrease the burden of mycobacteria and therefore the risk of emergence of resistant mutants (13).
- The rapid detection of drug resistance is key to prevent the use of unnecessary drugs and prevent inadequate treatment regimens. The diagnosis of drug resistance in TB has profoundly improved in recent years with the availability of targeted molecular tools (14, 15) and more recently extensive genomic analysis (16) which can rapidly provide information on the presence of drug resistance, allowing treatment to be modified for these patients, increasing the probability of success of therapy.
- Adherence to treatment is key to prevent under-dosage of drugs and consequently the selection of drug resistant bacterial mutants. Adherence to treatment results from a complex interaction between the patient, their social environment and medical personnel. Side effects resulting from the treatment, stigma or lack of understanding of the disease and its treatment can result in poor adherence to treatment.

0.3.3. Second line treatment regimen

In 2015, 3,5% of all people developing TB disease globally were infected with MDR-TB, representing approximately 480.000 individuals. The majority of these people had not been previously exposed to TB drugs.

It was estimated that globally, only 52% of people initiated on TB treatment in 2013 for RIF-resistant TB were cured, while the others had a negative outcome (figure 0.2).

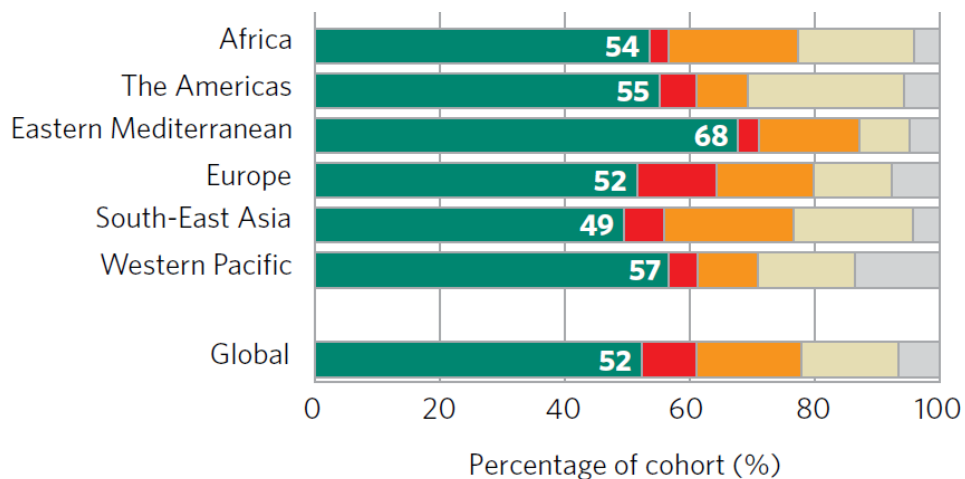


Figure 0.2: Treatment outcomes for RIF-resistant TB cases started on treatment in 2013. Green: treatment success; Red: treatment failure; Orange: died; Yellow: lost of follow-up; Grey: not evaluated. (Source: WHO Global Tuberculosis report 2016)

The WHO still recommends two empirical treatment regimens for MDR-TB (17). The recently endorsed shorter 9 months regimen is successful in approximately 90% of cases, much less toxic for patients and significantly cheaper compared to the 2-year regimen (1.000 USD versus 20.000 USD) (18). Today, MDR-TB treatment is therefore highly effective and affordable, but the main obstacle for accessing this treatment is the inability of many high burden countries to rapidly identify those patients who require a second line treatment. Today, in programmatic conditions, the treatment success rate of Rif-resistant TB is still very low (52%) (19), and this situation is probably due to many factors including the availability of second line drugs and the complexity of the two-year treatment regimen still used in many countries.

Under-detection of MDR-TB is a global issue. For the Democratic Republic of Congo (DRC) in 2013, the estimated number of patients with MDR-TB was 4.000 per year (CI 95%: 2.300 - 5.700), but only 268 (6,7%) patients received a second line TB treatment regimen.

0.4. Epidemiology of TB

0.4.1. Reservoirs

Humans and nonhuman primates (20) are the only reservoirs of *Mycobacterium tuberculosis* sensu stricto (MTB), and this is also probably the case for *Mycobacterium africanum*. Other members of the *Mycobacterium tuberculosis* complex (MTBC) such as *Mycobacterium bovis* and *Mycobacterium caprae* are responsible for zoonotic TB, and can cause a significant proportion of TB, particularly in developing countries. Transmission of *M. bovis* usually happens through ingestion of unpasteurized dairy products, although airborne contagion is possible. Disease caused by MTB or *M. bovis* are clinically indistinguishable from each other, although *M. bovis* is more frequently associated with extra-pulmonary disease (21).

1.4.2. Infectiousness

MTB is not highly infectious: untreated people with a smear positive microscopy test are thought to be the most infectious but are to infect eight to ten people per year, of whom only a minority will progress to active TB disease (22). The duration of infectiousness is dependent on the time between the onset of symptoms and the initiation of treatment, which is estimated to be over one year in many high burden countries (23).

Infectiousness can be prevented by general hygiene interventions that prevent the airborne transmission of MTB. Nevertheless, the most efficient intervention to prevent transmission is to provide effective treatment. After only 3-9 days of adequate therapy, the infectiousness of patients with TB is greatly diminished (24).

0.4.3. Individual susceptibility to TB disease

HIV infection, which is present in 0,5% of the world population, is the strongest medical risk factor for TB (25, 26): 12% of all new individuals developing TB disease are coinfecting with HIV and a quarter of TB mortality occurs among these people.

Type 2 diabetes mellitus is a constantly progressing public health challenge in developing countries (27). This disease triples the risk of developing TB disease (28).

Excessive alcohol use and smoking are respectively associated with a three- and two-fold increased risk of developing TB disease (29, 30).

Other risk factors, despite being less strongly associated with the development of TB disease, affects a much larger proportion of the world population. It is estimated that 27% of people infected with TB worldwide are attributable to undernutrition and 22% to indoor air pollution (31, 32).

0.4.4. WHO definition of risk groups for TB

According to the WHO, a risk group for TB is any group of people within which the prevalence or incidence of TB is significantly higher than in the general population. A risk group may be a group of people sharing a specific individual-level risk profile (for example, being in close contact with a person who has active TB; or living with HIV or having diabetes; or being a migrant). A risk group can also be defined as all people living in a specific geographical location associated with a high burden of TB (for example, all people living in an urban slum) or a specific type of institution (such as all prisoners in a country)(33).

0.4.5. TB in the mining sector of sub-Saharan Africa

There is evidence that people exposed to the mining sector in sub-Saharan Africa constitute an important high-risk group for TB. Exposure to mining is significantly associated with higher population TB incidence, and this association is independent of HIV, possibly because of the independent risk of silicosis in the gold mining sector as well as the confined environment and living conditions in artisanal mines that contributes to TB transmission (34).

A study performed in South-Africa showed a high *MTBc* genetic diversity among gold miners, suggesting that although transmission is frequent among workers in this sector, it seems that occupational factors also increase the risk of reactivating latent TB infection (35).

0.4.6. A disease associated with poverty

Although high TB incidence has been associated with several bio-medical (e.g. HIV infection) or behavioural (e.g. people working in the mining sector) risk factors, poverty remains the main driver of the TB epidemic as illustrated by the strong association observed between the *per capita* gross domestic product (GDP) and TB incidence (36). Figure 0.4 illustrates this evident association.

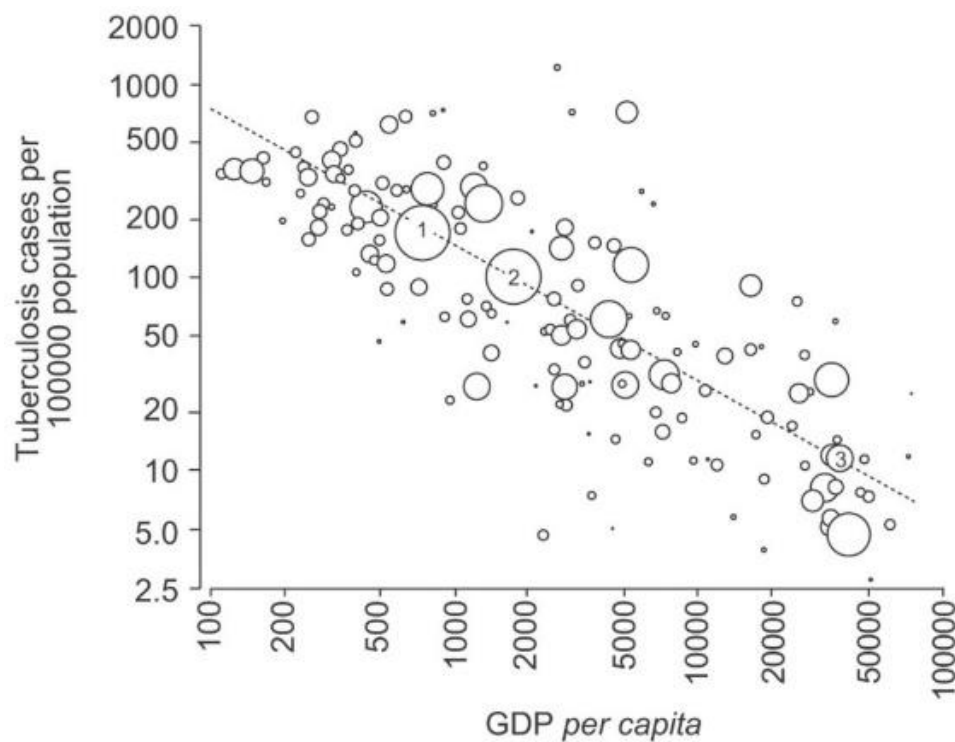


Figure 0.3: Relationship between per capita gross domestic product (GDP; Wold Bank data, 2005) and incidence of TB per 100,000 population. 1; India; 2: China; 3: UK. (Source : Janssens and Rieder, 2008)

Poverty is further associated with malnutrition, indoor air pollution and untreated diabetes which are further independent risk factors for TB.

In DRC, according to World Bank estimates, 88% of the population lives with less than 1.25 USD per day and 75% of the urban population lives in slums. In this country, the minimum wage is 65 USD per month.

When considering the micro-economic aspects related to TB infection and care, it appears that in many high TB burden countries, not only may diagnostic tests be unaffordable (e.g. chest X-rays) (37), but the whole process of disease and treatment generates catastrophic costs for the households of people infected with TB, increasing the poverty of these households (38)

0.4.7. Under-detection of TB and MDR-TB

Each year, millions of people with active TB do not reach the health system or remain undetected despite seeking care. The situation is worse in many African countries where it is estimated that half of the people with TB disease remain undetected, untreated and an important proportion of them remain infectious to their relatives and communities.

In DRC, where WHO estimates that approximately 50% of people with active TB disease remain undetected and un-diagnosed, representing an estimated 130.000 people per year that will not benefit from appropriate treatment and follow-up. When considering the mortality rate expected for these people, WHO estimates that 62.000 individuals die of TB each year in DRC without knowing the name of their disease.

The situation is even worse concerning MDR-TB. In many places, only 10% of patients with drug resistant TB will receive adequate treatment (figure 0.4). In DRC, where 4.000 patients are believed to develop MDR-TB each year, only 413 were started on treatment. Under-detection of MDR-TB among children is thought to be even greater (39).

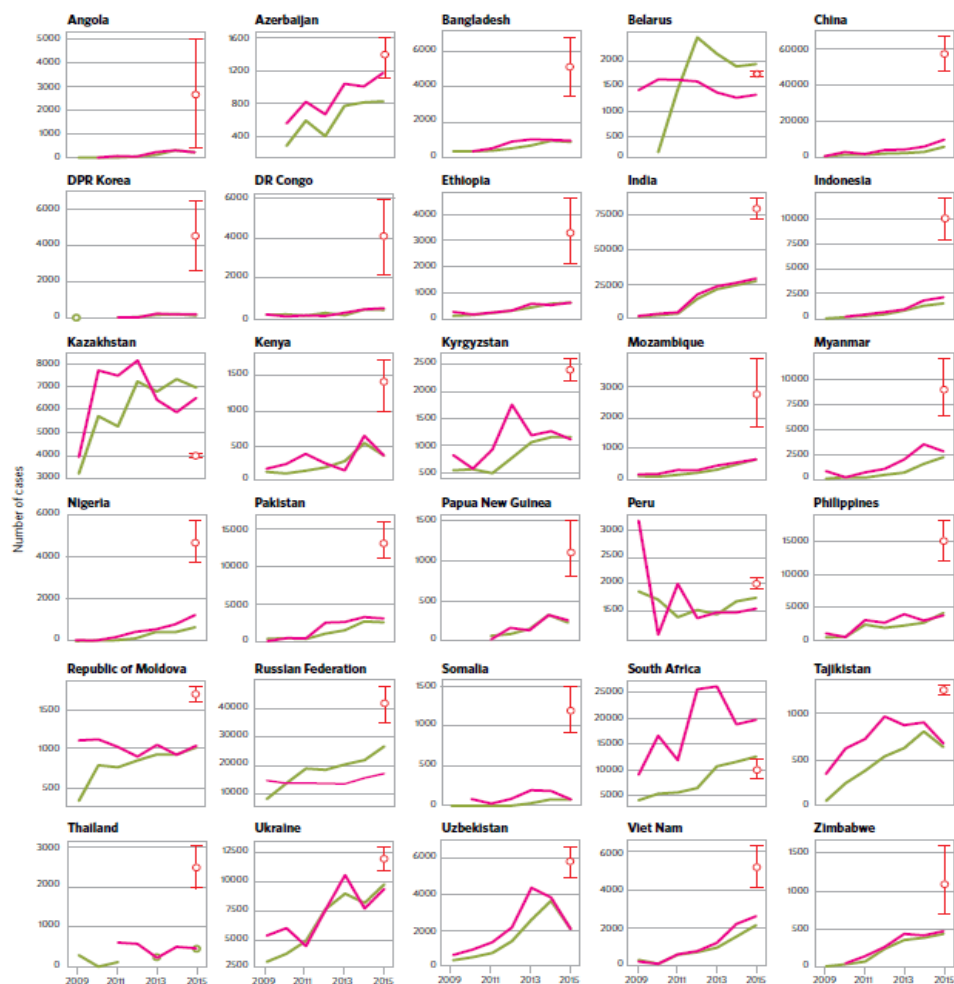


Figure 0.4: Under-detection of drug-resistant tuberculosis. Number of MDR/RR-TB cases detected (pink) and enrolled on MDR-TB treatment (green) compared with the estimated number of MDR/RR-TB cases among notified TB cases in 2015 (uncertainty interval shown in red), 30 high MDR-TB burden countries (Source: WHO. Data from 2009 to 2015. In Global Tuberculosis Report 2016)

0.5. Challenges and limits associated with the WHO estimates for TB incidence

0.5.1. National estimates of TB incidence

Each year, WHO publishes an estimate of the burden of TB and MDR-TB for each country. These estimates are the result of a multitude of surveys, notification data and expert opinions. These estimates have a major impact on public health policies and research priorities.

In 2015, there were an estimated 10.4 million new (incident) people with active TB disease worldwide. The burden of diseases is nevertheless highly variable between countries, and the Africa region includes the highest number of countries with incidence rates of over 300 new people infected with TB per 100.000 people and per year (figure 0.5).

With an estimated annual incidence of 324 (95% CI: 210 - 463) new people with TB disease per year and per 100.000 inhabitants, DRC has one of the highest incidences of TB.

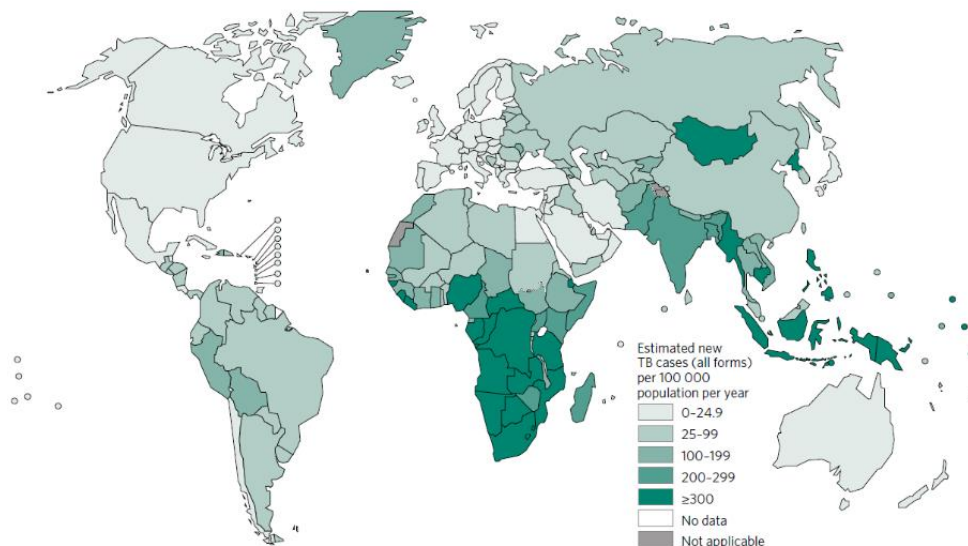


Figure 0.5: Estimated TB incidence per country (Source: WHO. Data: 2015. In Global Tuberculosis Report 2016)

0.5.2. Methodology for estimating TB incidence in Africa

As illustrated in the map below, the estimated incidence of TB in African countries is largely determined by case notification rates and expert opinions (figure 0.6). There is an inherent risk of error associated with this methodology, particularly when considering the proportion of undetected (and therefore un-notified) people with active TB in these countries. Few African countries have conducted TB prevalence surveys.

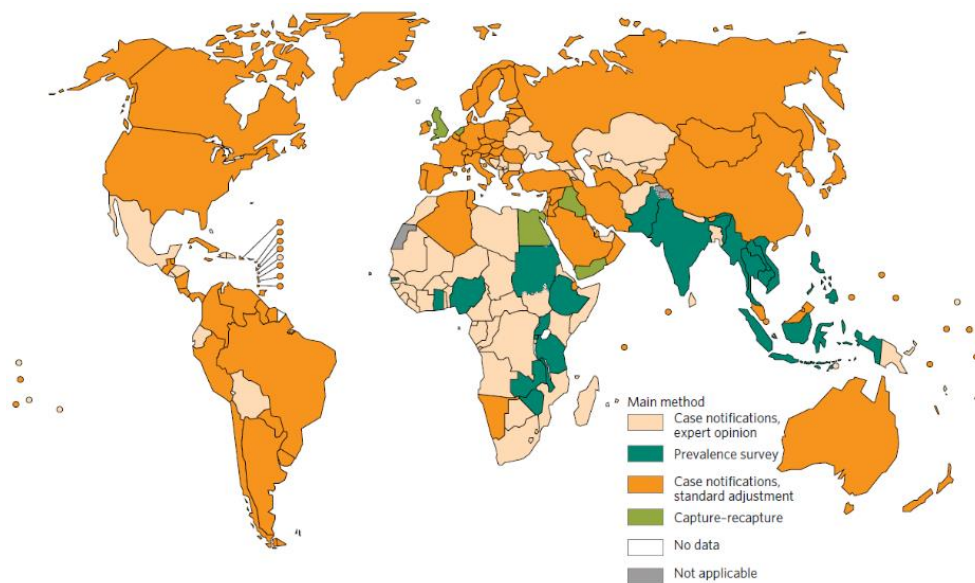


Figure 0.6: Main methods used to estimate TB incidence. (Source: WHO. In Global Tuberculosis Report 2016)

The WHO compared the results of the prevalence surveys with the previous estimates calculated from case notification and expert opinion. The figure 0.7 illustrates the discrepancies between the two methods.

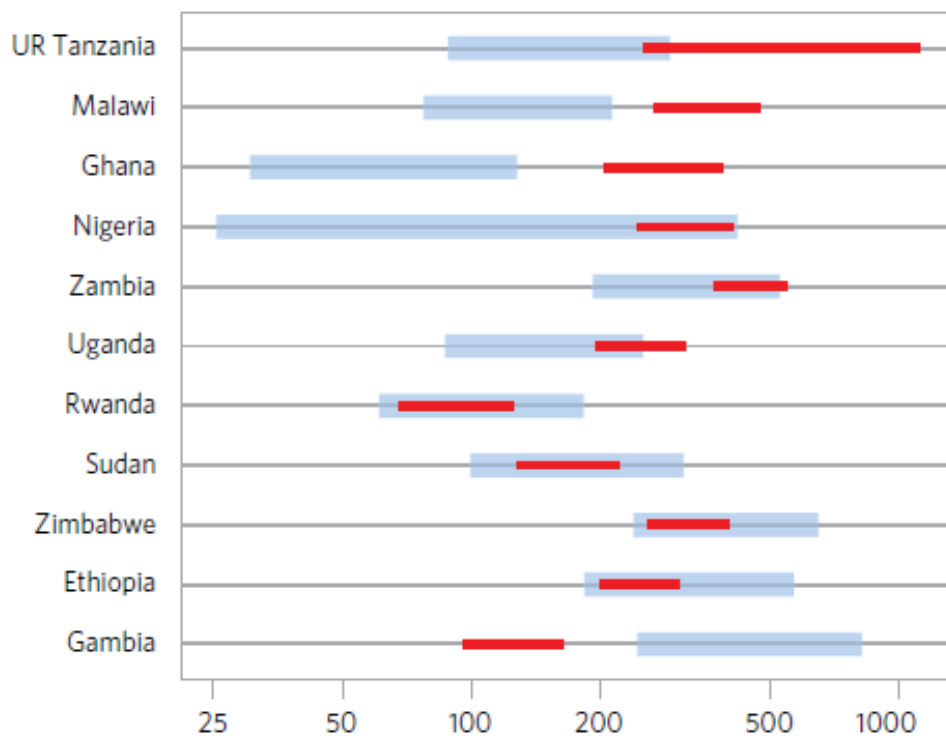


Figure 0.7: Estimates of TB prevalence in African countries before (blue) and after (red) results from national TB prevalence surveys became available. (Source: WHO. In Global Tuberculosis Report 2016)

0.5.4. The lists of TB high burden countries

The WHO has defined the lists of priority countries based on the burden of respectively TB, MDR-TB and HIV-TB that these face (figure 0.8). DRC, together with seven other African countries and six Asian countries, compose the group of 14 countries that are on the top of these three lists.

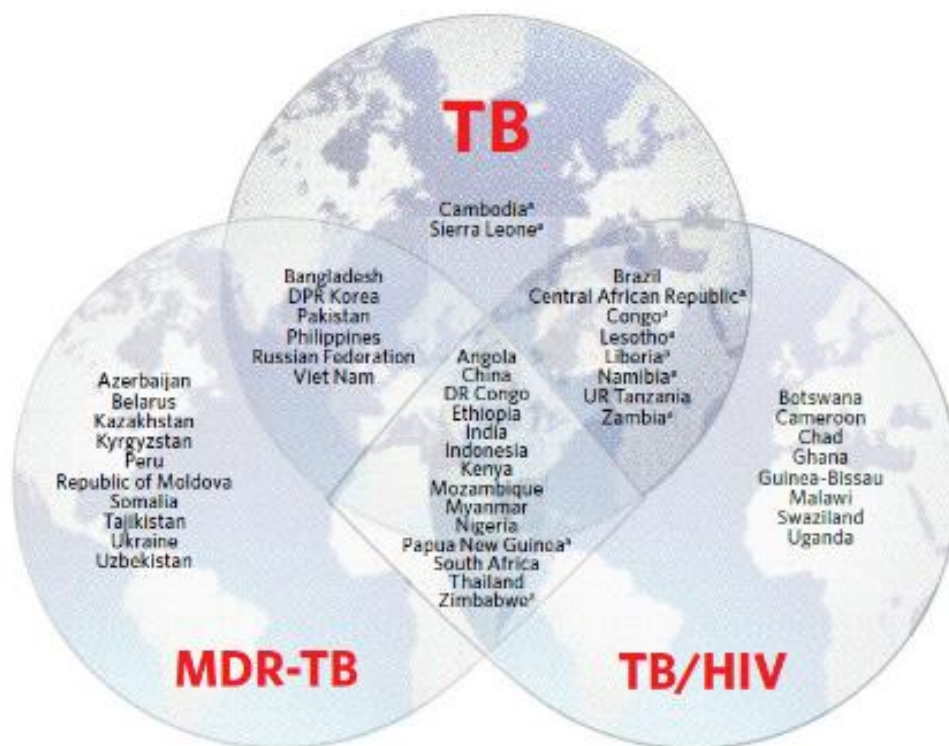


Figure 0.8: Countries in the three TB high-burden country lists and their areas of overlap.
(Source: WHO. In Global Tuberculosis Report 2016)

0.5.5. Limits of national estimates for addressing local hotspots of TB

A major limitation of these national estimates is that they do not take into account the geographical and social diversity that may exist in a country. Therefore, these national estimates cannot serve to prioritize interventions at the local level.

The DRC is a country with 77 million inhabitants and extreme variations in population density, distribution of high-risk groups and accessibility of health services.

0.6. Historical perspective with regard to international strategies for TB control

In 1994, the United Nations recognized that TB was a global emergency. Since then, WHO has released three major documents that have directed the global effort to control and eradicate the TB epidemic. While richer countries have the possibility to adapt these recommendations to their medical and technical capacities, the countries that have fewer resources dedicated to public health spending are more inclined to stick to these recommendations.

In addition, the STOP TB PARTNERSHIP, composed by 1500 partner organizations and patient groups, has led an effort to stimulate innovation (TB REACH initiative) and has provided an operational plan to end the TB epidemic¹.

Proposing an historical perspective to these major documents, we illustrate how the fight against TB has evolved from a highly normative and paternalistic vision to a holistic strategy that includes a deep involvement of civil society and research. A better understanding of this context should not only ensure that the solutions proposed are integrated in a global effort, but should also eventually help understand forces resisting change when considering the adoption of innovative solutions.

¹ <http://www.stoptb.org/global/plan/plan2/>

0.6.1. Framework for Tuberculosis control (1994-2006)

The WHO released a first document named “Framework for Tuberculosis control” in 1994² that has been considered as the reference during 12 years, until 2006. The strategy proposed was based on the belief that the epidemic would be controlled by focusing mainly on people presenting the most contagious form of TB, and that these people would seek care spontaneously. From a laboratory point of view, this strategy relied nearly exclusively on smear microscopy and aimed to prevent the emergence of MDR-TB by encouraging treatment adherence.

Today, these principles are considered to have been insufficient and, on the contrary, may have contributed to our inability to control TB and MDR-TB.

The concept of passive case finding

The concept of “passive case finding” relies on the principles that all people with symptoms of TB would present spontaneously to the health system and that the first line health professionals would diagnose and treat the disease.

The main limitations of this approach are that the households and communities in a population which presents the highest risk of TB infection are often marginalized from public health services due to financial, geographical or cultural factors (40). It has been demonstrated that even when treatment is free, TB may generate catastrophic costs for people and their families (38). Therefore, it is a natural behaviour for the poorest to delay or avoid these costs by not seeking healthcare.

Limits of smear microscopy for the diagnosis of TB

Smear microscopy is a simple diagnostic test that allows people to be diagnosed with a very high burden of mycobacteria in their sputum, and thus those who are believed to be the most contagious. Although the test has major limitations, it is rapid and inexpensive. These characteristics are probably the reasons why this test has been promoted as the solution of choice in poor countries.

The main limitations of microscopy are its inability to:

- detect up to 50% of patients with active pulmonary TB. Children and HIV-infected people are those for whom the test is less sensitive.

² http://apps.who.int/iris/bitstream/10665/58717/1/WHO_TB_94.179.pdf

- detect most forms of extra-pulmonary TB, as it requires a targeted biological sample
- detect drug resistance.
- differentiate MTB from other pathogenic non-TB mycobacteria (NTM).

Directly Observed Treatment (DOT)

Adherence is a key element of any drug-based treatment. Following demonstrations that this general concept applied to TB and that treatment success increased when patients benefited from continuous adherence, the concept of “Directly Observed treatment” (DOT) was introduced (42).

An early evaluation of the DOT strategy performed in 1997 in South-Africa supported this principle of treatment supervision, as the early discharge of patients from hospital decreased the overall cost of TB treatment (43).

At that time, the medical community generally believed poor adherence to treatment was the main cause of the emergence of drug resistance, and therefore that DOT would prevent MDR-TB. Today, we know that drug-resistant TB is more frequently acquired in hospitals through nosocomial infections than caused by poor adherence to treatment (44).

A key criticism of the DOT strategy is that it presented the complex issue of adherence as a sole issue of the patient (45, 46). However, adherence is also a matter of treatment toxicity and education of the patient who has the right to understand his disease. DOT may have contributed to an erroneous perception among health practitioners that patients are not reliable and that treatment failure was due to the patient rather than the inability of the health system to recognize its limits or identify drug resistance (47, 48).

0.6.2. Surveillance of anti-tuberculosis drug resistance (since 1994)

In 1994, the Global Tuberculosis Program of the World Health Organization (WHO), with the support of the International Union against Tuberculosis and Lung Disease (the Union), established the Global Project on Anti-Tuberculosis Drug Resistance” to measure the magnitude of drug resistance and to monitor trends. This project remains the oldest and largest initiative on the surveillance of antimicrobial resistance in the world (41).

In their paper published in 2016, Zignol et al. claimed to have representative data on antituberculosis-drug resistance from 153 countries, covering 96% of the world’s population and incident tuberculosis cases (figure 0.9).

Most of the countries for which no surveillance data was available were from Africa (data from DRC were only available in 2017), and the authors recognized that the understanding of the evolution of the MDR-TB epidemic in this continent was very limited.

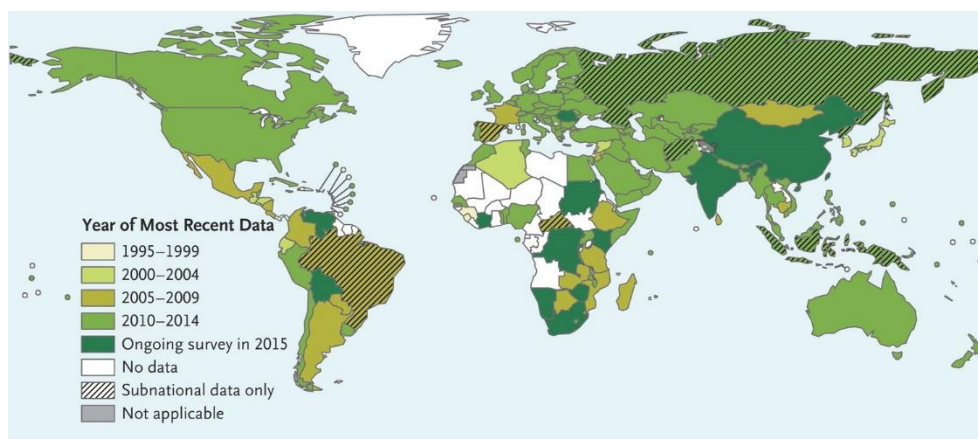


Figure 0:9: Global Coverage of Surveillance Data on Resistance to First-Line Antituberculosis Drugs, 1995–2015. (Source: Zignol et al. 2016)

0.6.3. The Stop TB strategy (2006-2015)

In 2006, the World Health Organisation released a second framework document entitled the “Stop TB Strategy”³. The release of this document was an indirect acknowledgement that the previous strategy was failing to achieve TB control.

The solution proposed by the WHO proposed major improvements and included the necessity to monitor the success of the strategy based on the outcome of patients rather than on the level of adoption of the strategies.

- The strategy insisted on the necessity to address the barriers preventing access to care by the vulnerable populations and high-risk groups. It further highlighted the need to engage the patients⁴, the civil society and research in the global effort to control TB.
- From a clinical perspective, the strategy included the need to improve the skills of front-line health professionals and integrate TB in a global approach to lung health
- The strategy insisted on the utility of culture and DST for the diagnosis of smear negative TB and drug-resistant TB.

0.6.4. Focus on innovation: the TB REACH initiative (2010-2020)

The Stop TB Partnership launched the TB REACH initiative in 2010. The aim of this initiative supported by Global Affairs Canada is to stimulate innovation that would address the major challenge of undetected TB. This grant-based system supports projects that focus on reaching people with limited or no access to TB services for one to two years.

The idea behind TB Reach is that innovation and moving away from just a passive approach is needed to reach those people in need. Sensitive diagnostic tests alone

³ http://apps.who.int/iris/bitstream/10665/69241/1/WHO_HTM_STB_2006.368_eng.pdf

⁴ http://www.who.int/tb/publications/2006/patients_charter.pdf

cannot be considered as an answer to under-detection of TB. It is moving care (and diagnostic tests) closer and more accessible to people in need.

Through five successive waves of funding, this initiative has supported numerous projects around the world. To date, the innovations and operational research have led to the publication of 43 scientific papers⁵.

This initiative has largely contributed to generate the evidence that the evolution of the TB epidemic could be changed based by introducing new strategies, in particular targeted active case finding programs and more sensitive diagnostic tests.

0.6.5. The End TB strategy (2015-...), a paradigm shift

In 2015, the WHO released the END TB Strategy (49), which was rapidly followed by a costed plan by the Stop TB Partnership for implementing the first five years⁶. This ambitious strategy was introduced following a very dynamic period mainly characterized by the successful deployment of rapid molecular diagnostic tests (RDT) in high burden countries (14).

The increased sensitivity of these scalable RDTs and their ability to detect drug resistance provoked a drastic change in the possibility and necessity to address TB among HIV-infected people, paediatric TB and drug-resistant TB. With these “point of care” assays, the diagnosis of these complex forms of TB would not require anymore the timely process of sample transportation, culture and DST.

The context was further characterized by the observations that MDR-TB was mainly caused by transmission and that half of the patients with MDR-TB had no prior history of treatment. This pushed the necessity to introduce universal DST for all patients diagnosed with TB.

Finally, the End TB strategy integrated the necessity to address the complex issues of undetected TB and the accessibility of health services for the poorest communities.

⁵ <http://www.stoptb.org/global/awards/tbreach/publications.asp?pYear=2017>

⁶ <http://www.stoptb.org/global/plan/plan2/>

0.7: Context of the South-Kivu province in DRC

The South-Kivu province lies along the eastern border of DRC and has a population of over 6,6 million inhabitants, mainly living in rural areas. Health delivery is provided to the population through over 500 health facilities (HF), among which 78 (years 2010-2013) to 113 (from year 2014) provide TB services: Ziehl-Neelsen microscopy and provision of first-line anti-TB drugs. Since 2012, 10 laboratories located in urban clinics have been progressively equipped with GeneXpert MTB/RIF technology. The province does not have a laboratory able to perform mycobacterial cultures—the most sensitive diagnostic method—and diagnostic samples are usually not sent to the National Reference Centre in Kinshasa unless drug resistance is suspected.

The South-Kivu province is divided in 34 health zones (HZ), and each HZ has one to six HF. Each HF is responsible for a median of 51,500 habitants (percentiles 10 and 90: 17,800 and 97,300). The number of patients notified with active TB disease by each HF is variable: 10% of these report less than 7 cases per year, while 10% report more than 140 cases, the median being 32.

Among the 34 HZ, five are mainly urban (Bagira, Kadutu, Ibanda, Kamituga, and Uvira), while the remaining 29 HZ are located in rural areas. Some of the latter are relatively well served in terms of infrastructure and communications, while 11 are difficult to reach due to the absence of roads or on-going armed conflicts. Most of these isolated HZ are located in the centre and west of the province.

This province has experienced over two decades of conflicts which importantly affected the population (50), leading to major displacements (51). The majority of these conflicts are directly or indirectly associated with the distribution and exploitation of the numerous mining resources (52) present in this region. Apart from the impoverishment of the population, a consequence of this dramatic context is that the health system is under-resourced to face many challenges, including the TB epidemic. This is exacerbated by the presence of many high risk groups for this disease, including people living in mining camps (53), military camps (54, 55) and prisons (56, 57), which are consequent to the conflict.

Because of the long-lasting humanitarian crisis in this region, the vast majority of the 34 HZ receive external support from non-governmental organisations (NGOs) for the delivery of primary health care services. This support is generally integrated within the existing public system and fully consistent with the national guidelines. Four HF located

in the HZ Shabunda, Fizi and Kimbi-Lulenge (since 2012), are nevertheless considered as exceptions: these are supported by Médecins Sans Frontières (MSF) which applies a more “independent” policy with regard to the organisation of health services, including supplementation of the health structures with qualified medical staff, clinical guidelines and technical resources including laboratory equipment.

In 2013, the South-Kivu province had 408 doctors (among which 14 had a specialization degree) and 2.862 nurses for a population of over 6.6 million. In this context, nurses, laboratory technicians or the so-called “Rapidly Trained Agents” form the front line of primary health-care delivery and are inevitably sometimes faced with medical conditions that they have not been trained to recognize. Furthermore, these professionals and their patients have very limited access to complementary tests such as X-Rays and accurate laboratory tests, which could partially palliate these deficiencies.

0.8. Priorities for research

0.8.1. Overcoming socio-economic barriers to care

Although it is impossible to precisely determine the proportion of people with active TB disease that remain undetected, it is reasonable to estimate that over 50% of people infected with TB are currently left undetected in the South-Kivu province.

There are multiple socio-economic reasons that can explain why people do not access the health system. The stigmatization surrounding TB infection (58) and the catastrophic costs for people infected with TB and their household (38, 47) are believed to be among the main reasons that explain treatment abstention and delay.

To prevent the medical and socioeconomic consequences of TB disease, transmission must be stopped and TB-affected households must receive adequate support (59, 60).

Chapter 1 describes how we engaged TB-affected populations in active case-finding (ACF) strategies. The principal objectives of this intervention were:

- to provide direct economic support to TB-affected households by providing them socially-useful jobs
- to alleviate the burden of TB in the community of these screeners by accelerating the referral of people with TB related symptoms
- to increase the cost-effectiveness of ACF by better targeting high-risk groups and decreasing the cost of conventional ACF campaigns led by hired personnel.

In 2013, the WHO modelled yield of different screening algorithms being used for ACF (Table 0.1) (33). These models illustrate the performance of the different two-steps algorithms that can be used based on the local technical capacities (availability of Xpert and chest X-Rays). In this model, a cough lasting for more than 15 days is expected to be present among 35% [CI 95%: 24%-46%] of people with active TB disease.

In our setting, due to the local context, we performed ACF activities by performing symptomatic screening followed by smear microscopy.

Screening	Final diagnostic test	Outcome of screening ^a				Outcome of diagnosis in persons with positive screening ^a						
		TN	FN	NPV ^b (%)	Screened positive ^c	TP	Detected of true cases (%)	FP	PPV ^d (%)	TN ^e (n)	FN ^f	NPV ^g (%)
Cough lasting >2 weeks	SSM	93 753	649	99.3	5 598	214	21	105	67.1	5 142	137	97.4
	Xpert	93 753	649	99.3	5 598	324	32	52	86.0	5 195	27	99.5
1st screen: cough >2 weeks 2nd screen: (if 1st screen positive): chest radiography	SSM	96 691	684	99.3	2 625	193	19	46	80.7	2 263	123	94.8
	Xpert	96 691	684	99.3	2 625	291	29	23	92.7	2 286	25	98.9
Any TB symptom	SSM	67 023	230	99.7	32 747	470	47	640	42.3	31 337	300	99.1
	Xpert	67 023	230	99.7	32 747	710	71	320	68.9	31 657	60	99.8
1st screen: any TB symptom 2nd screen (if 1st screen positive): chest radiography	SSM	84 930	307	99.6	14 763	423	42	281	60.0	13 788	270	98.1
	Xpert	84 930	307	99.6	14 763	639	64	141	82.0	13 929	54	99.6
Chest radiography: abnormality suggestive of active TB	SSM	88 506	132	99.9	11 362	529	53	210	71.6	10 284	339	96.8
	Xpert	88 506	132	99.9	11 362	800	80	105	88.4	10 389	68	99.4
Chest radiography: any abnormality compatible with TB	SSM	74 646	22	100.0	25 332	597	60	487	55.1	23 867	381	98.4
	Xpert	74 646	22	100.0	25 332	902	90	244	78.7	24 110	76	99.7

Table 0.1: Modelled yield of different algorithms when screening 100 000 persons in a population with a 1% prevalence of culture positive pulmonary TB (1 000 cases). (It is assumed that the final diagnosis uses results from sputum-smear microscopy or the Xpert MTB/RIF test, and there is no further diagnostic evaluation) (Source: Systematic screening for active TB, WHO 2013)

0.8.2. Identification and management of high risk groups for TB

According to the WHO, systematic screening in high risk groups is a possible complement to any approach to improve the patient-initiated pathway to TB diagnosis (that is, diagnosing TB among people who themselves seek care for their TB symptoms, also called “passive case-finding”) (33). Chapter 2 describes the process of identification and characterization of high-risk groups in the South-Kivu province of DRC.

The WHO further provides an estimate of the number needed to screen (NNS) per category of population, as represented in Table 0.2. This table highlights several important considerations:

- In a high burden population, defined as having an incidence of over 300 new TB cases per year, it is estimated that 100 [16-6355] people need to be screened to find one new patient with active TB. The important variability of results reported highlights the necessity to better understand the determinants of TB and the impact of ACF interventions in these populations with the highest burden of TB.
- These estimates are based on a review of the literature and aggregate a wide range of screening approaches. Using a screening algorithm based on the detection of cough lasting for more than 15 days followed by a smear-microscopy confirmatory test, it is expected that the NNS will be much higher than the real prevalence of the disease. This is due to the high proportion of true cases missed as a consequence of the low sensitivity of these approaches.
- There exist an important lack of information available concerning high-risk groups from high burden countries.

Population screened (No. of studies)	Low incidence (<30/100,000)	Moderate incidence (30-100/100,000)	Medium incidence (100-300/100,000)	High incidence (>300/100,000)
General population (98)	3922 (137–30865) ^a	669 (15–5594)	603 (25–4286)	100 (16–6355)
Infants in vaccine trial (3)	NS	NS	140 (7–343)	NS
Immigrants (26)	235 (3–1262) ^a	NS	1206 (198–6250)	NS
Refugees (38)	108 (6–1630) ^a		120 (57–291)	
Military (6)	1159 (134–492) ^a	NS	1280 (73–1440)	NS
Health-care workers (16)	1613 (30–5550) ^a		506 (25–842) ^a	NS
Miners (8)	48 (–) ^b	154 (–) ^b	NS	36 (21–93)
Other occupations (14)	1565 (47–5235) ^a	NS	109 (4–778)	NS
Homeless people (18)	133 (22–1778)	NS	NS	NS
Prisoners (44)	1180 (4–2945) ^a	155 (19–191)	110 (7–2762)	
General inpatients (4)	NS	NS	795 (6–3364)	
General outpatients (14)	758 (42–30 000)		269 (19–806)	
Nursing homes (7)	120 (68–137) ^a		NS	7 (–) ^b
Psychiatric facilities (3)	1049 (32–1275)		111 (–) ^b	NS
Pregnant women (9)	536 (88–3843) ^a	NS	36 (25–143)	
People with diabetes (6)	NS	2223 (–) ^b	35 (17–54)	
Drug users (8)	158 (108–252) ^a	5 (–) ^b	20 (8–20)	NS
VCTC (5)	NS		37 (8–120)	
People living with HIV (74)	30 (8–391) ^a	61 (5–316)	13 (2–120) ^a	10 (3–64)
Other clinical groups (6)	290 (10–2846) ^a	NS	4 (–) ^b	NS
Gynaecology clinics (5)	18 (–) ^b	NS	13 (5–38)	
Household contacts (89)	54 (5–430) ^a	40 (7–355) ^a	25 (3–568) ^a	17 (2–129)
Community contacts (78)	104 (3–4200) ^a	85 (6–137)	NS	
Health-care contacts (17)	276 (7–223) ^a	25 (–) ^b	NS	NS

Table 0.2: Weighted mean and range number needed to screen (NNS) to find one case of TB in selected risk categories. All screening approaches are aggregated within each risk group. NS: No study. VCTC: Voluntary counselling and testing for HIV. (Source: Systematic screening for active TB, WHO 2013)

0.8.3. Evaluation of the clinical skills of first-line health care providers. Focus on paediatric TB.

Despite the major improvements recently observed in the field of laboratory diagnosis, clinical skills remain the cornerstone of TB diagnosis, in particular when sensitive laboratory tests are not available. Nowadays, particularly with regard to the complex clinical manifestations of TB such as paediatric TB (PedTB), TB among HIV-infected people and extra-pulmonary TB, diagnosis usually rely on the ability of the clinician to suspect and initiate empirical treatment without bacteriological confirmation. According to the WHO, DRC is among the countries that notify the highest proportion of bacteriologically confirmed TB despite the fact that the utilization of sensitive diagnostic tools is rare in many areas. This observation suggests that clinicians fail to diagnose and treat many people presenting smear-negative active TB disease.

Assessing the clinical skills of first-line health professionals in such a large country is something nearly impossible. There is thus an urgent need to develop new tools that would simplify the process of evaluation and allow the implementation of targeted interventions. These aspects are discussed in Chapter 3.

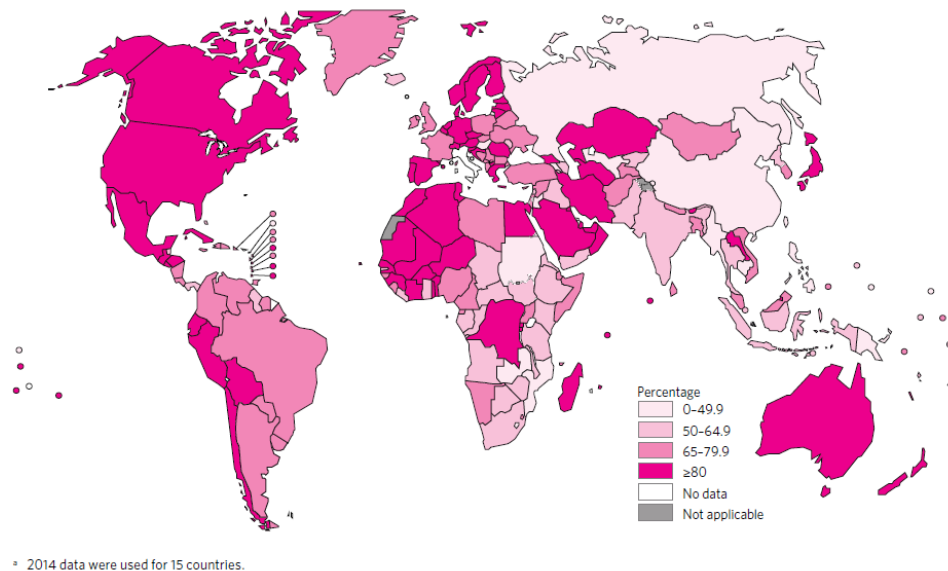


Figure 0.10: Percentage of new and relapse pulmonary TB cases with bacteriological confirmation, 2015 (Source: Global Tuberculosis Report, WHO 2016)

0.8.4. Introduction and evaluation of rapid molecular tests

When the Xpert MTB/RIF test was endorsed by the WHO in December 2010, the expectations were that this simplified PCR technology would be used as a point of care test in high burden countries.

In Chapter 5, we explore the technical aspects of the different laboratory methods available. We illustrate the limits of current diagnostic strategies by the detection of a multi-country outbreak of MDR-TB that has been undetected by algorithms supported by the WHO.

From 2012, we introduced the first Xpert machines in DRC and experienced numerous logistical challenges. This experience is discussed in chapter 5.

Except for line probe assays (LPA) performed in Kinshasa, DRC did not have any experience in using molecular tools for the diagnosis of TB, and it was not clear how this technology would be integrated in the health system and impact the detection of TB and drug-resistant TB.

0.8.5. Improving disease surveillance and quality management of TB laboratories

Both from the clinical and epidemiological perspective, it appears that the paper-based medical and surveillance reports for TB used in many high TB burden countries fail to provide high standard level of care and useful information that could guide the epidemiological response.

Chapter 6 describes how the utilization of Xpert MTB/RIF revealed the potential of connected diagnosis for improved patient management and real-time disease surveillance.

We developed an innovative IT solution that demonstrated the utility and feasibility of connected medicine in the context of TB high burden countries.

Chapter 1: Overcoming barriers to patient care and empowerment

1.1. General limitations of active case-finding interventions

Active case-finding (ACF) of people with TB disease is thought to be a useful intervention to overcome the challenge of under-detection. A review published by the World Health Organization (WHO) revealed that the impact and cost-effectiveness of ACF on TB detection was highest when high-risk groups could be specifically targeted.

Some ACF interventions failed to demonstrate their utility and sustainability in programmatic conditions (61). This failure to demonstrate impact is probably due to the inability of authors to evaluate their interventions at the level of the communities where they intervened rather than mixing their findings in national figures (62-64).

1.2. Recruitment of TB-affected people for performing the community screening

We proposed to recruit TB-affected people to perform ACF in the South-Kivu province of DRC. There were several reasons for this choice:

- First, the dispersion of these people in the community was expected to be comparable to the distribution of TB, meaning that most of the screeners would be familiar with the poor households and communities where there is an active circulation of TB. This inherent integration within these hard-to-reach populations was considered as a strong argument to recruit these TB –affected people rather than qualified staff such as nurses.
- Second, because these people have experienced TB themselves or through close relatives, they have a concrete knowledge of symptoms and treatment of TB. They have also experienced the socio-economic effects of TB disease.
- Third, we wished that the financial incentives and positive social repercussions associated with a socially useful screening work would affect positively TB-affected people.
- Last, initial discussions with TB-affected people revealed that they were highly motivated to perform such work

In figure 1.1, we illustrate the process a diagnosis, treatment, recruitment and training of people infected with TB who are being asked to perform ACF activities in their communities.

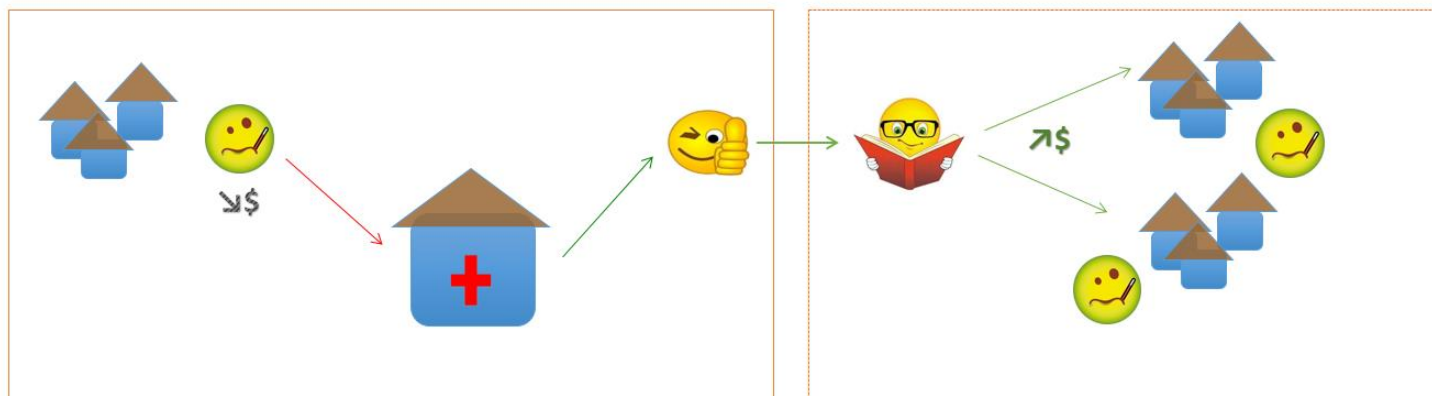


Figure 1.1: Illustration of the baseline services and proposed intervention. The baseline TB services (left) consists of passive case-finding (the patient presents spontaneously to the clinic), laboratory diagnosis and treatment. In the intervention (right), TB-affected people and their relatives are trained to perform active case-finding activities in their communities and refer patients presenting a cough lasting for more than 15 days to the clinic. These people are offered a TB test. The financial incentives allow compensating part of the financial consequences associated with TB disease (loss of income, medicines, transports ...).

1.3. External funding and the challenge of sustainability

The activities were initially supported by a TB REACH grant that allowed us to demonstrate the feasibility and early results of the initiative. During this period, the ex-TB patients structured their activity as a non-profit local organization and performed provincial workshop to consolidate their strategy based on experience from the different local groups (figure 1.2). This organization was later supported by a CHALLENGE TB grant, which, despite being more limited in amount, allowed a multi-year intervention.

In 2016, the organization has structured a parallel for-profit activity of guarding services that offers sustainable jobs to TB-affected people and covers an important part of the administrative costs such as the provincial office and supervision visits to the groups dispersed around the province. This activity does not yet cover the costs of the TB-related activities.



Figure 1.2: Workshop held during the TB REACH grant with representatives of the different local screening groups. Discussions allowed defining the screening strategies, the distribution of financial incentives and the reporting of activities (Photo: Emmanuel André).

1.4. Complementing the biomedical approach through patient-led tuberculosis active case finding. Evaluation of a large-scale intervention in the Democratic Republic of Congo.

(manuscript in preparation, subject to further modifications)

Emmanuel André^{1,2,3*}, Olivier Bahati Rusumba³, Carlton A Evans⁴, Philippe Ngongo⁵, Pasteur Sanduku⁵, Elvis Marhegane Munguakonkwa³, Celestin Ndwanyi Habimana³, Alain Ishara³, Eric Mulume Musafiri⁵, Jean-Pierre Kabuyai⁶, Olivier le Polain de Waroux⁷, Nadia Aït-Khaled⁸, Michel Delmée^{1,2} and Francis Zech⁹

1: Pôle de microbiologie médicale, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium

2: Service de microbiologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium

3: Ambassadeurs de Lutte contre la Tuberculose (ALTB), Bukavu, Democratic Republic of Congo

4: Imperial College London, London, United Kingdom

5: Coordination Provinciale Lèpre et Tuberculose, Bukavu, Democratic Republic of Congo

6: Challenge TB, Kinshasa, Democratic Republic of Congo

7: London School of Hygiene and Tropical Medicine, London, United Kingdom

8: The International Union against Tuberculosis and Lung Disease, Paris, France

9: Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium

* Corresponding author: emmanuel.andre@uclouvain.be

1.4.1. Abstract

Background: Under-detection of tuberculosis (TB) is a major challenge globally. In many high TB burden countries, including the Democratic Republic of Congo (DRC), it is estimated that half of people with TB disease do not receive appropriate diagnosis and treatment. Community-based active case finding (ACF) has been proposed as a mean to tackle TB under-detection and overcome the barriers to patient care. ACF is cost-effective among high-risk groups. The aim of our study was to evaluate the feasibility, quality and cost of a large-scale community-based ACF intervention driven by screeners directly affected by TB, principally people with a history of TB disease.

Material and methods: We evaluated the ACF intervention between 2014 and 2016. We collected the screening reports of the “Ambassadeurs de Lutte contre la Tuberculose”, which were validated against the official TB notification reports. We evaluated the quality of the intervention based on (1) the number needed to screen (NNS) to find one new sputum smear-positive pulmonary TB (SS+PTB) case, and (2) the cost-effectiveness of the intervention.

Results: Over 650.000 individuals were screened and the intervention contributed to the detection of 4.300 new SS+PTB cases. Overall, 42% of the SS+PTB cases at provincial level were identified through the ACF intervention. The NNS was 128 and the cost per new SS+PTB case found was 44 USD.

Conclusion: Both the low NNS and the cost-effectiveness of this peer-led ACF intervention complied with WHO criteria. To achieve TB eradication in hard-to-reach communities, we propose that TB affected people should actively engage in ACF interventions as a mean to increase the accessibility of health services and decrease the cost of TB in these populations. In the South-Kivu province, this intervention has become a major driver of TB detection.

1.4.2. Background

Under-detection of Tuberculosis (TB) is a major global challenge. Globally, among the 10.4 million people who developed active TB disease in 2015, it is estimated that a third of them remain undetected or unreported, and receive inappropriate treatment. In many high-burden countries, the level of under-detection can reach 50%, as it is the case in the Democratic Republic of Congo (DRC), a country of 77 million inhabitants with an estimated annual incidence of 324 TB cases per 100,000 inhabitants (19). The World Bank estimated in 2013 the gross domestic product (GDP) per capita in DRC to be 484 USD, and the minimal wage is 65 USD per month.

Active case-finding (ACF) of people with TB disease is thought to be a useful intervention to overcome the challenge of under-detection. Nevertheless, to date, the evaluation of ACF interventions failed to demonstrate their utility and sustainability in programmatic conditions (61, 65). A review published by the World Health Organization (WHO) and later studies (66) revealed that the impact and cost-effectiveness of ACF on tuberculosis detection was greatest when the baseline risk of TB in the targeted population was high. The definition of high-risk groups proposed by the WHO was relatively vague, as it refers to sub-populations that: present a higher burden of TB compared to the general population; sub-populations that have very poor access to health care; and populations for which the annual incidence of TB is over 1%. The WHO strongly recommends ACF among these high-risk groups (33).

In 2013, people with TB and TB-affected communities of the South-Kivu province of DRC created a non-profit organisation called “Ambassadeurs de Lutte contre la Tuberculose” (ALTB). The main objectives of this organisation and its external partners are: (1) to alleviate the burden of TB in the community by accelerating the referral of people with TB related symptoms, (2) to provide direct economic support to TB-affected households by providing them socially-useful jobs, and (3) to bring ACF-costs to an acceptable level.

We evaluated the NNS of this ACF intervention by assessing the ability of the screeners to (i) identify and reach high-risk populations (ii)

convince symptomatic people to be tested for TB and (iii) detect new TB cases.

1.4.3. Material and methods

Study setting

The South-Kivu province lies at the Eastern border of DRC and has an estimated population of 6,6 million inhabitants. With regard to the health system organisation in the South-Kivu province, it is composed of 34 health zones (HZ) containing over 500 health facilities (HF). TB services are provided by 113 of these HF (78 until 2014), which include free Ziehl-Neelsen microscopy and first-line anti-TB drugs, but do not include the cost of the first medical visit. In the province, 10 of these HF perform Xpert MTB/RIF testing, principally among retreatment cases and HIV-infected populations. Chest X-rays are only available in very few HF and their cost (20 USD) is borne by the patients. As a consequence, X-rays are not routinely performed for TB diagnosis due to their prohibitive cost.

The Kivu region of DRC has experienced over two decades of conflicts that had major consequences on the local populations (50, 51), and explains the presence of a sizeable military population. The province has important gold-mining resources that are mainly exploited by the so-called “artisanal mining sector”. These miners live in remote camps, with some of them composed of thousands of miners living in very difficult sanitary conditions and very difficult access to care (52).

In this province, the main high-risk groups for TB were identified as people living in: the households of people with TB; prisons (7, 8); military camps (9, 10); and mining camps (11) were identified as the main high-risk groups for TB.

Data collection and validation

We collected reports from the community ACF screeners that included four key operational indicators of their activities: the number of individuals screened for symptoms in the community; the number of symptomatic people identified with a cough lasting for more than 15 days; the number and identity of these symptomatic people who presented for testing; and their test results. Data were also collected from each HF. These official reports included the number and the identity of people diagnosed with bacteriologically-confirmed TB. Confrontation of these two reports allowed independent validation of the screeners reports.

Creation of local screening groups

Activities started at the end of 2013 and continue at the time of writing (May 2017). The ALTB organisation is structured by local groups composed by one to over 20 screeners, which work in the catchment area of the local HF to whom they refer symptomatic people. To create each local group, we invited up to ten cured patients with TB to participate in a three-day workshop together with the staff of the HF. This workshop covered fundamentals of TB disease, transmission and treatment. We further explained the problem of TB under-detection and the necessity to identify diagnose and treat patients with TB before further TB transmission. We explained why some people are at higher risk of TB, including the main high-risk groups described above. Finally, we explained to the participants how ACF would benefit people with TB disease, their families and communities by preventing death and further transmission.

Organization and financial support for ACF activities

Each local group was asked to autonomously define its internal organization and the planning of ACF activities to be conducted. Limited financial incentives were provided to each group following a quarterly report, subject to the availability of funds and external validation by the NTP staff. The funding system evolved during the intervention, but has always been dependent on the number of symptomatic people referred that were tested for TB.

Briefly, each local screening group receives 10 USD per months to support their costs (administration, transport of screeners to remote areas) and 0.5 USD per symptomatic patient referred to the HF. The intervention further pays 0.5 USD per diagnostic test performed by the HF. Expenses associated with this intervention are illustrated in Supplementary Table 1.

Evaluation of the quality and the sustainability of the ACF activities

Symptomatic screening was based only on the presence for more than 15 days of a cough lasting. The evaluation of the ACF activities followed the WHO criteria, and were (i) the ability of screeners to reach high-risk groups, (ii) their ability to convince symptomatic people to be tested for TB and (iii) their ability to detect new TB cases. We further evaluated the sustainability and the evolution of these indicators over time among the groups that reported a sustained activity over the 12 quarters of the evaluation period. We defined groups with a sustained activity those who reported at least nine quarters of activity, for which there was no reported period of inactivity lasting longer than one quarter and for which two periods of inactivity were separated by at least two quarters of activity.

The impact of ACF activities on TB detection at the provincial level was not used as a quality indicator because of the high variability of the activities during the study period. Rather, we used the provincial SS+PTB notification data to illustrate the proportion of TB cases referred by this intervention during the evaluation period.

Statistical analysis

Screening activities and TB notification for each health facility and quarter were studied as 647 separate units, with an internal correlation for the successive results of the same health facility. The screening activities and the TB notification reports by quarter and health facility followed a negative-binomial ("gamma-Poisson") distribution due to hyperdispersion of results among different areas. Similarly, percentages followed a beta-binomial distribution. Therefore, results are expressed not as counts or percentages of the sums of the populations but as the averages of negative binomial or beta-binomial distributions. This

procedure is mandatory according the structure of the data. It gives a weight to each unit different from the crude count of their population, minimizing the influence of units with exceptionally high counts. The relations between the reported screening activities, TB notification and time were examined using a generalised linear regression of a negative binomial (“gamma-Poisson”) variable. The screening activities and the TB notification reports by health facility followed a negative-binomial distribution, due to hyperdispersion of results between health areas. Similarly, for percentages, we used a generalised linear regression of a beta-binomial variable. We used quasi-least squares, which is suitable for calculating internal correlations to adjust the regression, and chose the hypothesis of constant between-year correlation matrix. The validity of the regressions was checked by screening of the Anscombe’s transformed residuals. To calculate statistical significance, we used the “sandwich variance matrix”. We gave the same weight to each unit for the numerations, percentages, and regressions to prevent an excessive influence of some units high situated in the gamma distribution and to minimize the effects of absence of some HF during some quarters.

Cost-effectiveness

The cost of this ACF intervention was evaluated based on the direct expense costs associated with these activities, including financial incentives for screeners, cost of diagnostic tests plus operation costs of the ALTB organisation.

The cost per new SS+PTB case detected is calculated based on the total cost of the intervention divided by the number of SS+PTB found through ACF.

Ethics statement

We report here a retrospective evaluation of a public health intervention. This was not a trial, nor a research project. Since no human subject’s research was done, ethical approval was not sought and consent was not requested.

1.4.4. Results

ACF activity reported

On average, per quarter, 69 groups (range: 24-100) and 775 screeners (range: 197-1713) participated to the intervention during the 12 quarters of the evaluation period (2014-2016).

In total, 650.434 individuals screened in their communities for a cough lasting for more than 15 days. On average, 14% (± 0.66 , $n=73,418$) of individuals screened reported positive symptoms and 69% (± 1.67 , $n=50,368$) of those with symptoms presented to the health post for a TB test.

Ability to target high-risk populations

On average, 10% ± 0.76 (total count = 4,300) of the patients that received a TB test were confirmed with SS+PTB. The number needed to undergo symptom screening in order to diagnose one person with SS+PTB (NNS: number needed to screen) was 128 (± 14.1) for the average HF.

The NNS was similar among people living in the household of people infected with TB (135 SS+PTB cases among 17.693 individuals screened; NNS=131). The NNS was significantly ($p<0,0001$) lower in: mining camps (508 SS+PTB cases among 10.876 individuals screened; NNS=83), in military camps (195 SS+PTB cases among 3.953 individuals screened; NNS=71) and among prisoners (7 SS+PTB cases among 480 individuals screened; NNS= 68.5).

Screening groups with a continuous activity

Over the 12 quarters of intervention evaluated, 24 out of 100 local groups of screeners met our definition of continuous activity described above. These groups represent 37% of the total number of screeners, 36% of the population screened, 40% of people reporting a cough, 47% of the symptomatic people tested for TB and 59% of the SS+PTB cases notified through this intervention.

The 24 groups with continuous activity caused a significantly higher ($p<0.0001$) proportion of symptomatic screened individuals to undergo TB testing than other groups (Odds ratio: 1.33, 95% CI: 1.30 to 1.36). Among the 24 screening groups with a continuous activity, the mean level of acceptance for the mean HF was 77.11% ($\pm 0.41\%$) and a positive trend was observed over 3 years: +50.25% (± 17.22 ; $p=0.00066$). This evolution is illustrated in Figure 1.3.

Ability to detect additional TB cases

The presence of even a limited number of screeners was associated with an increase in the number of people diagnosed with SS+PTB at the HF level ($p=0.005$). As illustrated in Figure 1.4, the number of screeners involved in each group further tended to be associated with SS+PTB notification.

The average number of SS+PTB cases found per screener and per quarter pooled for the whole period was 0.71. Analysing the individual performance of the screeners, we observed among the 24 groups with continuous activity that the number of SS+PTB cases detected increased by 118% ($\pm 47.5\%$) over three years ($p=0.0051$). This trend is illustrated in Figure 1.5.

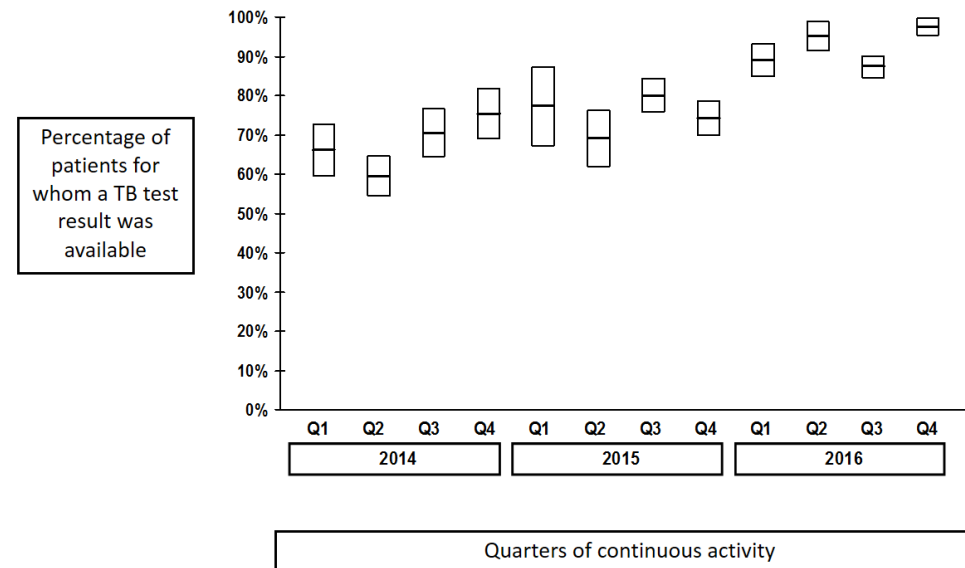


Figure 1.3: Acceptability of TB testing for people identified by community screeners with a cough lasting for more than 15 days. Box plot showing the percentage and standard error of the mean (SEM) of symptomatic people identified for whom a TB test result was available. The results represented are those from the 24 screening groups that reported continuous activity over the evaluation period. In this group, the mean level of acceptance was 77% (± 0.41) and a positive trend was observed over 3 years ($+50.25\% \pm 17.22$; $p=0.00066$).

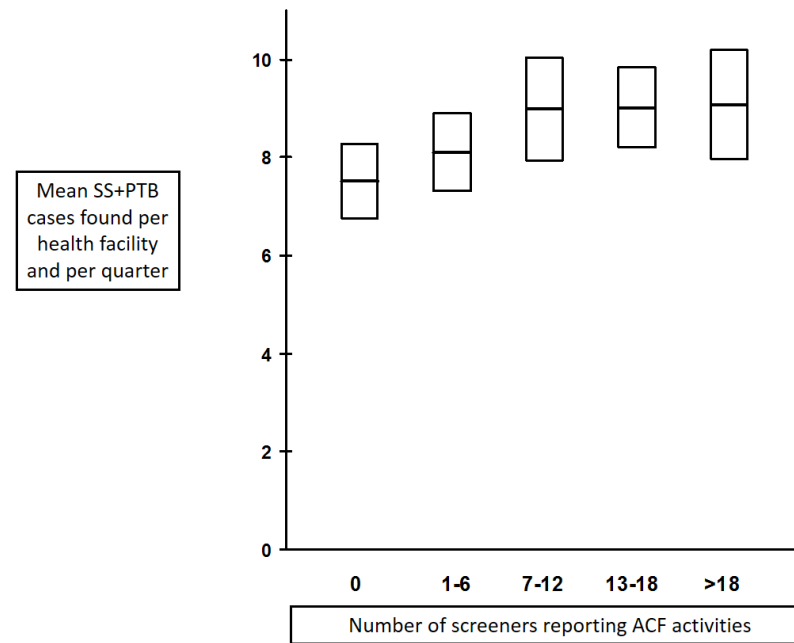


Figure 1.4: Mean SS+PTB cases found (vertical axis) per health facility and per quarter depending on the number of active screeners (horizontal axis). Box plot showing the mean and standard error of the measure. The results represented are those from the 113 health facilities.

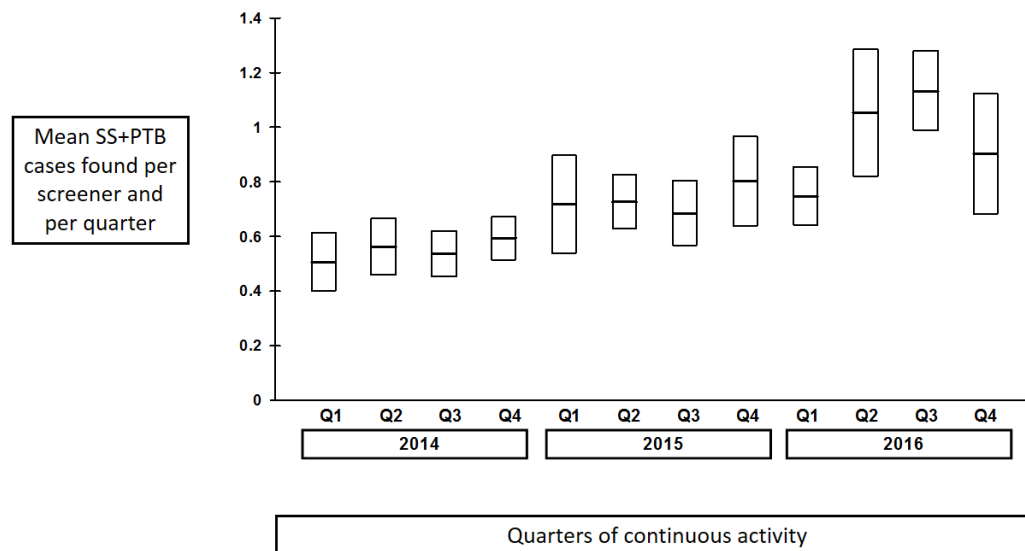


Figure 1.5: Evolution of the number of SS+PTB cases found (vertical axis) per individual screener and per quarter (horizontal axis). Box plot showing the mean and standard error of the measure. The results represented are those from the 24 screening groups who reported a continuous activity over the evaluation period. In this group, the mean number of SS+PTB found by screener and per quarter was 0.71, with a trend of +117.6% over 3 years ($p=0.0051$).

Cost-effectiveness

For evaluating the annual operational cost and the cost-effectiveness associated with this intervention, we took into account the direct expenses associated with the financial incentives distributed to the screeners (0,5 USD per symptomatic patient tested), the structural costs of the ALTB organization and the cost of the laboratory diagnostic tests.

Proportion of TB cases notified through ACF intervention at the provincial level

Between 2014 and 2016, the proportion of SS+PTB cases notified in the South-Kivu province found through this ACF intervention was 42% (4,300/10,247). Although there was a statistically significant increase in the annual number of TB cases notified at the provincial level ($p=0.04$, Figure 1.6), this increase was not statistically significant if adjusted by an estimated 3.1% annual increase in the population of the province. We could not observe a contribution of this intervention with regard to the notification rate of smear-negative pulmonary TB or extra-pulmonary TB.

nostic tests paid to the HF. The total cost of the intervention over 3 years was 189 USD, and the calculated cost per new SS+PTB case found was 44 USD. Details of these expenses are available in Tables 1.1 and 1.2.

	Variable cost per patient tested	Variable cost per number of active local screening groups	Fixed annual supervision and coordination expenses
Financial incentives distributed to the screeners	0.5 USD	NA	NA
Support for local ACF groups	NA	30 USD	NA
Cost of diagnostic tests, paid to the local HF	0.5 USD	NA	NA
Supervision costs (5 full-time staff and field visits)	NA	NA	24,000 USD
Overhead costs (3 full-time staff and office expenses)	NA	NA	15,600 USD

Table 1.1: Budgeted expenses associated with the patient-driven ACF intervention. NA: not applicable; USD: United States Dollars

	Incentives distributed based on the number of symptomatic people tested (n=50.368)	Incentives distributed quarterly for each screening group reporting ACF activity (n=647)	Supervision and coordination expenses (3 years)
Active screeners	25.184 USD	NA	NA
Local screening groups	NA	19.410 USD	NA
Health facility	25.184 USD	NA	NA
Field supervision	NA	NA	72.000 USD
Overhead costs	NA	NA	46.800 USD
Total	188.578 USD		
Cost per new SS+PTB	44 USD		

Table 1.2: Cost of the 3-year ACF intervention and cost-per new SS+PTB case found. NA: not applicable; USD: United States Dollars

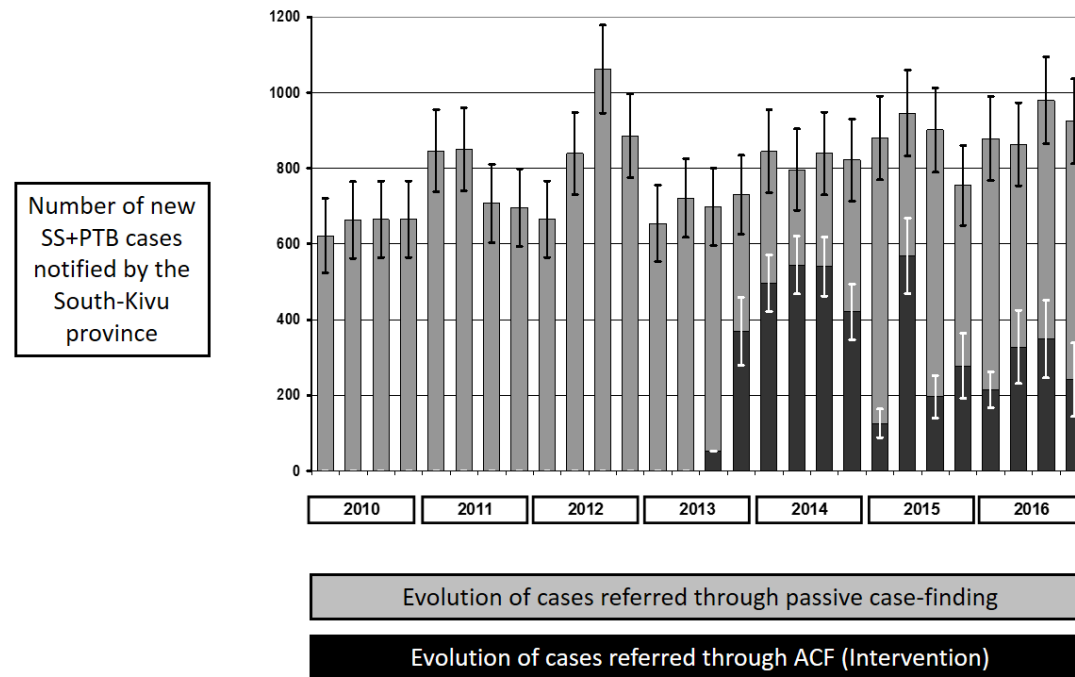


Figure 1.6: Number of new SS+PTB people notified by the South-Kivu province of DRC between 2010 and 2016 (light grey bars). The proportion among these referred through the ACF intervention between 2013 and late 2016 are represented (dark grey bars).

1.4.5. Discussion

Predicted sensitivity of the screening algorithm applied

The choice of the screening protocol was determined by the simplicity of the symptomatic screening at the community level and the availability of diagnostic tests in the HF. The people infected with TB and TB-affected populations that performed the community screening did not have a medical background. It was therefore proposed that they would focus on a prolonged cough, lasting for more than 15 days. This symptom is expected to be present in only 35% [CI 95%: 24%-46%] of people that have active pulmonary TB disease. When followed by a smear microscopy or Xpert test, this algorithm is expected to detect only 21% to 32% of all active TB cases participating in the screening intervention (3).

For these reasons, it is probable that this intervention missed the majority of TB cases included in the screening process.

Number needed to screen to find one positive case

The NNS in the general population was 128 ± 0.41 . This number is comparable with the results reported in populations with an annual incidence higher than 300 per 100.000 people (33). The NNS was similar in the total population screened and among those living in the household of people infected with TB. This observation suggests that the screeners perform ACF interventions in households and communities where the risk of TB is higher than in the general population. The NNS was lower among people living in some pre-identified high-risk populations, particularly those living in mining camps, military camps and prisons, supporting WHO policy that those groups should be prioritized for ACF.

Acceptability of screening

The proportion of symptomatic people that accepted TB testing was 77% ($\pm 0.41\%$), and this proportion increased over time. The positive evolution observed during the evaluation is probably associated with the cumulated experience of the screeners and

the amelioration with regard to their ability to convince symptomatic people to be tested for TB. Non-acceptance for TB testing can be explained by (i) the distance to the nearest HF, which can be considerable for people living in rural areas; (ii) the level of trust that the general population has towards non-health professionals in regard to their ability to identify TB; (iii) the stigma surrounding TB and (iv) the anticipated socio-economic repercussions of TB for the patients and their households.

Positive evolution of quality indicators over time

Among the 24 screening groups that were evaluated throughout the evaluation, we found a positive evolution of several quality indicators. The number of people screened, the level of acceptability and the number of new SS+PTB cases identified increased significantly over time. These figures demonstrate that the individual ability to perform ACF increases with experience. Further, these observations support the possible positive impact of the professionalization of people performing ACF compared to short-term involvement of screeners.

Variability among screening groups

We observed a high variability among the screening groups with regard to their indicators of ACF, including the number of people screened and the subsequent number of people detected with SS+PTB. This could be explained by a variation in the baseline incidence of TB among different zones and a variability of the clinical skills of local medical staff, as this has been suggested before (67).

The level of external funding available for the ALTB organization appeared to be the most important factor influencing the number of local groups and consecutively the number of screeners involved during this evaluation. This effect can be observed in Figure 1.7.

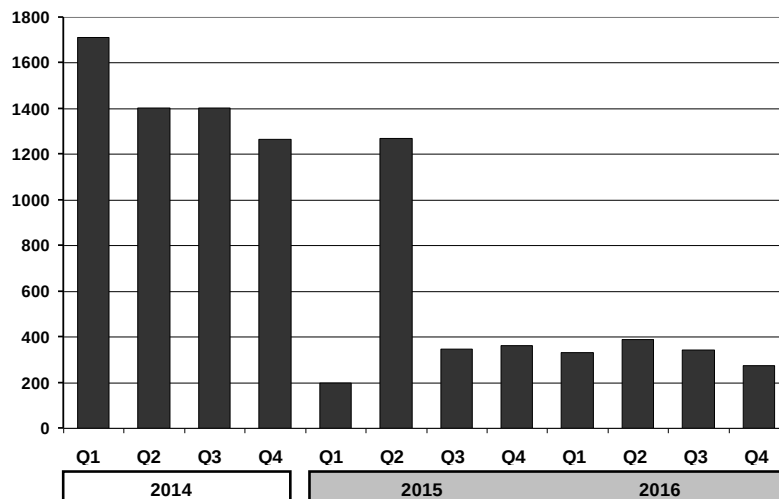


Figure 1.7: Number of screeners participating to the ACF intervention between 2014 and 2016. The difference observed between 2014 (white block) and 2015-2016 (grey block) followed a decrease in funding available.

Impact on TB-affected populations

This intervention had several positive impacts on TB-affected populations. First, people infected with TB and their relatives were directly involved as screeners and received direct financial support for the work performed. Although this support was limited, it was as a major driver of performance in this very poor setting. Outside the financial aspect, people infected with TB participating in the ACF intervention reported a positive social impact associated with the possibility to perform a socially useful work for their community. Interestingly, we found a correlation between the number and the long-lasting activity of screeners involved in a particular area and the proportion of SS+PTB cases found among the people screened. This may be due to a spontaneous adaptation of the intensity of the activity by local groups based on the local utility and yield of their screening activities. When a group finds more patients, it tends to increase its screening activities and better target the subpopulations with the highest risk of TB. Over the 12 quarters of intervention evaluated, 24 out of 100 local groups of screeners met the definition of continuous activity as described above. These groups represented had higher performance indicators in regard to the number of screeners, the population

screened, the number of symptomatic patients identified, the proportion of symptomatic patients tested for TB and the number of SS+PTB cases found.

Secondly, because this intervention contributed to the diagnosis of 4.300 individuals with SS+PTB prior to their self-referral to the HF, it possibly prevented death and TB-related morbidity in a proportion of these patients. By accelerating access to diagnosis and treatment, this intervention may have reduced transmission of TB in the household and communities of the people infected with SS+PTB.

Finally, through the screening of over 650.000 individuals living in a high TB burden setting, it is reasonable to hypothesize that this intervention possibly increased the general awareness of TB and its symptoms in the general population of the South-Kivu province. This intervention may therefore have positive long-term repercussions.

Impact on TB notification

The impact of this intervention on SS+PTB notification by the HF was associated with the number of screeners involved in each local screening group. We could not demonstrate that the increase of SS+PTB notification observed at the provincial level was associated with this intervention considering the estimated natural demographic increase in this population. We could not observe an impact of this intervention on the notification of smear-negative pulmonary TB or extra-pulmonary TB. Nevertheless, the pertinence of evaluating a limited ACF intervention by looking at provincial notification is limited due to the fact that only a fraction of HF benefited from the support of local screening groups. This non-randomized retrospective evaluation was not designed to assess the cost-effectiveness or long-term impact of this ACF intervention. Further studies will be needed to address these important elements.

1.4.6. Conclusion

This study highlighted the utility of performing ACF in the general population of the South-Kivu Province of DRC. It further demonstrated the ability of people infected with TB and TB-affected populations to perform ACF, to improve their skills over time, and to target ACF to populations at high-risk for TB.

In this evaluation, the low NNS and the limited costs are compatible with WHO criteria for recommending ACF as a cost-effective complement to the biomedical approach. This NNS could have been further decreased if highly sensitive diagnostic tools were available in local HF and if the clinical skills of local health staff allowed better identifying TB disease in the absence of a bacteriological confirmation (67).

The originality of the present intervention is the fact that the screeners involved are people infected with TB and their relatives, organised as a local non-profit association. This characteristic is probably associated with the ability of this intervention to intervene in high-risk groups and hard to reach populations.

The success of this community-led intervention was associated with (i) the high number of people screened in the community; (ii) the low NNS and (iii) the impact on SS+PTB notification at the HF level. In the scientific literature, we found several successful peer-led interventions that reported positive impact on the control of global pandemics including HIV (68, 69), malaria (70) and TB (71, 72). The present study supports the conviction that peer-led interventions can become a valuable complement to the biomedical approach in the global effort to control major pandemics.

To achieve TB eradication, we propose that people infected with TB and their communities should actively engage in ACF interventions at a global level. Their ability to perform these tasks among the most vulnerable and hard-to-reach populations allow increasing the accessibility of health services and complement the biomedical approach. The financial support provided to these people infected with TB becoming active screeners could further help to overcome the individual costs associated with TB disease (38).

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1.5. Identification and characterization of high risk groups for TB

Identifying high-risk groups for TB within the general population is a critical step towards disease control, as it allows interventions that have the highest impact to be prioritized. At the international level, a number of high-risk groups have been rigorously studied (33) and consequently receive the adequate attention through guidelines and funding of specific activities. As discussed in the introduction (Table 0.2), few high-risk groups have been studied in high incidence settings. These are miners, people living with HIV, prisoners and household contacts of people infected with TB.

According to UNAIDS estimates, HIV prevalence in DRC was 0.8% [0.7% - 1.1%] in 2015. This low prevalence in the general population, accompanied by the probable level of under-detection of HIV among infected patients makes it complex to develop targeted interventions in this population.

The peer-led ACF intervention described above resulted in a NNS of 128 in the population targeted by these screeners. Looking at the results for specific populations, we found that the NNS was significantly lower in mining camps (NNS=83), in military camps (NNS=71) and among prisoners (NNS= 68.5).

We proposed to investigate deeper the situation in the military population, as this population was the only high-risk group which had not been described before in the context of high burden countries.

1.6. Understanding the burden of TB in the military population

1.6.1. Context

The South-Kivu province has a long history of armed conflicts. Among the consequences of this situation, there is an important military population in the province, including in the capital city Bukavu. The major military camp of this city is the “Camp Saïo” where soldiers and their families live together, generally in tents. This camp constitutes approximately 12,000 individuals.



Figure 1.8: The camp Saïo in Bukavu is the largest military camp in South-Kivu. Most soldiers live with their families in tents. (Photo: Emmanuel André)

1.6.2. TB and HIV notification in the military health facility

We analysed retrospectively the TB and HIV notification data collected at the military hospital of Bukavu between 2009 and 2011, and compared the results with the civil reference hospital of the same city. We observed the following indicators:

- HIV infection among TB-infected patients was 60%, compared to 36% in the civil reference hospital of the same city.
- Over 50% of people infected with TB were lost to follow-up before completing treatment.
- The HIV-positivity rate among patients tested for HIV infection at the military hospital was 27% (558/2050), compared to 18% in the general population (361/2052).
- 55% of the soldiers tested for HIV in the 20-40 years age group had a positive result.

Between 2011 and 2013, 468 samples from soldiers clinically suspected of having pulmonary TB despite a negative smear result were tested with GeneXpert MTB/Rif. HIV status of patients were recorded when available.

- 30 patients (6%) tested positive for TB, with a proportion decreasing from 16% (year 1) to 2,25% (year 3). Among TB-positive patients, 8 (27% (95%CI 14 – 46%)) had Rifampicin resistant TB.
- HIV test results were available for 25% (116/468) of the patients, HIV prevalence among all patients tested for TB was 19% (95%CI 12 – 27%). HIV prevalence among TB-positive patients was 71% (95%CI 40 – 90%).

1.6.3. Semi-qualitative study

We performed semi-structured interviews with 14 individuals living in the military camp of Bukavu, and 2 interviews among the medical staff of the military hospital. This method is well validated in the domain of social sciences and consists of having a list of prepared themes to be explored, without constraining the discussion to rigid forms. In our study, we questioned soldiers about their knowledge, perception and behaviors regarding HIV and TB. The sociologist E. Goffman defines “total institutions” as places where people are all treated alike and behaviour is regulated. In these closed environments, individuals develop secondary adjustments, or “abnormal” behaviours as a way to express their remaining capacity of auto-determination.

These concepts can be transposed to soldiers and their relatives living in military camps. Our hypothesis was that the high rates of HIV and TB infection could be partly explained by the presence in these camps of “non-responsible” sexual and social behaviours exposing them to increased risk of diseases transmission and increased morbidity associated with these diseases.

These interviews highlighted “abnormal” behaviours that increase the risk of exposure to TB and HIV.

The denial of HIV

- 13/14 reported unprotected sexual intercourses with multiple partners.
- 10/14 did not consider being tested for HIV in the future.
- 5/14 reported using condoms to light the fire or clean their shoes.
- 3/14 believed that HIV testing has a negative impact on health

The ignorance of TB diagnosis and treatment

- 9/14 reported knowing someone who had TB disease
- 8/14 did not know that TB was a curable disease
- 7/14 considered prolonged cough a sign of TB
- 2/14 considered hemoptysis was the only symptom of TB

This qualitative study helped us to understand why the military population in Eastern DRC were at higher risk of HIV and TB despite the fact that these people had access to free care, free condoms and posters describing the symptoms of TB. We found that beliefs and perceptions of these contagious diseases were a major obstacle to early detection, effective treatment and disease control.

1.6.4. Cross-sectional survey in a military camp of Bukavu (Submitted manuscript)

Cisse Geleyn^{1*}, Emmanuel André^{3,5}, Bertin Bisimwa², Olivier Bahati Rusumba³,
Matthew Saunders[?], Jean-Paul Chirambiza², Denis Kikobya⁴, Zacharia Kashongwe²,
Steven Callens^{1,6*}

Affiliations:

1: Faculty of Medicine, Ghent University, Ghent, Belgium

2: National Tuberculosis Program, South Kivu, Democratic Republic of the Congo

3: Ambassadeurs de Lutte contre la Tuberculose, Democratic Republic of the Congo,
Former TB association

4: National Army, Democratic Republic of the Congo

5 : Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Université
catholique de Louvain, Belgium

6: Dept Internal Medicine, Ghent University Hospital, Ghent, Belgium

* Corresponding authors

E-mail: cisse.geleyn@ugent.be

Keywords: tuberculosis; prevalence; resource-limited settings; high-risk population;
military; Xpert MTB/RIF; screening; epidemiology

Abstract

Introduction: The military population in the Democratic Republic of Congo (DRC) is suspected to be a high-risk group for tuberculosis (TB). No prevalence study has yet assessed the burden of disease in this particular population.

Methods: We performed a cross-sectional, observational survey in a military camp of Bukavu, in the South-Kivu Province. Soldiers and their relatives were evaluated for TB-associated symptoms and asked to provide information on TB risk factors using questionnaires. All suspects were asked to provide a sputum sample, that was tested using smear microscopy and the Xpert MTB/RIF® PCR assay.

Results: 4047 individuals from 2630 households within the Saïo military camp were evaluated. 4% (n=158) of the persons interviewed were undergoing TB treatment, and 24.4% (n=987) had symptoms associated with pulmonary TB. Bacteriological investigation identified 6 additional, previously undiagnosed, pulmonary TB cases. Definite TB cases were less likely to have access to electricity (OR 0.41, P=0.003) and tap water (OR 0.35, P=0.010) and were more frequently exposed to indoor smoking (OR 1.71, P=0.009).

Conclusion: We found a high burden of TB disease within a Congolese military camp. In this camp, TB was more frequent among individuals with the lowest socio-economic status. Improved access to health services, regular screening for TB and introduction of preventive therapy should be prioritized in order to decrease the TB burden in this vulnerable population.

Chapter 2: Evaluation of the clinical skills of first-line health care providers. Focus on paediatric TB

2.1. Context

Globally, an estimated 10% of people infected with TB are aged less than 15 years, but two third of these are believed to be un-diagnosed or un-notified (8). The challenge of under-detection of TB is thus even more important among children than adults for whom approximately one third of people infected with TB are currently missed. Paediatric TB (PedTB) can rapidly evolve towards life-threatening manifestations such as miliary TB or meningitis (73). Improving the capacity of health care providers to detect TB among children will not only save lives, but also have major repercussions with regard to the control of the epidemic.

In DRC, according to the Department of Economics and Social Affairs of the United Nations in 2015, children aged less than 15 years represent 46% of the population compared to 26% globally. Despite the over-representation of this age group, only 11.4% of people infected with TB were less than 15 years in 2015. Clinical guidelines developed by the National TB Programme of DRC include recommendations for the clinical diagnosis of PedTB. These recommendations are relatively scanty, as they take into account the current situation of the vast majority of HF which lack technical resources such as X-rays, molecular tests or tuberculin skin test (TST). Therefore, the diagnosis of PedTB is principally based on non-specific clinical symptoms and the notion of TB contact in the household. In practice, the use of rapid molecular diagnostic tests, which have been progressively scaled-up in the country since 2012, is currently limited to patients with presumptive drug resistant TB or HIV-TB coinfection. Due to this particular context, and considering that no drug resistance survey has been performed in DRC to date, the incidence of drug-resistant TB (and particularly among children) cannot be precisely estimated. Regular supervision activities are performed by the national and provincial teams, although some areas are inaccessible due to insecurity or the high costs associated with travelling in the most remote areas.

2.2. Impact of resources on PedTB detection

Unlike most HF of rural DRC under-resourced in material and qualified staff, the few HF actively supported by MSF implement a more pro-active attitude concerning the diagnosis of PedTB. MSF recommends a documented growth assessment of children suspected or exposed to TB. Furthermore, MSF supports sample transportation and provides technical resources such as X-rays, HIV testing, TST or investigations for EPTB. During the study period, there was no significant difference in the population covered by each HF between rural, isolated (enclaved), or MSF-associated HF ($p = 0.14$).

We observed that the proportion of PedTB cases varied significantly among the health zones (HZ). HZ with the highest concentration of medical doctors seemed to better detect TB among children. The Figure 2.1. represents the 34 HZ of South-Kivu and the proportion of PedTB cases notified among all TB cases. The urban health zones with a higher concentration of doctors (black cross) and the health zones supported by MSF (grey diamonds) showed the highest proportion of children among TB cases.

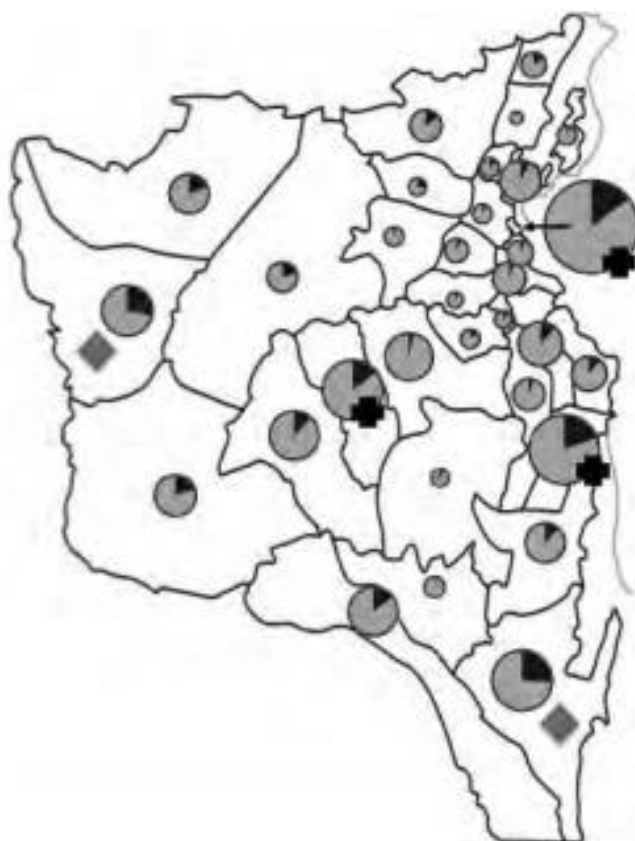


Figure 2.1: Proportion of PedTB (black) among all TB cases in the 34 health zones of the South-Kivu province. The urban health zones with a higher concentration of doctors (black cross) and the health zones supported by MSF (grey diamonds) showed the highest proportion of children among TB cases.

2.3. Impact of clinical skills on PedTB detection

Insufficient clinical skills among health professionals has been reported as the main reason behind the frequent missed opportunities to diagnose and treat patients with TB that present to health facilities (74). A qualitative study performed in Tanzania (75) reported that “inadequate awareness of the burden of childhood TB, limited knowledge of the wide spectrum of clinical presentation and lack of clinical decision support strategies is detrimental to the health staff’s central responsibility of suspecting and referring children with TB especially in the early disease stages”. The study authors stressed the importance of implementing strategies focusing on awareness among health staff and clinical skills in order to improve diagnosis.

We performed a retrospective of 28,878 cases of TB notified in the South-Kivu province between 2010 and 2015. Our aim was to better understand the determinants of PedTB under-detection and to develop a method to rapidly identify health facilities (HF) which have a higher probability to be facing PedTB under-detection as a result of the inability of health-care providers to recognise this clinical entity. Such method would allow the design and implementation of targeted interventions, and to evaluate their impact on PedTB detection.

We found that the reported incidence is highly variable between HF, and particularly when looking at the different forms of the disease which are notified through quarterly reports. This observation suggests that there are considerable inequalities between HF with regard to the ability to diagnose complex clinical presentations of TB, including PedTB and smear negative pulmonary TB (SS-PTB) among adults.

In the South-Kivu province of DRC, we found a very strong association between under-detection of SS-PTB among adults and under-detection of PedTB. This can be explained by the fact that recognizing these complex clinical entities require (particularly in the absence of radiological investigations and laboratory assays) higher clinical skills than the common smear positive pulmonary form of the disease. Interestingly, four rural HF supported by MSF had the highest proportion of TB cases diagnosed (mean 26.7%), and seemed to perform better than urban facilities with regard to PedTB detection. The explanation for these high figures is probably the highly qualified and adequately supported health staff and free access to X-rays or laboratory testing.

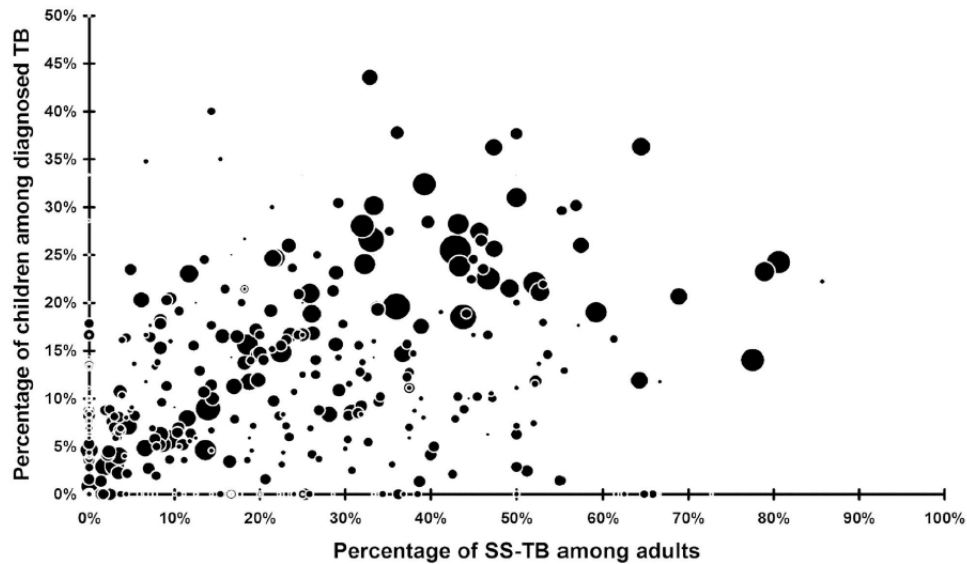


Figure 2.2: Correlation between the percentage of PedTB and the percentage of SS-TB among adults. Circles represent the number of TB cases notified for each HF during one year (6 years are represented). A strong correlation ($p < 0.000001$) was found between the proportion of SS-TB among adults and the proportion of PedTB.

In this analysis, we also observed that the HF reporting the highest number of TB cases (all forms of the disease), tended to detect a higher proportion of children.

In the South-Kivu province, the clinical skills and the clinical experience of the front-line health professionals have an important impact on the detection of PedTB. The negative impact of insufficient clinical skill is probably further exacerbated in the absence of radiological and laboratory resources.

2.4. Prediction of under-detection of PedTB in the Democratic Republic of Congo: Experience of six years in the South-Kivu Province

Based on the observations described above, we propose that the proportion of SS-TB among adults can be used as an indirect, but reliable indicator of PedTB under-detection.

When looking at different HF located from a same epidemiological and socio-economical entity,

$$\text{Level of underdetection of PedTB} \sim \frac{\text{SS} - \text{PTB among adults}}{\text{SS} + \text{PTB among adults}}$$

This indicator should enable TB control programs and their partners to identify those settings requiring additional support to tackle under-diagnosis of PedTB and other complex clinical presentations of the disease. The interventions should focus in parallel on the awareness of PedTB, active case finding strategies, strengthening of clinical skills oriented towards PedTB and finally the implementation of adequate technical resources such as X-rays, TST and rapid molecular laboratory assays.

Although improving clinical skills of health professionals should not be considered as the only priority, the importance of these human skills should not be under-estimated as they remain the cornerstone of rational use of diagnostic and treatment resources.

The full text is available online (open access)

RESEARCH ARTICLE

Prediction of Under-Detection of Paediatric Tuberculosis in the Democratic Republic of Congo: Experience of Six Years in the South-Kivu Province

Emmanuel André^{1,2*}, Yvette Lufungulo Bahati³, Eric Mulume Musafiri⁴, Olivier Bahati Rusumba⁵, Dimitri Van der Linden², Francis Zech¹

1 Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium, **2** Cliniques Universitaires Saint-Luc, Brussels, Belgium, **3** Hôpital Provincial Général de Référence de Bukavu, Bukavu, Democratic Republic of Congo, **4** Coordination Provinciale Lèpre et Tuberculose, Bukavu, Democratic Republic of Congo, **5** Ambassadeurs de Lutte contre la Tuberculose, Bukavu, Democratic Republic of Congo

* emmanuel.andre@uclouvain.be



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Abstract

Introduction

In the field of tuberculosis (TB), and particularly in regard to paediatric TB (PedTB), clinical skills of health professionals play an important role in determining quality of care. In an era where novel diagnostic technologies and efficient treatment regimens are being made available for the poorest, we must not divert our attention from the importance of clinical skills, as this deliverable remains the cornerstone of individualized patient care and ultimately the best assurance for optimal use of resources. The aim of our work was to study the epidemiology of PedTB and the determinants of PedTB under-detection in the South-Kivu Province of the Democratic Republic of Congo (DRC), a setting with nearly no technical resources allowing to support the clinical diagnosis of PedTB, i.e. chest X-rays, rapid molecular tests or culture laboratories.

Methods

We collected TB notification data from 2010 to 2015 and analysed the space-time variations in notification for the different forms of TB among the 113 health facilities (HF) the South-Kivu Province, a region with a low HIV incidence. The different forms of TB notified were: smear positive pulmonary TB (SS+PTB), smear negative pulmonary TB (SS-PTB) and extra-pulmonary TB (EPTB). We further analysed the distribution of these different forms of the disease per age group and explored the possibility to predict the detection of PedTB.

Results

Significant differences were observed between HF in regard to the proportion of paediatric TB and the proportion of SS-TB among adults. We found a strong correlation between the proportion of PedTB and three major factors: the proportion of TB cases with no

Abbreviations: DRC, Democratic Republic of Congo; EPTB, Extra pulmonary tuberculosis; HZ, Health Zone; HF, Health facility; INH, Isoniazid; MSF, Médecins Sans Frontières; MTB, *Mycobacterium tuberculosis* complex; NGO, Nongovernmental organization; PedTB, Paediatric tuberculosis; RIF, Rifampicin; SS+PTB, Smear-positive pulmonary tuberculosis; SS-PTB, Smear-negative pulmonary tuberculosis; SS-TB, Smear-negative tuberculosis (SS-PTB and EPTB); TB, Tuberculosis; WHO, World Health Organization.

bacteriological confirmation (SS-TB) among adults, the number of TB cases notified by the HF and the fact that the HF was supported or not by Médecins Sans Frontières (MSF). The proportion of SS-TB among adults was found to be a valid indicator for predicting the level of detection of PedTB at the same HF.

Conclusion

Our observations strongly suggest that under-detection of PedTB is associated with insufficient clinical skills and technical resources at the HF level which similarly affects other forms of the disease, in particular SS-TB. We demonstrated that, in the specific context of South-Kivu, under-detection of PedTB can be predicted by a low SS-TB/SS+PTB ratio in the adult population. In the context of severely under-resourced settings, this ratio could be used to rapidly identify HF that should benefit in priority from deeper evaluation, and eventually targeted interventions.

Introduction

The World Health Organisation (WHO) estimates that there were 9.6 million new patients infected with tuberculosis (TB) in 2014. Globally, an estimated 10% of TB patients are children less than 15 years, but two third of these are currently un-diagnosed or un-notified. The challenge of under-detection of TB is thus much more important **among children than adults** for which approximately one third of TB patients are currently missed [1]. Paediatric TB (PedTB) can rapidly evolve towards life-threatening manifestations such as miliary TB or meningitis [2]. Improving the capacity of health care providers to detect TB among children will not only save lives, but also have major repercussions in regard to the control of the epidemic.

The Democratic Republic of Congo (DRC) lies among the few high TB burden countries which did not meet any of the 2015 targets in regard to TB control: incidence is not falling and neither prevalence nor mortality was reduced by 50% in comparison with 1990 figures. In 2015, DRC notified 111,683 TB cases (146.8/100,000), roughly half of the estimated numbers of cases estimated for this country by WHO. In this low-income country, children aged less than 15 years represent 46% of the population compared to 26% globally [3]. Despite the over-representation of this age group, only 11.4% of TB patients were less than 15 years in 2015.

The aim of our work was to develop a method to rapidly identify health facilities (HF) which have a higher probability to be facing PedTB under-detection as a result of the inability of health-care providers to recognise this clinical entity. Such method would allow the design and implementation of targeted interventions, but also to evaluate their impact on PedTB detection.

Materials and Methods

Study setting

The DRC presents a very large network of HF constituting the public health system. TB-related services are well integrated within primary health care services, which offer free access to TB diagnostics—including rapid molecular tests in a minority of HF—and treatment [4].

Clinical guidelines developed by the National Tuberculosis Programme of DRC (17) include recommendations for the clinical diagnosis of PedTB. These recommendations are relatively scanty, as they take into account the current situation of the vast majority of HF which

lack technical resources such as X-rays, molecular tests or tuberculin skin test (TST). Therefore, the diagnosis of PedTB is principally based on aspecific clinical symptoms and the notion of TB contact in the household. In practice, the use of rapid molecular diagnostic tests, which are being progressively scaled-up in the country since 2012, is currently limited to patients with presumptive drug resistant TB or HIV-TB coinfection. Due to this particular context, and considering that no drug resistance survey has been performed in DRC to date, the incidence of drug-resistant TB (and particularly among children) cannot be precisely estimated. Regular supervision activities are performed by the national and provincial teams, although some areas are inaccessible due to insecurity or the high costs associated with travelling in the most remote areas.

The South-Kivu province lies along the eastern border of DRC and presents a population of over 6.6 million inhabitants, mainly living in rural areas. Health delivery is provided to the population through over 500 HF, among which 78 (2010–2013) to 113 (2014–2015) provide TB services: Ziehl-Nielsen microscopy and provision of first-line anti-TB drugs. Since 2012, ten laboratories located in urban HF have been progressively equipped with GeneXpert MTB/RIF technology. The province does not have a laboratory able to perform mycobacterial cultures—the most sensitive diagnostic method—and diagnostic samples are usually not sent to the National Reference Centre in Kinshasa unless drug resistance is suspected.

The province is divided in 34 HZ, and each HZ has 1 to 6 HF. A HF is responsible for a median of 51,500 habitants (percentiles 10 and 90: 17,800 and 97,300). The number of TB cases notified by each HF is largely variable: 10% of these report less than 7 cases per year, while 10% report more than 140 cases, the median being 32. Among the 34 health zones (HZ), five are mainly urban (Bagira, Kadutu, Ibanda, Kamituga, and Uvira), while the remaining 29 HZ are located in rural areas. Some of the latter are relatively well served in terms of infrastructure and communications, while 11 are difficult to reach due to the absence of roads or on-going armed conflicts. Most of these isolated HZ are located in the centre and west of the province. As a result of the long-lasting humanitarian crisis in this region, the vast majority of the 34 HZ receive external support from non-governmental organisations (NGOs) for the delivery of primary health care services. This support is generally integrated within the existing public system and fully consistent with the national guidelines. Four HF located in the HZ Shabunda, Fizi and Kimbi-Lulenge (since 2012), are nevertheless considered as exceptions: these are supported by Médecins Sans Frontières (MSF) which applies a more “independent” policy in regard to the organisation of health services, including supplementation of the health structures with qualified medical staff, clinical guidelines and technical resources including laboratory equipment [5]. In regard to the diagnosis of PedTB, MSF recommends a documented growth assessment of the children suspected or exposed to TB. Furthermore, MSF supports sample transportation and provides technical resources such as X-rays, HIV testing, TST or investigations for EPTB. During the study period, there was no significant difference in the population covered by each HF between rural, isolated (enclaved), or MSF-associated HF ($p = 0.14$).

Data collection and statistical analysis

We collected standard notification reports provided by the National TB Program. These reports include the notification of the different forms of TB, including smear positive pulmonary TB (SS+PTB), smear negative pulmonary TB (SS-PTB) and extra-pulmonary TB (EPTB). In this analysis, SS-PTB and EPTB among adults are considered jointly as smear-negative TB (SS-TB), while PedTB includes all forms of the disease among children under 15 years.

We analysed the changes in the incidence and proportion of different clinical TB presentations for each HF over a period of 6 years from 2010 to 2015. Each HF and each year was

studied as a separate unit, with an internal correlation for the successive results of the same HF. The relations between the percentages of PedTB and diverse factors were examined using a generalised linear regression of a beta-binomial variable. Actually, the percentages of paediatric case followed a beta-binomial distribution, due to hyperdispersion of results between health areas. We used quasi-least squares, which is suitable for calculating internal correlations to adjust the regression, and chose the hypothesis of constant between-year correlation matrix. To calculate statistical significance, we used the "sandwich variance matrix" augmented by the bias correction proposed by Morel, Bokossa and Neerchal [6], which may be evaluated by the normal distribution. To study the relation between the percentages of PedTB cases with other factors, we first explored the curves of non-linear regressions and further selected the adequate expression to include these factors in linear univariate and multivariate regressions. We used the logarithm for the total number of TB cases reported for the populations surveyed by the HFs and the percentages of SS-TB adults. We report here only the results of linear regressions.

The data being anonymised, ethical approval was not required for this retrospective study.

Results

Notified incidence of TB

Between 2010 and 2015, the South-Kivu province reported 28,878 cases of TB. Among the 26,337 patients for which clinical outcome was available, 3.73% deceased during the year following diagnosis. This proportion was higher among SS-PTB (6.22%) and EPTB (4.89%) compared to SS+PTB (2.79%). During this period, the mean incidence of TB per 100,000 people per year was 84.5 new cases, among which 63.6% were adults with a pulmonary disease confirmed by microscopy. A transient increase in notification was observed due to the intensification of active case-finding strategies supported by two TB REACH grants [7].

The notified incidence was largely dependent on the notification of adults with SS+PTB, accounting for up to 100% of all TB notified in some HZ.

Diagnosis of smear negative and extra-pulmonary TB among adults

Adults with SS-TB accounted for 21.8% of all cases notified and 28.5% of adult cases.

With regard to the yearly notified cases of SS-TB for each HZ during the 6 years, the percentage of SS-TB among adult cases was highly variable from one HF to the other. Moreover, we observed significant differences of the average percentages between rural HZ (15.7%), rural enclaved (19.4%), urban (37.5%), or MSF-supported HZ (34.7%).

Diagnosis of paediatric TB

The proportion of children less than 15 years accounted for 14.6% of all TB cases notified in the South-Kivu province, compared to 11.4% at the national level ($p < 0.00001$). In total, 4,219 PedTB cases were notified, among which 18.1% had a bacteriologically confirmed diagnosis and only two children were diagnosed with MDR-TB. In total, 56 MDR-TB cases were reported during the same period.

The proportion of PedTB cases notified varied significantly between HZ, with a ratio varying between 0% and 40%. This important variation cannot be explained only by demographic particularities, and rather suggests an important heterogeneity in the ability to diagnose PedTB between HF.

Looking at the yearly notified cases of PedTB for each HF during the 6 years, we could not find any significant difference between urban, rural and isolated (enclaved) HZ ($p = 0.074$). The strongest association was found between the ratio of PedTB and the ratio of SS-PTB

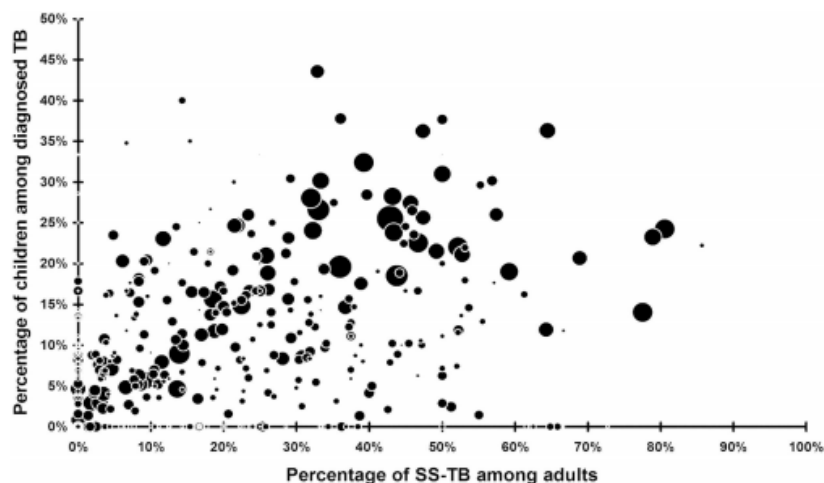


Fig 1. Correlation between the percentage of PedTB and the percentage of SS-TB among adults. Circles represent the number of TB cases notified for each Health Facility (HF) during one year (6 years are represented). A strong correlation ($p < 0.000001$) was found between the proportion of SS-TB among adults and the proportion of PedTB.

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among adults (Fig 1) ($p < 0.000001$). Furthermore, strong correlations were observed between PedTB detection and the total number of TB cases diagnosed ($p = 0.000016$) and with the presence of MSF in the HF ($p < 0.00001$). The size of the population covered by each HF has no significant correlation with the ratio of PedTB.

Development and validation of a model predicting under-detection of paediatric TB

For the final multivariate linear regression, we used the results as a model to predict the number of PedTB cases for each HF and each year (Fig 2). This model is based on 3 variables easily available through the standard notification reports: the percentage of SS-TB among adults, the total number of TB cases notified by the HF during the year and an external support received by MSF. In the multivariate regression, these three factors remain independently significant: being in a HF with a low or great percentage of adults with SS-TB ($p < 0.000001$), being or not in a MSF-supported HF ($p < 0.000001$) and being in a HF with a low or great number of TB cases notified ($p = 0.0011$). The validity of this model was further confirmed by the analysis of the normalized Anscombe's residues (data not shown).

Discussion

Particularities of the study setting and implication for TB surveillance

The South-Kivu province reports an incidence of 80.7/100,000 for all forms of TB, much lower than the 325/100,000 national incidence estimated by WHO. We found that the reported incidence is highly variable between HF, and particularly when looking at the different forms of

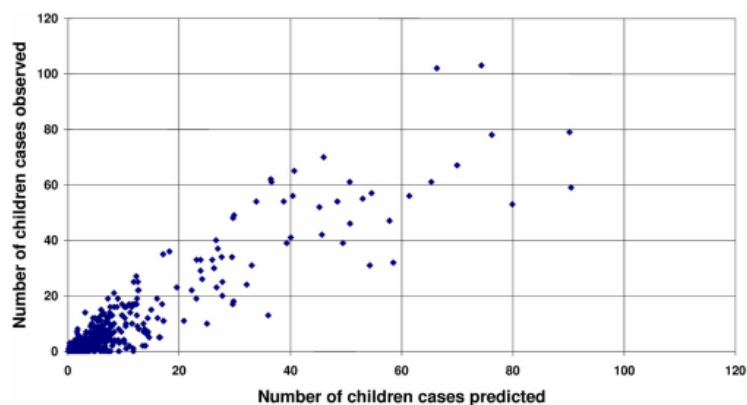


Fig 2. Application of the model predicting under-detection of PedTB at the HF level based on 3 informations: percentage of SS-TB among adults, external support received by MSF and the total number of TB cases notified by the HF during the year.

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the disease which are notified through quarterly reports. This observation suggests that there is a high inequality between HF in regard to the ability to diagnose complex clinical presentations of TB, including PedTB and SS-TB among adults. We could demonstrate with a strong level of evidence that the main factors which impact the detection of PedTB in South-Kivu, a setting with scarce technical resources, are linked with the clinical skills and the clinical experience of the health professionals. Indeed, the HF which receive from MSF an important input in terms of qualified health professionals tend to diagnose more PedTB and other complex forms of the disease. Furthermore, HF which diagnose many cases of TB (all forms of the disease), tend to be better skilled to recognise children affected by the disease. Of note, this critical importance of clinical skills may be exacerbated by the very poor infrastructure available in most HF: overall, only a very small proportion of TB suspects have access to X-ray, rapid molecular diagnostic or TST.

Importance of clinical skills

The very strong association between under-detection of SS-TB among adults and under-detection of PedTB is therefore to be explained by the fact that recognizing these complex clinical entities require (particularly in the absence of performant radiological investigations and laboratory assays) higher clinical skills than the common smear positive pulmonary form of the disease. Interestingly, four rural HF supported by MSF had the highest proportion of TB cases diagnosed (mean 26.7%), and seemed to perform better than urban facilities in regard to PedTB detection. The explanation of these high figures are probably the result of highly qualified and adequately supported health staff and free access to X-rays or laboratory testing.

Insufficient clinical skills among health professionals has been reported as the main reason behind the frequent missed opportunities to diagnose and treat patients with TB that present to health facilities [8]. A qualitative study performed in Tanzania [9] reports that "inadequate

awareness of the burden of childhood TB, limited knowledge of the wide spectrum of clinical presentation and lack of clinical decision support strategies is detrimental to the health staff's central responsibility of suspecting and referring children with TB especially in the early disease stages". The study authors stressed the importance of implementing strategies focusing on awareness among health staff and clinical skills in order to improve diagnosis.

Conclusion

There is a critical lack of medical doctors in rural areas of Africa. In 2013, the South-Kivu province counted 408 doctors (among which 14 had a specialization degree) and 2,862 nurses for a population of over 6.6 million. In such context, nurses, laboratory technicians or the so-called "Rapidly Trained Agents" form the front line of primary health-care delivery and are inevitably faced to medical conditions that they have not been trained to recognize. Furthermore, these professionals and the patients have very limited access to complementary tests such as X-Ray and accurate laboratory tests, which could eventually palliate part of these deficiencies.

The direct consequences of this lack of clinical skills are under-detection, increased mortality and continued transmission of the disease in the community [10]. As expected, SS-TB among adults are similarly affected by these insufficient clinical skills. In the South-Kivu province, these patients show an increased mortality during the first year after diagnosis.

In such context, performing a continuous assessment of all provider's level of "knowledge, attitudes and practices" ("KAP") related to TB would be the best option, but is not something that can be implementable in many difficult contexts such as the rural health zones of the South-Kivu province. Our findings suggest that a first screening of the facilities which would benefit the most from a complete assessment can be done based on the—already collected—data. We propose to use the ratio between SS-TB and SS+TB notification among adults as an indicator for insufficient clinical skills and under-detection of TB among children.

This indicator should enable TB control programs and their partners to precisely and promptly identify those settings requiring additional support to tackle under-diagnosis of PedTB and other complex clinical presentations of the disease. The interventions should focus in parallel on the awareness of PedTB, active case finding strategies, strengthening of clinical skills oriented towards PedTB and finally the implementation of adequate technical resources such as X-rays, TST and rapid molecular laboratory assays. Improving clinical skills of health professionals should not be considered as the only priority, but the importance of these human skills should not be under-estimated as they remain the cornerstone of rational use of diagnostic and treatment resources. In DRC, availability of X-rays, TST, rapid diagnostic tests are still anecdotal when considering the thousands of health facilities located in rural areas. Furthermore, a recent study performed by the National Tuberculosis Programme revealed that only 47% of HF had access to anti-TB treatments with adapted paediatric drug formulation [11].

Continued efforts focusing on the quality of clinical skills are a keystone for tackling the dramatic burden of infectious diseases among children in low-resource settings [12–14]. We propose an innovative approach to rapidly identify those HF facing alarming indicators and which would benefit the most from additional human and technical resources.

Supporting Information

S1 Fig. Validity of the model predicting under-detection of PedTB. Graphical representation of normalized Anscombe-s residues.

(TIF)

S1 Dataset. Dataset.
(XLS)

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Author Contributions

Conceptualization: EA FZ YLB DVDL.

Data curation: EA EMM FZ.

Formal analysis: FZ.

Funding acquisition: EA EMM OBR YLB DVDL FZ.

Investigation: EA EMM OBR YLB.

Methodology: EA FZ.

Project administration: EA.

Resources: EA EMM OBR YLB DVDL FZ.

Software: FZ.

Supervision: FZ DVDL.

Validation: EA EMM OBR YLB DVDL FZ.

Visualization: EA FZ DVDL.

Writing – original draft: EA.

Writing – review & editing: EA EMM OBR YLB DVDL FZ.

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Chapter 3: Limits and opportunities associated with the different TB tests

3.1. Conventional laboratory methods for the diagnosis of TB

3.1.1. Microscopy

Mycobacteria differ from most other bacterial pathogens in regard to the composition of their outer layer, which contains a high concentration of mycolic acids (76).

Mycolic acids are very long chain fatty acids and are essential for the viability and virulence of mycobacteria (77-79). These waxy compounds are responsible for the staining characteristics of mycobacteria, which are not coloured by the Gram staining procedure largely used in bacteriology laboratories. Two staining procedures are widely used: the Ziehl-Neelsen staining and fluorescence-based Auramine O staining.

Microscopic diagnosis of TB is a rapid, inexpensive and relatively simple. In settings facing a high burden of TB, the specificity of this technique for diagnosing TB is very high, while it is probably much lower in settings where non-TB (NTM) mycobacteria represent a higher proportion of causative agents among mycobacterial infections.

The main limitation of conventional Ziehl-Neelsen (ZN) microscopy is its low sensitivity, which has been estimated between 20 and 60% (80, 81). LED fluorescence microscopy (LED-FM) increases the sensitivity of front-line microscopic diagnosis of TB, including among HIV-coinfected patients (82). However, LED-FM is associated with higher material and reagent costs (83) and still has poor sensitivity compared to the gold standard technique. Both techniques have inadequate performance because they miss an important number of patients with active TB disease.

The second main limitation of microscopy is its inability to identify drug resistance. Delaying the detection of drug resistance has a direct impact on patient outcome and on the transmission of resistant strains, including in hospitals (84).

Due to these major limitations, countries such as South-Africa (85) and Brazil (86) have questioned the pertinence of using microscopy as a unique front-line test for the diagnosis of TB and have implemented alternative strategies.

3.1.2. Culture

Culture of mycobacteria is the most sensitive test for the diagnosis of TB and is used to perform drug susceptibility testing (DST) and monitor treatment response (87). There exist two major types of culture media, traditionally named “solid” and “liquid”. Loewenstein-Jensen solid media require limited laboratory infrastructure and can be produced locally by trained laboratory staff. MGIT (Beckton Dickinson, USA) is a liquid medium which can be incubated in a Bactec laboratory instrument allowing automated growth detection. This second option is recommended by the WHO since 2007 and has a higher sensitivity for TB diagnostic but requires regular electrical supply and commercial procurement of reagents and equipment.

Unfortunately, this technique could not be scaled-up in most high burden countries for three main reasons listed below:

- First, this technique is complex and biohazardous. It requires strict biosafety measures that prevent the risk of contamination to the environment and the infection of laboratory technicians. Building these laboratories require specialized professionals and complex equipment.
- Second, the performance of this technique is dependent on the capacity to strictly control the pre-analytical steps, including sample transportation and decontamination. If these are not mastered, an important proportion of results will be uninterpretable due to bacterial overgrowth of the culture media. Since 1975, CPC (cetyl-pyridinium chloride) was progressively introduced to facilitate sample transportation (88, 89). This technique has shown a good level of efficiency to prevent the overgrowth of the oral flora during the pre-analytic phase, and has a minimal mycobacteriolytic activity. Unfortunately, this method is not compatible with the automated liquid-based culture system MGIT (90). Furthermore, deploying CPC containers to the different TB clinics can be a major logistical challenge. As an illustration, DRC has a network of approximately 1.500 TB clinics distributed in the country, but only three culture laboratories.

- Third, it takes several weeks or months for the laboratory to provide a culture or DST result. This timeframe is incompatible with a utilization of these results to promptly treat patients with the correct therapy. For this reason, culture results have a moderate impact on patient management.

3.1.3. Phenotypic drug susceptibility testing (DST)

Different commercial and non-commercial WHO-endorsed methods (91-94) allow the drug susceptibility of MTBc strains to the main anti-TB drugs to be determined.

Together with the technical complexity and the time required to perform these tests, one major issue associated with these techniques is the lack of concordance observed between the different techniques and with the *in vivo* observations (95).

- Concerning RIF resistance, it has been shown that a significant proportion of patients with rifampicin resistance and consecutive negative treatment outcome were undetected by phenotypic techniques, particularly the automated MGIT 960 system (BD, Sparks, MD, USA) (96, 97).
- With regard to INH resistance, there is still an ongoing debate about how to interpret the different levels of resistance observed (minimal inhibitory concentration, MIC). Although it is clear that INH resistance has a negative impact on treatment outcome (98), giving high dose INH to patients with documented *in vitro* resistance seems to have a positive effect (99).
- Concerning other drugs, a recent study from South-Africa revealed that the concordance between the BACTEC MGIT 960 system and the proportion method was only 44% for ethambutol. Compared to the reference method, the MGIT system only detected 27% of Kanamycin-resistant strains (100).

As a result, although DST is recommended for all patients with TB, a result is available for only 12% of newly infected patients and 58% of retreatment cases globally in 2016. In the African region, these figures reduce to 6,4% and 33% respectively.

3.2. Molecular diagnostic methods

3.2.1. Detection of MTBc by PCR

The principle of polymerase chain reaction (PCR)-based diagnosis in infectious diseases involves amplifying short DNA sequences of a specific pathogen. This amplification is performed by adding exogenous nucleotides, primers and a DNA polymerase enzyme to the sample. Together, these reagents generate a copy of the microbial DNA sequence circumscribed between the two primers that bind to their complementary sequence on the bacterial genome. Cyclic modifications of the temperature allow sequential repetition of the steps of the reaction and generate sufficient short DNA amplicons to be detected.

Different techniques can be used to detect the PCR products, including gel electrophoresis or the use of fluorescent dyes. The latter strategy allows detection in real-time during the reaction and provide quantitative information with regard to the concentration of pathogens based on the number of reaction cycles required to obtain a detectable level of fluorescence.

Two types of fluorescent dyes can be used. Non-specific dyes intercalate in any double-stranded DNA and therefore could detect non-specific PCR products. Using sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reported increases the specificity of a PCR.

With regard to TB, most PCR techniques are based on the detection of a MTBC-specific genetic sequence called IS6110 (101). This sequence is characterized by the fact that it is inserted multiple times in the genome of MTBc, and most of the strains harbour eight to 20 copies of this transposed element (102). This high number of targets increases the sensitivity of the PCR compared to a target that would only have one copy in the genome. Furthermore, the fact that this sequence is repeated a variable number of times in variable location of the MTBC chromosome has been exploited to develop the gold-standard typing method for MTBC called “IS6110 – RFLP” (restriction fragment lengths polymorphism). For the latter technique, a restriction enzyme targeting the IS6110 region is used to divide the DNA in a variable number of fragments having variable size. Using gel electrophoresis, these fragments give a unique profile to each

strain, and therefore allows the identification of identical strains associated with direct transmission (103). A few MTB strains have been reported to lack this IS6110 region.

3.2.2. The Xpert MTB/RIF assay

The Xpert MTB/RIF PCR test is an all-in one test that targets the *rpoB* gene of MTBC (104) (105). This gene codes for the RNA polymerase of all bacteria, but the nucleotide sequence varies among different species. This variability can therefore be used for species identification, including among mycobacteria (106).

The Xpert MTB/RIF was endorsed by the WHO in December 2010⁷, just three months after the validation results were published (14, 107). This endorsement was followed by a massive financial support to countries with limited resources, which aimed to allow rapid implementation and scale-up of this tool.

The GeneXpert platform on which the MTB/RIF test runs is composed of individual modules that each have a heating block and an optical receptor. The PCR reagents are located in the cartridge, which is sealed once the sample has been introduced. When a test needs to be performed, a cartridge is inserted through the door of a module. The PCR reaction itself takes place in a small transparent compartment of the cartridge which allow the detection of the PCR products by the module's optical reader.

The Xpert MTB/RIF test was the first rapid PCR-based assay endorsed by the WHO for the molecular detection of TB. Unlike other tests targeting the IS6110 region, it detects TB by detecting the *rpoB* gene.

⁷ http://www.who.int/tb/features_archive/new_rapid_test/en/

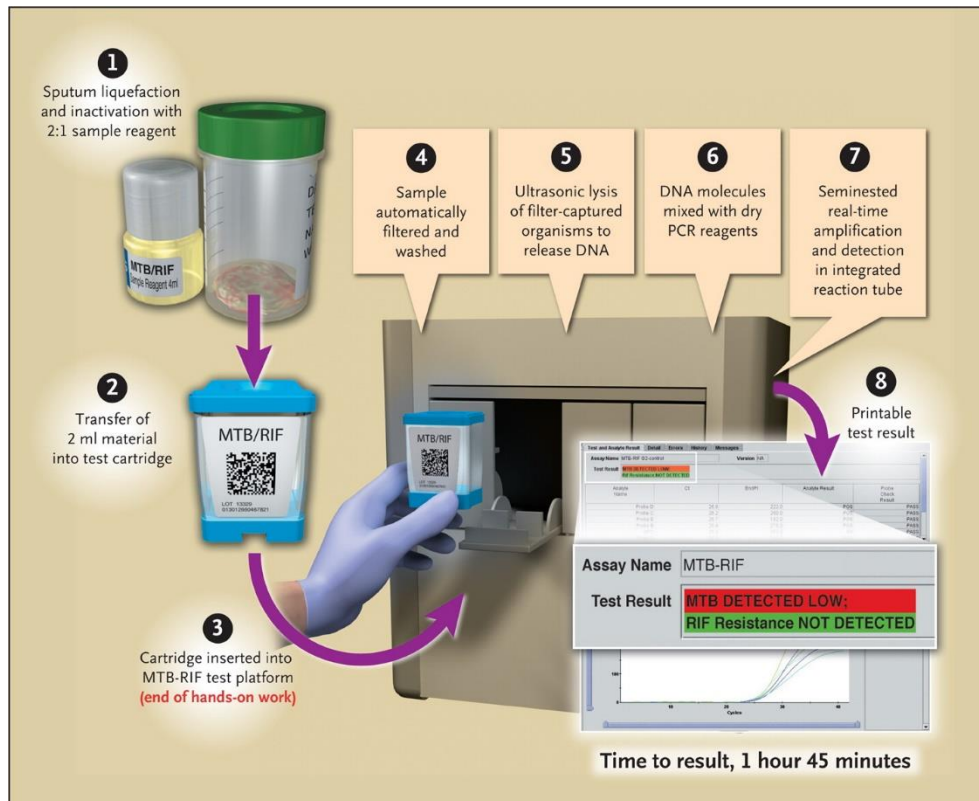


Figure 3.1: Principle of the Xpert MTB/RIF assay (Source : Boehme et al, 2010)

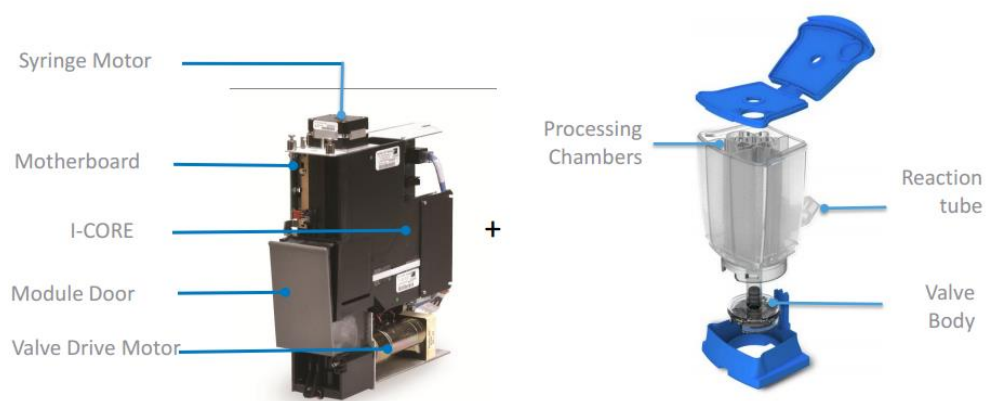


Figure 3.2: Analytical module (left) and PCR cartridge (right) are the main components of the Xpert MTB/RIF assay (source : Cepheid)

3.2.3. Incremental yield of Xpert MTB/RIF compared to smear microscopy

We participated in a study in Rwanda that evaluated the performance of ZN, LED-FM and Xpert MTB/RIF compared to the gold-standard liquid culture (108).

The significant incremental sensitivity of Xpert from either smear method supports the WHO recommendation for using Xpert as an initial test for TB diagnosis. The overall incremental sensitivity of Xpert over smear microscopy was 32.3 %; $p < 0.0001$.

The full study is available online (open access)
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RESEARCH ARTICLE

Open Access



Diagnostic performance of smear microscopy and incremental yield of Xpert in detection of pulmonary tuberculosis in Rwanda

Jean Claude Semuto Ngabonziza^{1*}, Willy Sengooba^{2,3}, Florence Mutua⁴, Gabriela Torrea⁵, Augustin Dushime⁶, Michel Gasana⁶, Emmanuel Andre⁷, Schifra Uwamungu⁸, Alaine Umubyeyi Nyaruhirira⁹, Dufton Mwaengo⁴ and Claude Mambo Muvunyi¹⁰

Abstract

Background: Tuberculosis control program of Rwanda is currently phasing in light emitting diode-fluorescent microscopy (LED-FM) as an alternative to Ziehl-Neelsen (ZN) smear microscopy. This, alongside the newly introduced Xpert (Cepheid, Sunnyvale, CA, USA) is expected to improve diagnosis of tuberculosis and detection of rifampicin resistance in patients at health facilities. We assessed the accuracy of smear microscopy and the incremental sensitivity of Xpert at tuberculosis laboratories in Rwanda.

Methods: This was a cross-sectional study involving four laboratories performing ZN and four laboratories performing LED-FM microscopy. The laboratories include four intermediate (ILs) and four peripheral (PLs) laboratories. After smear microscopy, the left-over of samples, of a single early-morning sputum from 648 participants, were tested using Xpert and mycobacterial culture as a reference standard. Sensitivity of each test was compared and the incremental sensitivity of Xpert after a negative smear was assessed.

Results: A total of 96 presumptive pulmonary tuberculosis participants were culture positive for *M. tuberculosis*. The overall sensitivity in PL of ZN was 55.1 % (40.2–69.3 %), LED-FM was 37 % (19.4–57.6 %) and Xpert was 77.6 % (66.6–86.4 %) whereas in ILs the same value for ZN was 58.3 % (27.7–84.8 %), LED-FM was 62.5 % (24.5–91.5 %) and Xpert was 90 (68.3–98.8 %). The sensitivity for all tests was significantly higher among HIV-negative individuals (all test $p < 0.05$). The overall incremental sensitivity of Xpert over smear microscopy was 32.3 %; $p < 0.0001$. The incremental sensitivity of Xpert was statistically significant for both smear methods at PL (32.9 %; $p = 0.001$) but not at the ILs (30 %; $p = 0.125$) for both smear methods.

Conclusions: Our study findings of the early implementation of the LED-FM did not reveal significant increment in sensitivity compared to the method being phased out (ZN). This study showed a significant incremental sensitivity for Xpert from both smear methods at peripheral centers where majority of TB patients are diagnosed. Overall our findings support the recommendation for Xpert as an initial diagnostic test in adults and children presumed to have TB.

Keyword: Tuberculosis microscopy in routine conditions, LED-FM versus ZN, Incremental yield of Xpert, Tuberculosis diagnosis in Rwanda

* Correspondence: cldsemuto@gmail.com

¹National Reference Laboratory Division, Rwanda Biomedical Centre, Kigali, Rwanda

Full list of author information is available at the end of the article



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Background

Despite the recommendations of the World Health Organization to use Xpert [1] as a first line diagnostic test, smear microscopy remains the most available and affordable test in low-income countries. Microscopy is inexpensive and highly specific in areas where there is a high prevalence of tuberculosis. However, it has several limitations including the fact that it is examiner-, technique-, and prevalence-dependent and in addition, it lacks sensitivity [2].

Studies evaluating the performance of LED-FM have shown that in addition to higher sensitivity (an average of 10 % higher than conventional ZN), it had qualitative, operational and cost advantages over both the conventional FM and ZN. On the basis of these findings, the World Health Organization (WHO) recommended in 2011 to replace conventional FM with LED-FM and phase in LED-FM as an alternative to ZN microscopy [3]. On the other hand, in 2010 WHO recommended that Xpert be used at district and sub-district levels as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated tuberculosis. The WHO further updated recommendations on the use of Xpert including a follow-on test for smear-negative patients in other settings [1]. In a Cochrane meta-analysis, sensitivity and specificity of Xpert compared with culture were 88 % (95 % CI 83 to 92 %) and 98 % (97 to 99 %), among smear-positive cases, and 98 % (97 to 99 %) and 68 % (60 to 75 %) among smear-negative cases [4].

In Rwanda, the national tuberculosis control program has started the phase-in of LED-FM as an alternative to ZN microscopy in peripheral (PLs) and intermediate health facility laboratories (ILs). To date, 40 % of laboratories have implemented LED-FM and 16 health facilities started using Xpert for TB detection in 2012. A sample transportation system was organized to facilitate transfer of samples from health facilities without an Xpert machine. The ILs or district hospital laboratories have an average of six qualified laboratory technologists and they are supervised, trained and mentored by the National Reference Laboratory (NRL) whereas peripheral laboratories or health center laboratories have an average of two laboratory technicians and they are subsequently supervised, trained and mentored by ILs. In regards to the workload, PLs test more than 75 % of pulmonary tuberculosis presumptive yet most of these facilities have very few technologists who also perform other requested laboratory tests. Based on the countrywide data of external quality assurance of smear microscopy (annual blind rechecking and proficiency panel test), the results of PLs tend to be better than those of ILs. However, no study of accuracy has been done to assess the significance of this difference. Therefore, this study aimed to determine the sensitivity of smear microscopy

and the incremental gain of Xpert for the detection of pulmonary tuberculosis at PLs and ILs to support the scale up of this new molecular technology. The present study assessed the performance of the two sputum smear microscopy techniques and the incremental yield of Xpert over microscopy among individuals with presumptive pulmonary tuberculosis, taking mycobacterial culture as the reference standard.

Methods

Setting, study design and population

This was a cross-sectional study involving eight health facilities which were purposively selected due to the high numbers of presumptive pulmonary tuberculosis recorded in the year 2013. Four PLs (two performing ZN and two performing LED-FM) and four intermediate laboratories (two performing ZN and two performing LED-FM). Based on quality control of smear microscopy (QC) data of 2012 and 2013 these eight laboratories performed equally well, though the QC for intermediate is performed by the NRL whereas QC for peripheral are subsequently done by ILs.

After smear microscopy, the left-over of the samples, of a single early-morning sputum from 648 new presumptive pulmonary tuberculosis patients, were tested using Xpert and mycobacterial culture as a reference standard.

Laboratory procedures

For each eligible participant, three to five mL of morning sputum specimen were collected in a clean plastic container with wide-mouthed, screw-capped and leak proof. A direct sputum smear was prepared, stained and examined by laboratory technicians at health facility laboratory. The left-over of sputum specimens and the examined corresponding sputum smear were immediately shipped to the tuberculosis laboratory of NRL. Sputum specimens not shipped immediately were refrigerated (4 to 8 °C). All sputum specimens collected were transported in a cool box (4–8 °C) and were processed on the same day at NRL TB laboratory.

At the NRL, sputum specimens were recorded and decontaminated using N-Acetyl-L-Cysteine Sodium hydroxide (NALC-NaOH) procedure followed by neutralization with phosphate buffer, centrifuged and the deposits (0.5 ml) inoculated in Mycobacterial growth indicator tube (BBL MGIT, Becton and Dickinson, Franklin Lakes, NJ USA) and two Home-made Lowenstein Jensen (LJ) tubes respectively. The remaining pellet was used to prepare a smear and to run Xpert. For Xpert 0.5 mL of decontaminated and concentrated sputum was added to 1.5 mL of the sample reagent (i.e., a ratio of 1:3). After 15 min, two mL of the mixture was added to the Xpert cartridge and then run in the machine in accordance

with manufacturer's guide (Cepheid, Sunnyvale, CA, USA). The smears prepared from pellet were stained using auramine for LED-FM examination and NRL results were considered final for those who tested negative at the health facility. Inoculated MGIT were incubated in an automated BD BACTEC 960 machine for up to 42 days according to manufacturer's guide (MGIT, Becton and Dickson, Franklin Lakes, NJ USA) while the two LJ tubes were incubated in manual incubator at 37 °C and inspected weekly for up to eight weeks. Positive cultures were confirmed for presence of acid fast bacilli by ZN microscopy and strain identification was done using an immunochromatographic test (SD MPT64TB Ag kit; SD Bioline, South Korea). Reexamination of smear from health facility at NRL, results of concentrated smear and Xpert provided preliminary results for treatment of pulmonary tuberculosis cases missed by health facility laboratories. The final results were provided by Mycobacterial culture.

Data management and analysis

As the presence of MTB cannot be excluded among contaminated cultures and cultures positive for non tuberculous mycobacterial (NTM), these results were excluded from the analysis as they may have led to an under-estimation of the sensitivity of Xpert or smear microscopy. The sensitivity and specificity was calculated for each method and type of health facility stratified by HIV-status using MGIT and/or LJ culture as gold standard. The incremental sensitivity of Xpert test to smear microscopy method was defined as the percentage of smear microscopy negative but Xpert positive by health facility among culture positive for *M. tuberculosis*. The McNemar statistical test was used to assess the significance of the differences in results obtained from smear microscopy using ZN versus LED-FM and the incremental sensitivity of Xpert. Based on these results, we compared the effectiveness of diagnostic strategies to propose the most accurate algorithm for the diagnosis of pulmonary tuberculosis at PLs and ILs. A *p*-value <0.05 was considered statistically significant. All data analysis was performed using SPSS version 21.0 software (Armonk, NY: IBM Corp.).

Results

Participants' characteristics and microbiological profile

Among the 648 patients enrolled, 48 were excluded for analysis due to incomplete results (23; 3.5 % contaminated cultures, 22; 3.4 % cultures positive for NTMs and 3; 0.5 % invalid Xpert results). Of the 600 included, 372 (62 %) were male and median age was 37 years (inter-quartile range 28–50). A total of 390 (65.0 %) and 210 (35.0 %) participants were from PLs and ILs respectively of whom, 318 (53 %) were from laboratories performing

ZN and 282 (47 %) from laboratories performing LED-FM microscopy, Fig. 1.

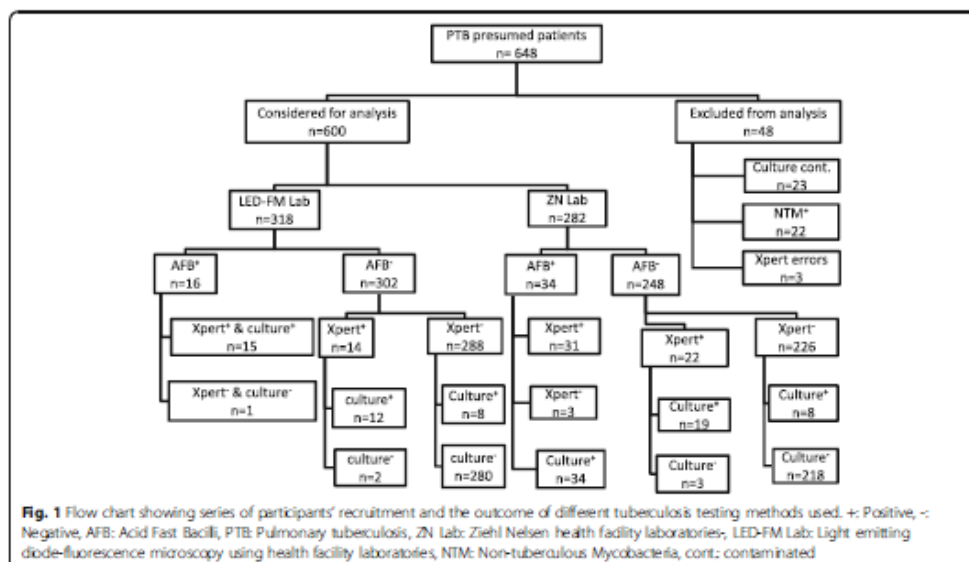
The prevalence of HIV in this study population was 162 (27.0 %). The smear positivity rates were 12.0 % and 5.0 % among ZN and LED-FM laboratories respectively, whereas the positivity rate for Xpert was 13.7 % of whom seven (1.2 %) had rifampicin resistant *M. tuberculosis*. A total of 96 (16.0 %) participants had culture confirmed TB of whom 28 (29.2 %) were HIV-infected, Table 1.

Sensitivity of smear methods and Xpert assay

Among 96 culture positive for *M. tuberculosis* by MGIT and/or LJ method, 47 (49.0 %) were smear negative of which 20 (42.6 %) were tested with LED-FM and 27 (57.4 %) with ZN. Among 82 patients found MTB positive with the Xpert assay, 36 (43.9 %) were smear microscopy negative of whom 13 (36.1 %) were HIV-infected. In regard to rifampicin resistance, seven rifampicin resistant MTB cases were detected by Xpert, among which only two were smear positive. The overall sensitivity of Xpert was 80.2 %, 95%CI (70.8–87.6). Xpert at PLs had a sensitivity of 77.6 %, 95%CI (66.6–86.4) as compared to 90 %, 95%CI (68.3–98.8) among ILs the overall sensitivity of smear microscopy was 51.0 %, 95 % CI (40.6–61.4 %). Overall sensitivity for smear microscopy among PLs was 48.7 %, 95 % CI (37.0–60.4) whereas for ILs was 60.0 %, 95%CI (36.1–80.9). The overall sensitivity of smear microscopy was 39.3 %, 95%CI (21.5–59.4) and 55.9 %, 95%CI (43.3–67.9) among HIV-positive and HIV-negative TB patients respectively. By smear microscopy method, smear by ZN at PLs had sensitivity of 55.1 %, 95 % CI (40.2–69.3) as compared to LED-FM 37.7 %, 95%CI (19.4–57.6). For ILs, the sensitivity for ZN smear microscopy was 58.3 %, 95%CI (27.7–84.8) as compared to LED-FM 62.5 %, 95%CI (24.5–59.4), Table 2. The overall specificity of smear microscopy 99.8 % 95 % C.I (99.4–100 %). The overall sensitivity of smear microscopy was 55.7 %, 95 % CI (42.4–68.5–63.2 %) and 42.9 % (26.3–60.6 %) among ZN and LED-FM using laboratories respectively, Table 2.

Incremental sensitivity of Xpert assay over the smear microscopy results

The overall incremental sensitivity (IS) of Xpert over smear microscopy at all HF was 32.3 %, 95 % CI (23.1–42.6 %). The overall IS of Xpert for either smear microscopy was 35.7 %, 95%CI (18.6–55.9) and 30.9 %, 95%CI (20.2–43.3) among HIV-positive and HIV-negative individuals respectively. The IS of Xpert among PLs using ZN was 32.7 %, 95 % CI (19.9–47.5); whereas for PLs using LED-FM was 33.3, 95%CI (16.5–54.0). Among ILs using ZN, the IS of Xpert was 25.0 %, 95%CI (5.5–57.2)



as compared to ILs using LED-FM 37.5 %, 95%CI (8.5–75.5), Table 2.

Discussion

In this cross-sectional study aimed at assessing the accuracy of smear microscopy and the incremental

sensitivity of Xpert in presumptive pulmonary tuberculosis patients at tuberculosis laboratories in Rwanda, we document low sensitivity of sputum smear microscopy in tuberculosis diagnostic laboratories, particularly in peripheral laboratories. The added value of Xpert was particularly important among HIV-infected patients and for detection of drug-resistant cases. We further confirm a significant gain from Xpert when used as an initial diagnostic test at health facility laboratories. For both health facility levels, the sensitivity of Xpert was significantly higher than either smear microscopy methods. As expected, the sensitivities of both smear methods including Xpert was higher among HIV-negative participants. Replacing ZN smear microscopy with LED-FM did not increase the detection of TB at both health facility levels. The incremental detection of Xpert from both smear methods was significantly higher among PLs but not at ILs.

The sensitivity found in this study was however in the range of findings from several studies where the sensitivities of conventional ZN microscopy ranged from 32 to 94 % and the sensitivities of fluorescence microscopy ranged from 52 to 97 %, with the fluorescent method being on average 10 % more sensitive than light microscopy [5]. In contrast to earlier findings, however, the current study did not find the sensitivity of LED-FM to be statistically different from ZN ($p = 0.371$). These results differ from several previous studies where LED-FM

Table 1 Participant characteristics ($n = 600$)

Characteristic	Frequency	Percentage
Sex		
Male	372	62.0
Female	228	38.0
Age category		
1 (15–35)	287	47.8
2 (36–55)	202	33.7
3 (≥56)	111	18.5
HIV status		
Positive	162	27.0
Negative	438	73.0
Mycobacterial testing results		
ZN smear positive	34	12.1
LED-FM smear positive	16	5.0
Xpert positive	82	13.7
Culture (MGIT and/or LJ) positive	96	16.0

MGIT mycobacterial growth indicator tube; LJ Lowenstein Jensen; LED-FM light emitting diode fluorescence microscopy; ZN Ziehl Nelsen Xpert Xpert MTB/Rif test; HIV human immunodeficiency virus

Table 2 Yield of smear microscopy versus Xpert among culture confirmed tuberculosis patients (n =96)

		Xpert		Sensitivity of SM % (95 % CI)	Incremental sensitivity of Xpert % (95 %CI)
		Positive	Negative		
Overall SM at all HF n =96	positive	46	3	51.0 (40.6–61.4)	32.3 (23.1–42.6)
	negative	31	16		
SM in all HIV positive n =28	positive	10	1	39.3 (21.5–59.4)	35.7 (18.6–55.9)
	negative	10	7		
SM in all HIV negative n =68	positive	36	2	55.9 (43.3–67.9)	30.9 (20.2–43.3)
	negative	21	9		
SM at all PL n =76	positive	34	3	48.7 (37.0–60.4)	32.9 (22.5–44.6)
	negative	25	14		
SM at all IL n =20	positive	12	0	60.0 (36.1–80.9)	30.0 (11.9–54.3)
	negative	6	2		
SM at ZN PL n =49	positive	24	3	55.1 (40.2–69.3)	32.7 (19.9–47.5)
	negative	16	6		
SM at LED-FM PL n =27	positive	10	0	37.0 (19.4–57.6)	33.3 (16.5–54.0)
	negative	9	8		
SM at ZN IL n =12	positive	7	0	58.3 (27.7–84.8)	25.0 (5.5–57.2)
	negative	3	2		
SM at LED-FM IL n =8	positive	5	0	62.5 (24.5–91.5)	37.5 (8.5–75.5)
	negative	3	0		

LED-FM light emitting diode fluorescence microscopy; ZN Ziehl-Nelsen; Xpert Xpert MTB/RIF test; HIV human immunodeficiency virus; CI confidence interval; SM smear microscopy; IL intermediate laboratories; PL peripheral laboratories; SM smear microscopy

increases an average of 10 % of sensitivity over the conventional ZN technique [6–10]. Other studies have shown equal sensitivity or low specificity of LED-FM compared to conventional ZN technique [9, 11–13]. In these studies, readers had no previous experience with fluorescence microscopy, which is the most likely explanation for sensitivity differences compared with other studies and indicating the importance of adapting training intensity according to the level of operator proficiency. Our findings showed that smear microscopy performed better at intermediate laboratories compared to peripheral; the sensitivity for LED-FM 62.5 % vs. 37.0 %, $p=0.023$ but not for ZN 58.3 % vs. 55.1 %; $p=0.265$. This may either be explained by the fact that LED-FM was implemented at IL prior to peripheral and therefore technologists acquired experience earlier compared to those at IL or due to small sample size at the ILs.

The sensitivity of sputum smear microscopy in HIV-infected participants was found to be low and are in agreement with findings of previous studies, where it ranges from 30 to 48 % [14–16]. The poor performance of sputum smear microscopy in HIV patients can be explained in part by the fact that pulmonary tuberculosis in these patients presents with paucibacillary TB and lack cavitation [16].

The overall prevalence of smear negative pulmonary tuberculosis using either smear method in HIV-infected PTB presumptive participants was found to be high and are in line with those of previous studies [17, 18]. The level of immunosuppression among HIV-infected patients affects significantly the results of the sputum smear; less severely immunocompromised HIV-positive patients tend to have classic cavitary tuberculosis with smear-positive results; as the level of immunocompromised increases with advancing HIV disease, atypical pulmonary features predominate and smear examinations prove less sensitive [17].

Although, the overall sensitivity of Xpert for the detection of *M. tuberculosis* was slightly lower, its specificity was consistent with those of previous studies in a Cochrane review even when stratified by HIV status [4]. The insignificant incremental sensitivity of Xpert test over smear microscopy at ILs is likely to be more explained by the small sample size we had at these facilities.

The significant incremental sensitivity of Xpert from either smear method at PLs supports the WHO recommendation for using Xpert as an initial test for TB diagnosis [19]. Although the cost per test and compulsory required maintenance of Xpert machine (annual calibration, replacement of modules, good and constant power

supply) may be not affordable by many poor resource settings given the limited health budgets [20], the savings from increased case detection and timely initiation of treatment due to early diagnosis, may be more cost-effective in terms of supplies savings as well as patient savings from repeat facility visits. In addition, early diagnosis may reduce the risk of TB transmission. It is worth noting that the effectiveness of Xpert testing is likely to depend on utilization as the test tends to be less effective in low workload settings [21] as the low numbers of patients tested at IL could have affected the strength of obtained significance measure.

Our study had some limitations; the level of immunosuppression for HIV positive PTB presumptive participants was not measured (CD4); this could lead to poor classification and consequently low differences in terms of smear sensitivity among HIV-infected participants. Secondary, the low samples size obtained from ILs which probably masks the obvious significant incremental detection of Xpert; among the 600 participants of this study, only 210 (35 %) participants and 20 (20.8 %) pulmonary tuberculosis confirmed cases were from ILs. The few numbers of HIV-infected patients in this study could not allow for meaningful comparison of incremental sensitivity by HIV-status, however, significant Xpert IS among HIV-infected patients was previously documented [22]. Lastly, excluding samples which became contaminated or positive for an NTM from the analysis may have led to an under-estimation of the sensitivity of Xpert and smear microscopy, as tuberculosis cannot be definitively excluded for these patients. TB-NTM coinfection has been reported, but is supposed to be a relatively rare clinical entity in Rwanda. However, excluding these participants may have over-estimated the specificity of microscopy, as smear-positive NTM infections were not taken into account in the analysis, while the specificity of Xpert would probably have been much less affected by the presence of NTM.

Conclusions

The findings from this study revealed a low detection rate of both LED-FM and ZN smear microscopy at health facility tuberculosis diagnostic laboratories in Rwanda. This study revealed a low sensitivity of LED-FM smear microscopy compared to ZN-microscopy among PLs whereas for ILs, the sensitivity of LED-FM was higher than that of ZN microscopy, indicating differences in skills requirements among microscopy methods. This study revealed a significant incremental detection gained from Xpert. Hence, the data from this study strongly support the conditional recommendation of WHO for Xpert; where the Xpert may be used as initial diagnostic test in adults and children presumed to have TB. Nevertheless, other studies of cost-effectiveness and feasibility of the proposed strategy at large scale are necessary.

Abbreviations

AFB: acid fast bacilli; BD: Becton Dickinson Company; FM: fluorescence microscopy; HIV: human immunodeficiency virus; ILs: intermediate laboratories; LED-FM: light emitting diode fluorescence microscopy; LJ: Löwenstein-Jensen; MDR-TB: multi-drug resistant tuberculosis; MGIT: mycobacterium growth indicator tube; MTB: *Mycobacterium tuberculosis* complex; NRL: National Reference Laboratory; NTM: Non Tuberculosis Mycobacterium; PLs: peripheral laboratories; PTB: pulmonary tuberculosis; RF: rifampicin; TB: tuberculosis; WHO: World Health Organization; ZN: Ziehl-Neelsen staining

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Availability of data and materials

All data supporting the findings are contained within the manuscript.

Authors' contributions

JCSN, FM, DM and CM were the primary researcher, conceived the study, designed the study, participated in data collection, conducted data analysis and drafted the initial manuscript. WS, SU and MG participated in data collection, data analysis and reviewed the initial and final manuscript. WS, AUN, AD, EA and GT revised critically the initial and the final manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval and consent to participate

Ethical approval was obtained from Rwanda National Ethical Committee and Kenyatta National Hospital/University of Nairobi Ethics and Research committee. Written informed consent was obtained from participants and the results were communicated via respective health facilities. All tuberculosis cases received appropriate treatment as per national guidelines.

Author details

¹National Reference Laboratory Division, Rwanda Biomedical Centre, Kigali, Rwanda. ²Department of Medical Microbiology, Makerere University College of Health Sciences, School of Biomedical Sciences, Kampala, Uganda. ³Department of Global Health and Amsterdam Institute of Global Health and Development, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. ⁴Department of Medical Microbiology, School of Medicine, College of Health Sciences, University of Nairobi, Nairobi, Kenya. ⁵Mycobacteriology Unit, Institute of Tropical Medicine Prince Leopold, Antwerp, Belgium. ⁶Tuberculosis and other respiratory diseases Division, Rwanda Biomedical Centre, Kigali, Rwanda. ⁷Pôle de microbiologie médicale, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium. ⁸Biomedical Laboratory Sciences Department, School of Allied Health Sciences, College of Medicine and Health Sciences,

University of Rwanda, Kigali, Rwanda. ⁹Management Sciences for Health, Pretoria, South Africa. ¹⁰Clinical Biology Department, School of Medicine and Pharmacy, College of Medicine and Health Sciences, University of Rwanda, Butare, Rwanda.

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3.2.4. How end-users could improve the sensitivity of the Xpert MTB/RIF assay

The sensitivity of PCR-based methods is dependent on several factors including the bacterial load present in the sample and the presence of PCR inhibitors. In the Xpert MTB/RIF assay, accurate TB detection is based on the amplification of the target genetic region followed by the binding of specific probes on the PCR products. It is technically possible that in some cases, the probe-binding step would fail.

To test this eventuality, we performed *rpoB* sequencing (109) on 93 samples from the South-Africa which had a negative result for TB with the Xpert MTB/RIF assay, but for which the raw data showed one to three positive probes.

Among these, we found 15 samples positive for MTBC (16%). Two of these MTB strains presented RIF resistance-conferring mutations. One of these two RIF-resistant strains (ref 717) was tested for other drugs using the Deeplex MycTB assay (Genoscreen, France). This test highlighted that this sample was MDR-TB, with confirmed RIF resistance and additional resistance to INH (*katG* S315T) and E (*embB* M306I).

These results suggest that, when Xpert MTB/RIF is used as a first-line test, increased sensitivity could be achieved by performing additional testing on Xpert negative samples that harbour a positive probe. This process would allow additional TB and MDR-TB strains to be identified.

3.2.5. Specificity of the Xpert MTB/RIF assay

Because the *rpoB* gene is present and relatively similar among the different mycobacterial species, neither the primers nor the probes of this test are 100% specific for MTBC. In the initial validation study, non-specific amplification signals were observed for some NTM species. However, in these cases, only one or two probes are generally detected, and the GeneXpert software only reports TB detection when at least three probes are detected (104). These considerations will be used later in the document (Chapter 7.1).

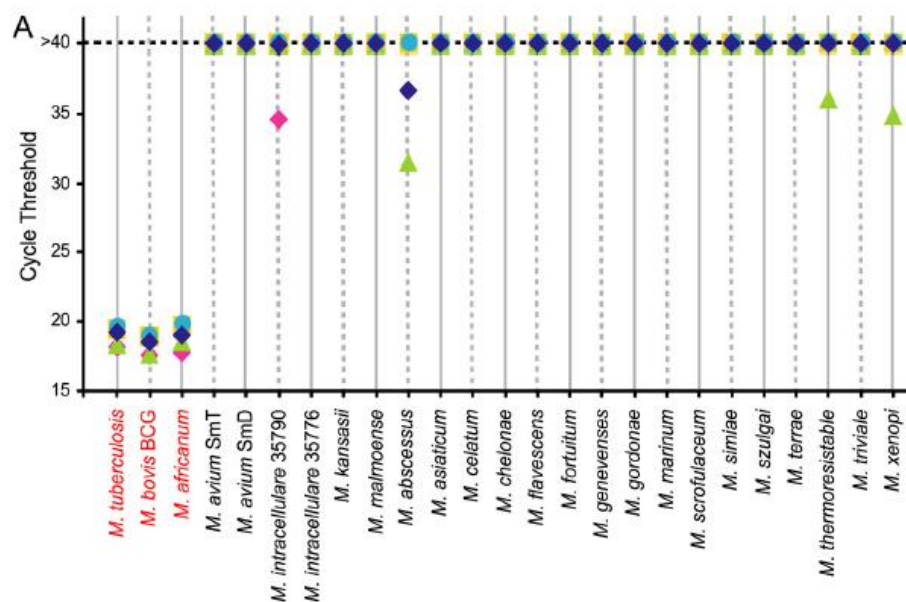


Figure 3.3: Xpert MTB/RIF results obtained with different mycobacterial species. The results produced by each sample are indicated by a single vertical line on which the Ct of each of the five molecular beacon probes is plotted. (Source: Helb et al, 2010)

3.2.6. How end-users could use the Xpert MTB/RIF assay for improving the diagnosis of NTM infections

NTM constitute a major reservoir for opportunistic pathogens, in particular among immunocompromised patients, and are an emerging cause of pulmonary infection, particularly among patients with underlying immunosuppression of chronic pulmonary disease (110). From a clinical perspective, patients presenting pulmonary NTM infections regularly present similar symptoms and risk factors when compared to people infected with TB. Unfortunately, these patients will not respond to first line TB standard regimen due to intrinsic resistance to most first-line TB drugs.

The burden of NTM infections is much lower than TB in most high burden countries. Nevertheless, they are not uncommon (111, 112), particularly among HIV-infected patients (113).

NTM infections can be confounded with TB due to the similarity of symptoms and the fact that they cannot be distinguished by microscopy. Therefore, the presence of an NTM infection can lead to the erroneous diagnosis of TB (i.e. a positive smear microscopy in a patient with pulmonary symptoms) or drug-resistant TB (i.e. persistent smear positive microscopy in a patient undergoing TB treatment).

The diagnosis of NTM infections is based on culture followed by identification of the species commercial molecular assays (114), MALDI-TOF MS (115) or sequencing (116). This approach is nevertheless hampered by the fact that many pathogenic NTM causing major public health attention such as *Mycobacterium leprae* or *M. ulcerans*, or less frequent species cannot be cultured with conventional methods used for the diagnosis of MTBc.

We observed in our clinical experience in Brussels that Xpert MTB/RIF tests consistently presented the same probe profile among different NTM when the test was performed from positive liquid cultures. The diagnosis of NTM infections requires species identification (110).

We analysed the raw probe results of 31 Xpert MTB/RIF tests performed on positive MGIT cultures for which an NTM was later confirmed and at least one Xpert probe was positive (A, B, C, D or E). Definitive NTM identification was performed by *rpoB* sequencing (117).

- Among the 8 strains presenting an amplification of Probe A only, all were later identified as *M. intracellulare* and closely related species such as *M. chimaera* and *M. marseillense*.
- Among the 9 strains presenting an amplification of Probe C and Probe E, all were identified as *M. avium*.
- Among the 14 strains presenting an amplification of Probe C only, different species were identified, including *M. xenopi*, *M. fortuitum*, *M. avium* and *M. goodii*.

These results suggested that Xpert could support the diagnosis of NTM-associated infections. The clinical significance of this system may be important for patients, as it should prevent the initiation of inappropriate therapies and orient towards more targeted diagnostic tests and treatment regimens.

This finding has been transposed to the DatatoCare (Savics, Belgium) application as an alert for the clinician.

3.2.7. Immune reconstitution inflammatory syndrome due to *Mycobacterium genavense* in an HIV-infected patient: Impact of rapid species identification by *rpoB* sequencing on clinical management

We developed a sequencing method that allows identifying NTM species directly from smear positive sputum samples, which was utilized for obtaining the results presented in section 3.2.6. and 3.2.8.

Sanger sequencing allows the reproduction of DNA sequences or genes that have been previously selected and amplified by PCR. Four reactions run in parallel. Each reaction uses a primer labelled with a specific colour, a DNA polymerase, nucleotides (A, T, G and C), plus a small concentration of one nucleotide analogue called dideoxynucleotides (ddNTP). When ddNTP are incorporated to the new DNA chain, elongation stops and fragments of different colour and length are generated. The products of the four reactions are then combined and analysed through capillary electrophoresis. This allows reproducing the original DNA sequence.

The full study is available online (open access)
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Case Report

Immune reconstitution inflammatory syndrome due to *Mycobacterium genavense* in an HIV-infected patient: Impact of rapid species identification by *rpoB* sequencing on clinical management

Emmanuel Andre,^{1,2†} Leila Belkhir,^{3†} Léonie Goeminne,¹
 Alexandre Colmant,² Jean-Yves Sottiaux,² Halil Yildiz,³ Jean Ruelle,¹
 Michel Delmee,^{1,2} Bernard Vandercam³ and Jean-Philippe Defour⁴

Correspondence
 Emmanuel Andre
 emmanuelandre@uclouvain.be

¹Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Place de l'Université, Avenue Hippocrate 54 bte B1.54.05, 1200 Bruxelles, Belgium

²Service de Microbiologie, Département de Biologie Clinique, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

³Service de Médecine Interne et Pathologies Infectieuses, Département de Médecine Interne, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

⁴Service d'Hématologie, Département de Biologie Clinique, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Bruxelles, Belgium

Introduction: Diagnosis of infections due to *Mycobacterium tuberculosis* has been recently facilitated with the worldwide implementation of rapid molecular tests. However, the diagnosis of non-TB mycobacteria (NTM) remains a major challenge for laboratories, as this group of bacteria contains over 150 different species with highly variable bacteriological properties. The development and implementation in clinical practice of techniques allowing rapid identification of NTM at the species level is, therefore, important for clinical laboratories. We report the case of an HIV-1 positive patient presenting with severe NTM infection who benefited from a novel rapid diagnostic technique, based on the direct sequencing of the *rpoB* gene from smear-positive clinical samples.

Case Presentation: A 43-year-old HIV-1 positive woman was hospitalized for bicytopenia in the presence of an inflamed supraclavicular lymph node. The diagnosis of disseminated *Mycobacterium genavense* infection, which involved the bone marrow and several lymph nodes, was missed by conventional mycobacteriological techniques. Identification was obtained by performing direct sequencing of the *rpoB* gene.

Conclusion: We describe a novel technique that allows the rapid diagnosis of NTM infections directly from biological samples. This technique is now implemented in our routine workflow for smear and culture-positive samples, after the exclusion of TB, by rapid molecular assays.

Keywords: ART; HIV; IRS; *Mycobacterium genavense*; NTM; rapid diagnosis.

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Introduction

The diagnosis of infections due to *Mycobacterium tuberculosis* (Mtb) has made an unprecedented step forward recently with

the worldwide implementation of rapid molecular tests. However, the diagnosis of non-TB mycobacteria (NTM) remains a major challenge for laboratories, as this group of bacteria contains over 150 different species presenting with variable bacteriological properties and clinical significance. Current molecular techniques lack sensitivity and are unable to distinguish mycobacterial species or subspecies to a level that would guide therapeutic management. Development of techniques allowing rapid identification of NTM at the species level is, therefore, important for clinical laboratories.

†These authors contributed equally to this paper.

Abbreviations: ART, antiretroviral therapy; FDG PET CT, fluorodeoxyglucose positron emission tomography computed tomography; HIV, human immunodeficiency virus; IRS, immune reconstitution inflammatory syndrome; Mtb, *Mycobacterium tuberculosis*; NTM, non-TB mycobacteria; TB, tuberculosis; VL, plasma viral load.

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 On: Mon, 20 Feb 2017 14:53:11

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We report the case of a patient presenting with disseminated infection due to *Mycobacterium genavense*, a rare and hardly cultivable mycobacterial species. The patient benefited from direct sequencing of the bacterial *rpoB* gene from smear-positive clinical samples.

Case Report

Here, we describe the case of a 43-year-old woman, hospitalized for bicytopenia, in the presence of an inflamed supraclavicular lymph node.

The patient was diagnosed with human immunodeficiency virus-1 (HIV-1) infection nine years previously and had been treated with antiretroviral therapy (ART) containing abacavir/lamivudine and nevirapine for the past six years. She was then lost to follow up for three years and ART was interrupted.

The patient was admitted for *Pneumocystis jirovecii* pneumonia. Her CD4 cell count was $4 \mu\text{l}^{-1}$ with a plasma viral load (VL) of 273,423 copies ml^{-1} . Antiretroviral therapy containing tenofovir/emtricitabine and dolutegravir was begun.

Two months later, she was readmitted for fever, chills and night sweats. Clinical examination revealed an inflamed supraclavicular lymphadenopathy. The CD4 cell count was 66 cells μl^{-1} , and VL was 144 copies ml^{-1} . Further blood tests revealed anaemia (Hb: 10.8 g l^{-1}) a low white blood cell count (3030 leukocytes μl^{-1}) and C-reactive Protein at 56 mg l^{-1} (NI value: $<5 \text{ mg l}^{-1}$).

A bone marrow aspirate was performed to exclude a haematological neoplasm. Cytological examination showed more than 60 % reactive plasmacytes locally, rare

epithelioid granulomas and a single Langhans giant cell with no clear cell border (Fig. 1).

An 18F-fluorodeoxyglucose positron emission tomography-computed tomography scan (FDG PET-CT scan) was performed and showed abnormal metabolic activity in the spleen, and in multiple lymph nodes located in the cervical, mediastinal, hepatic, periaortic and iliac regions (Fig. 2).

A biopsy of the supraclavicular lymph node was performed. Direct smears showed over 100 acidfast bacilli per microscopic field, as illustrated in Fig. 3. A TB-specific PCR (GeneXpert MTB/RIF, Cepheid) performed on the same day gave a negative result for the *Mtb* complex.

Despite these early microbiological results, anti-TB treatment composed of rifabutin (300 mg day^{-1}), isoniazid (300 mg day^{-1}), ethambutol (800 mg day^{-1}) and pyrazinamide (1000 mg day^{-1}) began rapidly. Rifabutin was preferred to rifampicin to avoid drug-drug interactions with dolutegravir (Miller *et al.*, 2015). Five days later, the patient developed a biological hepatitis with liver enzymes five times over the upper normal limit. Pyrazinamide and isoniazid were stopped and liver function improved rapidly.

In the meantime, additional bacteriological investigations were performed. DNA was extracted from the lymph node using a commercial Fluorolyse kit (Hain Lifescience). The DNA strip assay, GenoType Mycobacterium CM (Hain Lifescience), confirmed the presence of the genus *Mycobacterium*, but did not provide a species identification. We performed DNA sequencing of the *rpoB* gene as described below.

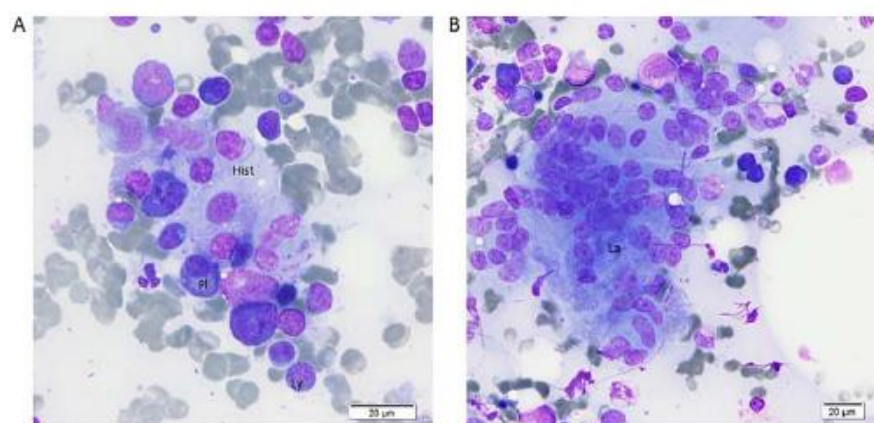


Fig. 1. Bone marrow. May–Grünwald Giemsa staining showing: (A) non-necrotizing epithelioid granuloma composed of epithelioid histiocytes surrounded by small lymphocytes (ly) and reactive plasmacytes (pl); (B) a multinucleated giant Langhans cell (La). Bars, 20 μm .

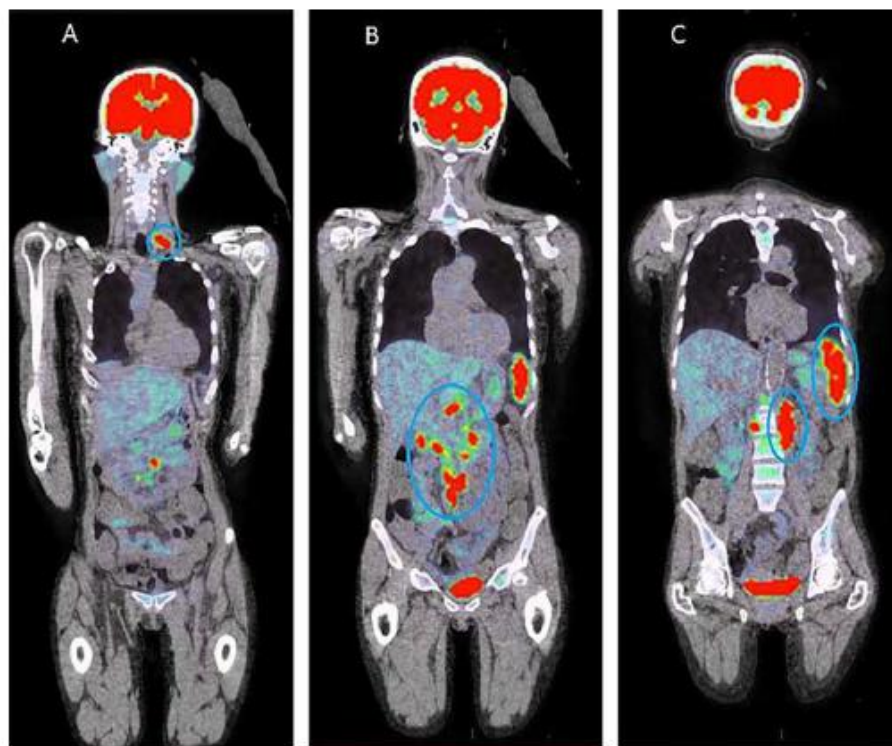


Fig. 2. FDG PET-CT scans indicating hypermetabolic lymph nodes (areas circled in blue): (A) the supra-clavicular lymph node; (B) and (C) multiple lymph nodes in the hepatic, periaortic and iliac regions. Note the high metabolic activity of the spleen (areas circled in red).

As a first step, PCR amplification of the *rpoB* gene was carried out using the KOD Hot start master mix (Merck Millipore) with the primers, My9 and MycoR. These primers amplify a 800 bp region, presenting significant variations between mycobacterial species. Amplicons were purified with a QIAquick PCR purification kit (Qiagen) and diluted to 10 ng μl^{-1} .

The second sequencing step was performed on an ABI 3130 system (Life Technologies) using a BigDye Terminator v3.1 Cycle Sequencing and BigDye XTerminator Purification kit (Applied Biosystems, ref. 4376486) with primers My10, My11 and MycoF (Table 1).

A sequence of 643 bp was obtained, for which analysis was performed using a Smartgene Bacteria module (Smartgene), which had 100 % homology with *Mycobacterium genavense* (accession number HM022216).

This technique was applied to fresh samples and to samples which had been fixed in paraffin.

Based on these results, clarithromycin (500 mg b.i.d.) was added rapidly to the treatment as recommended in the ATS/IDSA guidelines (Griffith *et al.*, 2007) with good clinical evolution after one month.

Bone marrow and lymph node samples were cultured in an MGIT 960 instrument at 37 °C for ten weeks and in Lowenstein-Jensen media incubated at 30 °C, 35 °C and 42 °C for eight weeks. All these media remained sterile.

Discussion

Immune reconstitution inflammatory syndrome (IRIS) is a common complication of ART initiation among HIV patients, particularly in those with an advanced state of immunosuppression (CD4 count $<50 \mu\text{l}^{-1}$). It typically occurs as CD4 cell numbers increase and it is linked to the paradoxical worsening of pre-existing subclinical opportunistic infections due to pathogens such as TB and NTM, *Cryptococci* and *Cytomegalovirus* (Barber *et al.*,

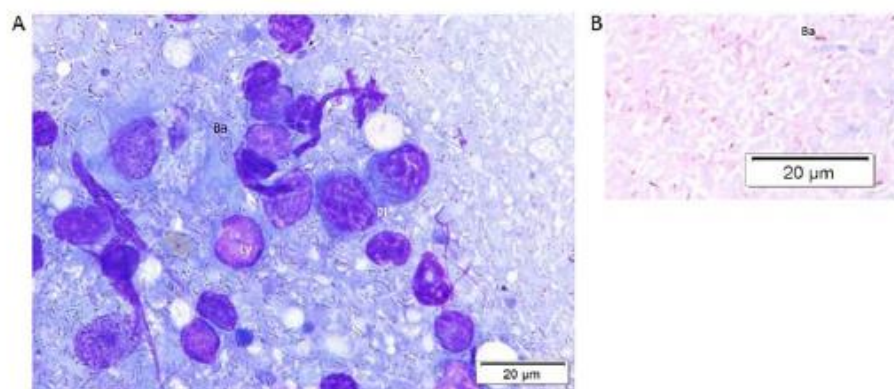


Fig. 3. Cervical lymph node. (A) May-Grünwald Giemsa staining showing poorly cohesive lymphocytes (Ly), reactive plasmacytes (Pl) and irregularly stained mycobacteria (Ba). (B) Ziehl-Neelsen staining showing numerous acid-fast bacilli (Ba) coloured in pink. Bars, 20 µm.

Table 1. Primers used for the amplification of the *rpoB* 'identification' region based on the *Mtb* H37Rv sequence (accession number AL123456.3)

Primer	Forward or reverse	Sequence (5' - 3')	Location	Source
My 9	F	ATCGGCGCCGAGGTCCGGAC	2356-2376	This paper
My 10	F	AAGGTGCCSCACGGTGAGTC	2494-2517	This paper
My 11	R	ATGTAGCCGACCGTSAACGGGT	3044-3065	This paper
MycF	F	GGCAAGGTCACCCGAAAGG	2392-2411	(Adékambi <i>et al.</i> , 2003)
MycR	R	AGCGGCTGCTGGGTGATCATC	3135-3152	(Adékambi <i>et al.</i> , 2003)

2014; DeSimone *et al.*, 2000; French, 2012; Griffith *et al.*, 2007; Walker *et al.*, 2015.). IRIS can be severe and require the rapid initiation of an effective treatment.

Although *Mtb* remains the most prevalent species, in our particular experience (low incidence of TB), NTM represent an emerging problem (up to 50 % of *Mycobacteria* identified in the lab are non-TB species). NTM can be particularly severe among immunosuppressed patients, requiring a rapid diagnosis and rapid initiation of treatment (Murthy *et al.*, 2015; van der Werf *et al.*, 2014). In the clinical case presented above, rapid identification of a species of NTM directly from a clinical sample permitted the definite exclusion of *Mtb* infection and the initiation of a treatment oriented towards *Mycobacterium genavense*.

Mycobacterium genavense is a clinically relevant pathogen among immunocompromised patients, causing disseminated disease with serious morbidity and mortality (Santos *et al.*, 2014). The prevalence of these infections may be underestimated due to the difficulties in recovering the bacteria from the usual culture media, such as MGIT

and LJ, used in clinical practice (Thomsen *et al.*, 1999). Reports suggest that *Mycobacterium genavense* may grow on these media after up to 90 days of incubation at 45 °C in liquid media with an acid pH and Mycobactin J as a supplement. These specific requirements are not implementable in routine practice.

Rapid and accurate diagnostic methods for this growing group of opportunistic pathogens should, therefore, be made widely accessible. Using the technique described above, we were able to perform rapid species identification directly from smear-positive clinical specimens.

This technique has since been implemented in our routine workflow for smear-positive samples with negative *Mtb* PCR.

Acknowledgements

The authors declare no conflicts of interest. This case report was approved by the local ethical committee of Université Catholique de Louvain (health sector).

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3.2.8. Missed opportunities for diagnosing NTM infections using Xpert raw data in South-Africa

We performed *rpoB* sequencing (109) on 115 DNA extracts from sputum samples originating from the Limpopo province in South-Africa. These samples had been previously tested negative by Xpert, but presented on to three positive probes.

We obtained an interpretable mycobacterial *rpoB* sequence for 93 samples (80,9%). In all samples, a mycobacterial species could be revealed by sequencing. Among these, 41 were slow-growing mycobacteria (44%), 15 were identified as MTBC (16%), 5 were rapidly growing mycobacteria (5%) and 32 were considered as mycobacteria with no

definitive species identification due to an insufficient identity score obtained using the Smartgene IDNS software (Smartgene, Switzerland). The results are presented in Figure 3.5.

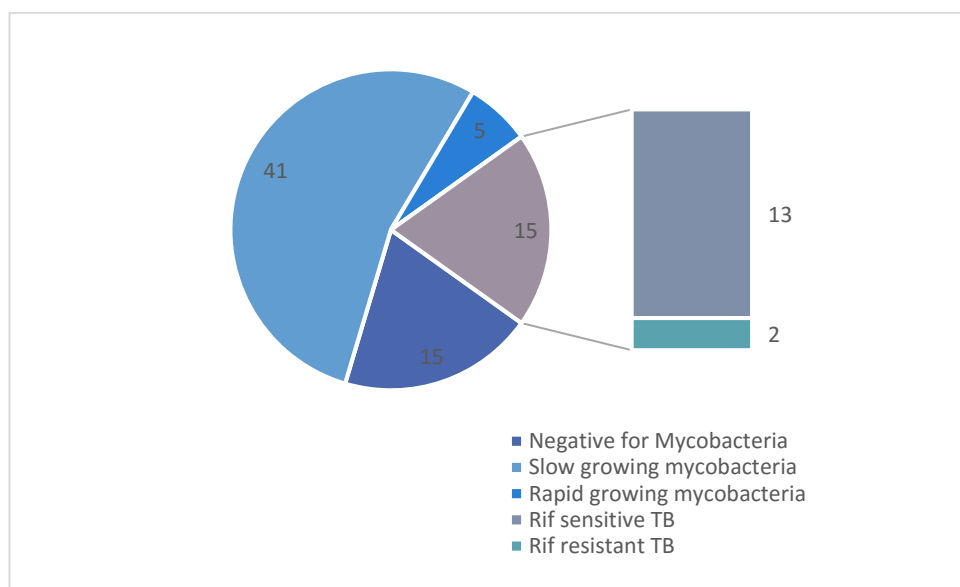


Figure 3.4: Detection of different mycobacterial species from biological samples presenting one to three positive probes on Xpert MTB/RIF test.

These data strongly suggest that raw data from rapid TB-oriented molecular assays are to be analysed carefully as they can reveal clinically important mycobacterial infection, including missed TB infections.

Rapid diagnostic tests which target the *rpoB* gene are of particular interest in this regard as this gene exists in all mycobacteria and is relatively conserved, allowing some TB-specific probes to link with NTM DNA. In the cohort of South-Africa, all samples with at least one positive probe had a detectable level of NTM.

These results suggest that, when Xpert MTB/RIF is used as a first-line test, improved diagnosis of NTM infections could be achieved by performing complementary tests on Xpert negative samples which harbour a positive probe.

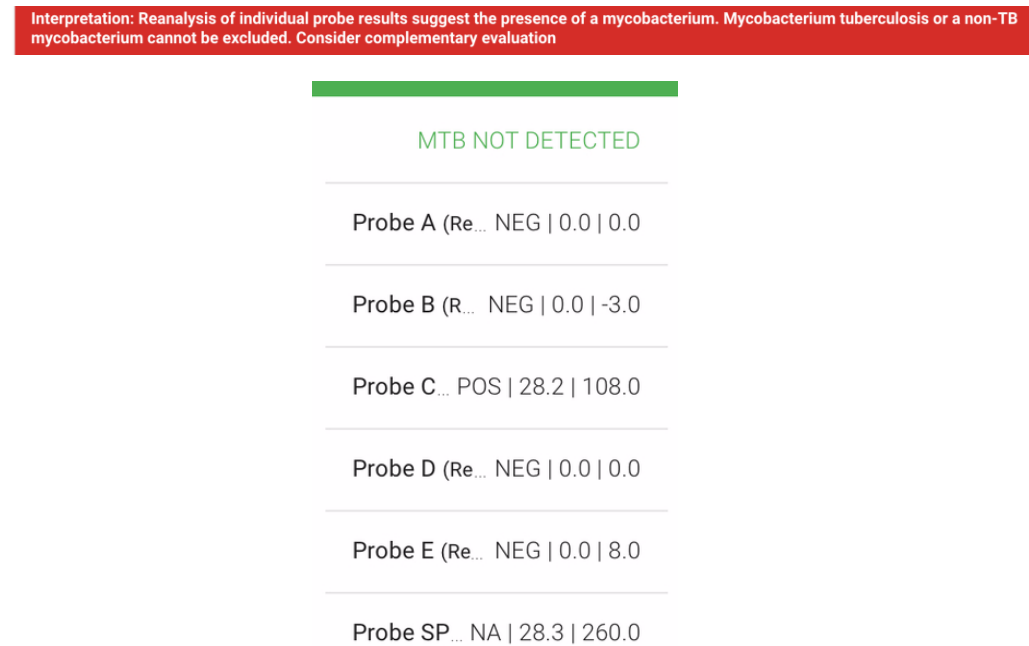


Figure 3.5: Automatic alert by DatatoCare (Savics, Belgium) when an Xpert MTB/RIF result suggests the presence of an NTM

The reference technique for detecting NTM infection is culture followed by the characterization of the *rpoB* or *hsp65* genes using sequencing techniques or commercial PCR kits (114, 116). Alternatively, sanger sequencing (118) or high throughput sequencing methods (119) can be used directly from biological samples.

3.3. Molecular diagnosis of drug resistance in TB

3.3.1. Rifampicin resistance

Mutations in the *rpoB* gene are associated with RIF resistance among several pathogenic mycobacteria, including MTBC, *M. leprae* and *M. kansasii* (120). These mutations alter the structure of the RNA polymerase and prevent the binding of RIF to its site of action. Most of these mutations occur in a small region of the *rpoB* gene called Rifampicin Resistance Determining Region (RRDR) or hotspot. Due to the similitude of the *rpoB* gene observed among different bacterial and mycobacterial species, the numbering system used to describe the precise nucleotide position of these mutations was initially the numbering of the *Escherichia coli* amino acid codons (121). This historical numbering system was later found to be imprecise, and we proposed a consensus number system for describing the resistance mutations found in the *rpoB* gene of MTBC and other pathogenic mycobacteria for which acquired RIF resistance has been reported (Figure 3.6).

The main mutations associated with RIF-resistant TB are represented in figure 3.7.



Figure 3.6: Alignment of the 81-bp RRDR of *E. coli*, MTBC, *M. leprae* and *M. kansasii*. Bars above the nucleotidic sequence indicate the position of seven codons frequently associated with RIF-resistance, using the numbering system proposed for each species. (Source: André E et al, 2017)

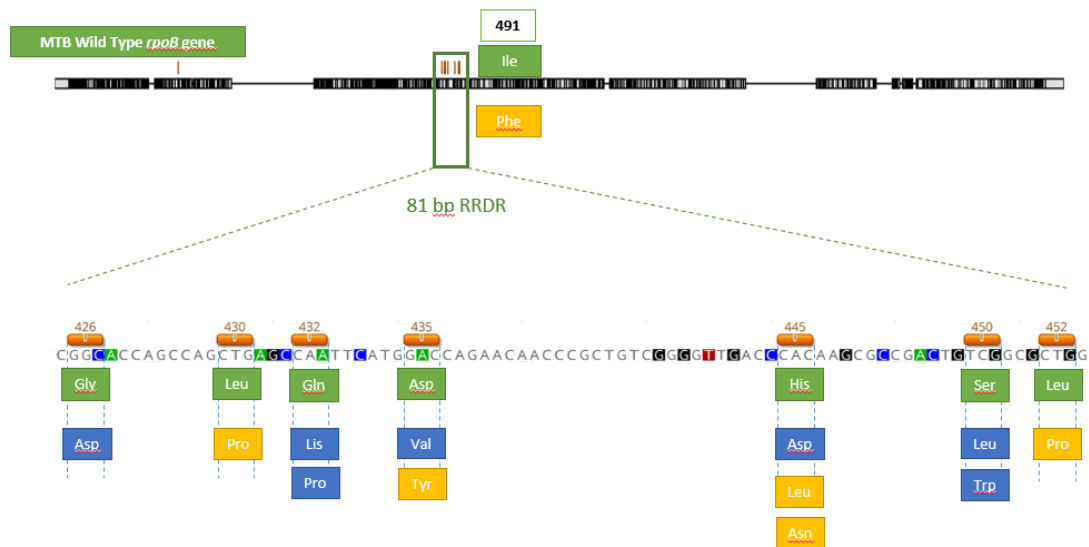


Figure 3.7: *rpoB* gene of MTBc and codons commonly associated with RIF resistance. Most RIF resistance conferring mutations occur in the rifampicin resistance determining region (RRDR). Phenotypic DST methods adequately detect mutations associated with high-level resistance *in vitro* (blue), but often misdiagnose low-level resistance (yellow boxes). These low-level resistance mutations represent approximately 10% of RIF resistance mechanisms and are associated with poor treatment outcome when patients are given first-line TB regimen.

3.3.2. Consensus numbering system for the rifampicin resistance-associated *rpoB* gene mutations in pathogenic mycobacteria

We found it very confusing that scientists used different numbering systems for describing RIF resistance conferring mutations. We proposed a consensus numbering system.

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Consensus statement

Consensus numbering system for the rifampicin resistance-associated *rpoB* gene mutations in pathogenic mycobacteriaE. Andre^{1,2,3,*}, L. Goeminne¹, A. Cabibbe⁴, P. Beckert^{5,6}, B. Kabamba Mukadi^{1,2}, V. Mathys⁷, S. Gagneux^{8,9}, S. Niemann^{6,10}, J. Van Ingen^{2,11}, E. Cambau^{3,11,12}¹ Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium² Microbiology Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium³ European Society for Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Mycobacterial Infections (ESGMYC), ESCMID, Basel, Switzerland⁴ Emerging Bacterial Pathogens Unit, Tuberculosis Supranational Reference Laboratory (SRL), IRCCS San Raffaele Scientific Institute, Milano, Italy⁵ Molecular and Experimental Mycobacteriology Group, Research Center Borstel, Borstel, Germany⁶ German Center for Infection Research, Borstel Site, Borstel, Germany⁷ National Reference Laboratory for Tuberculosis and Mycobacterial Diseases, Scientific Institute of Public Health (WIV-AISP), Brussels, Belgium⁸ Swiss Tropical & Public Health Institute, Basel, Switzerland⁹ University of Basel, Basel, Switzerland¹⁰ Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands¹¹ APHP, Hôpital Lariboisière, Laboratory of Bacteriology, National Reference Center for Mycobacteria and Antimycobacterial Resistance, Paris, France¹² University Paris Diderot, INSERM IAME UMR1137, Sorbonne Paris Cité, Paris, France

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ABSTRACT

The *rpoB* gene codes for the RNA polymerase β subunit, which is the target of rifampicin, an essential drug in the treatment of tuberculosis and other mycobacterial infections. This gene is present in all bacteria, but its length and nucleotide sequence vary between bacterial species, including mycobacteria. Mutations in the *rpoB* gene alter the structure of this protein and cause drug resistance. To describe the resistance-associated mutations, the scientific and medical communities have been using, since 1993, a numbering system based on the *Escherichia coli* sequence annotation. Using *E. coli* reference for describing mutations in mycobacteria leads to misunderstandings, particularly with the increasing use of whole genome sequencing, which brought an alternative numbering system based on the *Mycobacterium tuberculosis* *rpoB* sequence. We propose using a consensus numbering system for the reporting of resistance mutations based on the reference genomes from the species interrogated (such as strain H37Rv for *M. tuberculosis*). This manuscript provides the necessary figures and tables allowing researchers, microbiologists and clinicians to easily convert other annotation systems into one common language. **E. Andre, CMI 2016;•1**

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Introduction

Rifampicin, also named rifampin in the USA, is the key drug of the first-line treatment regimen for tuberculosis (TB), a disease affecting 9.6 million persons per year [1]. Multidrug-resistant TB (MDR-TB), defined as disease caused by *Mycobacterium tuberculosis* complex strains resistant to rifampicin and isoniazid, requires prolonged and

more complex administration of alternative treatment regimens including second-line anti-TB drugs, and is associated with poorer treatment outcome [2,3]. Isoniazid-resistance is caused by different mutations affecting different genes [4], but the molecular basis of RIF resistance is simpler, as virtually all resistant strains present a mutation in the *rpoB* gene, and 95% of these are located within a small 81-bp region named the rifampicin-resistance determining region (RRDR) [5]. Consequently, molecular assays were easily developed to detect rifampicin resistance, and several commercial kits are now available and used worldwide [6–8]. Furthermore, molecular detection of rifampicin resistance is used as a surrogate

* Corresponding author.

E-mail addresses: emmanuel.andre@uclouvain.be, emmanuel.andre@outlook.be (E. Andre).<http://dx.doi.org/10.1016/j.cmi.2016.09.006>1198-743X/© 2016 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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marker of MDR-TB in many countries, since <10% of rifampicin-resistant cases are not resistant to isoniazid [9–11]. The different species of the *M. tuberculosis* complex present similar resistance mutations [12–16].

Mutations in the *rpoB* gene are also associated with rifampicin resistance among other mycobacterial species, such as in *Mycobacterium leprae* causing leprosy [17,18] and *Mycobacterium kansasii* causing mainly respiratory infections in immunocompetent patients with also disseminated infections in immunocompromised patients [19]. Rifampicin resistance in *M. leprae* was described as associated with dapsone resistance in patients with MDR-leprosy. For these leprosy cases, the standard treatment regimen needs to be switched to a 2-year treatment combining daily second-line agents such as fluoroquinolones [20]. In *M. kansasii*, rifampicin is also a key component of treatment. Here *rpoB* gene mutations also lead to rifampicin resistance [19] and the need for alternative regimens [21].

Rifampicin resistance is caused by a structural alteration in the RNA polymerase β subunit, an enzyme coded by the *rpoB* gene. This mechanism was first described in 1981 for *Escherichia coli* [22,23], and it is therefore not specific to mycobacteria, although the clinical utility of *rpoB* mutations characterization is more important for TB and leprosy than for any other bacterial infections [24].

It was only in 1993 that Telenti et al. first suggested that rifampicin resistance in *M. tuberculosis* complex was associated with mutations in the *rpoB* gene, and observed a high similarity of the amino acids coded between several bacterial species including *E. coli*, *M. tuberculosis* complex and *M. leprae* [5]. Since the mutations described in that work were identified in an *rpoB* region that aligned with the RRDR of *E. coli*, Telenti et al. proposed an annotation system based on the *E. coli* genetic sequence. The authors probably underestimated how the diversity of the *rpoB* nucleotide sequence would later affect our ability to correctly report single nucleotide mutations causing amino acid substitutions [25–27]. Furthermore, although the authors recognized that resistance mutations may be located outside the RRDR, they did not pay attention to the low similarity between *E. coli* and *M. tuberculosis* complex in *rpoB* outside the RRDR. The existence and epidemiological importance of these ‘non-RRDR’ resistance mutations was later confirmed [28–30]. Nevertheless, this annotation system was rapidly adopted [31] and is still often used today for *M. tuberculosis* complex [32,33], *M. leprae* [34,35] and *M. kansasii* [19,36]. It was only recently that authors started using a species-specific numbering system [29,37–39]. This has caused some confusion because the same mutations are now named differently depending on the author, the year and the journal that published the work. For *M. leprae*, *rpoB* mutations were numbered first according to the gene sequence of *M. leprae rpoB* gene cloned in a cosmid, where the codon 425 corresponds to the codon 531 in *E. coli* and this was used to establish the WHO resistance surveillance network [40–42]. More recently, this expert group decided to switch to the numbering system of the *M. leprae* genome taking as a reference the genome sequence of the Tamil Nadu (TN) strain [37,43,44].

In this manuscript, we discuss the limitations of the traditional numbering system and propose to shift towards a more natural numbering system based on the mycobacterial reference sequences. This should allow better communication between laboratories, especially at a time when whole genome sequencing is becoming the standard for detecting drug resistance [39], improved disease surveillance [45,46] and faster spreading of scientific knowledge in the medical community.

Diversity of *rpoB* numbering systems

Among *M. tuberculosis* complex, *M. leprae* and *M. kansasii*, the nucleotide similarity lies between 86% and 88% for the entire *rpoB*

gene and is slightly higher in the 81-bp RRDR (between 88.9% and 92.6%). The similarity of these mycobacteria with *E. coli* is less (47% and 74% for the *rpoB* gene and the RRDR respectively) [47].

Furthermore, the *rpoB* genes of *E. coli*, *M. tuberculosis* complex, *M. leprae* and *M. kansasii* differ in length (4029, 3519, 3537 and 3540 base pairs, respectively). These differences explain the variable shift between the *E. coli*-based annotation and the mycobacterial sequence-based numbering systems. At present, resistance mutations outside the RRDR have only been characterized for *M. tuberculosis* complex. This situation leads to an even more complex situation, as the shift from the *E. coli* numbering system to the *M. tuberculosis* complex numbering system is different depending on the location of the resistance mutation (i.e. +24 for the *M. tuberculosis* complex codon 170, and –81 for the other codons of interest).

Table 1 and Fig. 1 represent the codons that are most frequently associated with resistance mutations across the three mycobacterial species. *Mycobacterium tuberculosis* complex and *E. coli* present identical amino-acids only for eight of the nine codons, and not nine as initially suggested by Telenti et al. [5]. Only three of the nine codons show the same sequence.

Reporting systems derived from the use of commercial assays

The commercial assays have developed several mutation detection systems as an alternative to gene sequencing. These assays report rifampicin resistance based on the detection of mutations in the wild-type (WT) sequence of the RRDR by molecular beacons [48,49] or hybridization [8,37,50]. These tests include probes that bind to WT sequences (Xpert MTB/RIF (*M. tuberculosis*/rifampicin), Cepheid), eventually combined with additional probes binding to mutated sites (line probe assays InnoLipa RifTB, Innogenetics, Ghent, Belgium; GenoType MTBDRplus and GenoType lepraDR, Hain Lifescience, Nehren, Germany), so silent mutations or mutations not associated with resistance can occasionally generate false-positive results [50–54].

The reporting format of these commercial assays can be ‘matched’ with the specific sequence covered by each probe (Xpert MTB/RIF), or the combination of WT and mutation bands (MTBDRplus, lepraDR) as illustrated in Fig. 2.

In practice, the majority of *rpoB* mutations are located at *M. tuberculosis* complex codons 435, 445 and 450 (also named 516, 526 and 531, respectively, using the *E. coli* numbering system) and at codons 456 and 441 in *M. leprae*. As an example, in the presence of the mutation Ser450Leu, the Xpert MTB/RIF assay will report a negative ‘Probe E’, signing the presence of a mutation located between positions 447 and 452. For the same mutation, the MTBDRplus V2.0 assay will report a negative ‘WT8’ band associated with a positive ‘MUT3’ band. Although complex laboratory networks generally use a combination of these tests, the concordance between the results of each test is rarely verified in routine conditions because of the difficulties in comparing between the numbering systems. The number of commercial assays available for TB diagnostics and detection of rifampicin resistance will increase in the future [55], and each of these tests will probably come with its own reporting format. If this is the case, the efforts required for understanding and solving discordant results will increase further [32].

Consequences of different coexisting numbering systems

The sequencing methods used to identify mutations are currently shifting from traditional Sanger sequencing to whole genome sequencing [56,57]. Although both methods are used today and will continue to coexist for some years, high-throughput sequencing technologies present multiple advantages including

Table 1

Conversion table between the two co-existing numbering systems for the most frequent *rpoB* mutations associated with rifampicin resistance among *Mycobacterium tuberculosis* complex, *Mycobacterium leprae* and *Mycobacterium kansasii*

<i>M. tuberculosis</i> H37Rv (GenBank AL123456)		<i>M. leprae</i> TN (GenBank AL583923)		<i>M. kansasii</i> ATCC 12478 (GenBank CP006835)		<i>Escherichia coli</i> (GenBank V00339)	
WT nucleotide sequence (amino acid)	Codon position (conversion from <i>E. coli</i> numbering)	WT nucleotide sequence (amino acid)	Codon position (conversion from <i>E. coli</i> numbering)	WT nucleotide sequence (amino acid)	Codon position (conversion from <i>E. coli</i> numbering)	WT nucleotide sequence (amino acid)	Codon position
GTC (Val)	170 (+24)					GTT (Val)	146
GGC (Gly)	426 (~81)	GGC (Gly)	432 (~75)			GTT (Gly)	507
CTG (Leu)	430 (~81)	CTG (Leu)	436 (~75)			CTG (Leu)	511
CAA (Gln)	432 (~81)	CAG (Gln)	438 (~75)	CAG (Gln)	442 (~71)	CAG (Gln)	513
GAC (Asp)	435 (~81)	GAT (Asp)	441 (~75)	GAC (Asp)	445 (~71)	GTC (Val)	516
CAC (His)	445 (~81)	CAC (His)	451 (~75)	CAC (His)	455 (~71)	CAC (His)	526
TCG (Ser)	450 (~81)	TGC (Ser)	456 (~75)	TCG (Ser)	460 (~71)	TCC (Ser)	531
CTG (Leu)	452 (~81)	CTG (Leu)	458 (~75)			CTC (Leu)	533
ATC (Ile)	491 (~81)					ATC (Ile)	572

WT, wild-type. The homology in regard to the nucleotide and the amino acid sequences of the different mycobacteria and *E. coli* is highlighted in green. Differences are highlighted in red.

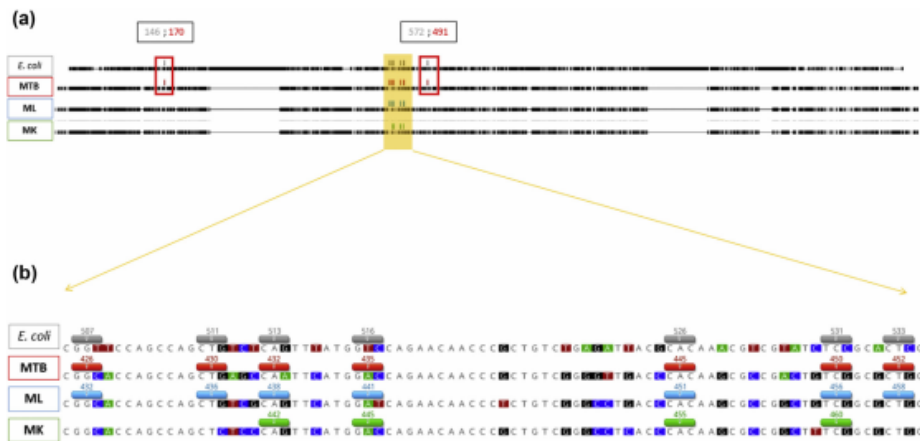


Fig. 1. (a) Alignment of the nucleotide sequences for the *rpoB* genes of *Escherichia coli*, *Mycobacterium tuberculosis* H37Rv (MTB), *Mycobacterium leprae* TN (ML) and *Mycobacterium kansasii* ATCC 12478 (MK). Rifampicin-resistance-determining region (RRDR) is highlighted in yellow. Red boxes show rifampicin resistance mediating mutation outside the RRDR. (b) Alignment of 81-bp RRDR of *E. coli*, *M. tuberculosis* complex, *M. leprae* and *M. kansasii*. Bars above the nucleotide sequences indicate the position of the seven codons frequently associated with rifampicin resistance, using the annotation system specific to each species. Nucleotide and amino acid differences are highlighted. Graph created using Gouxos, Biomatters, version 9.1).

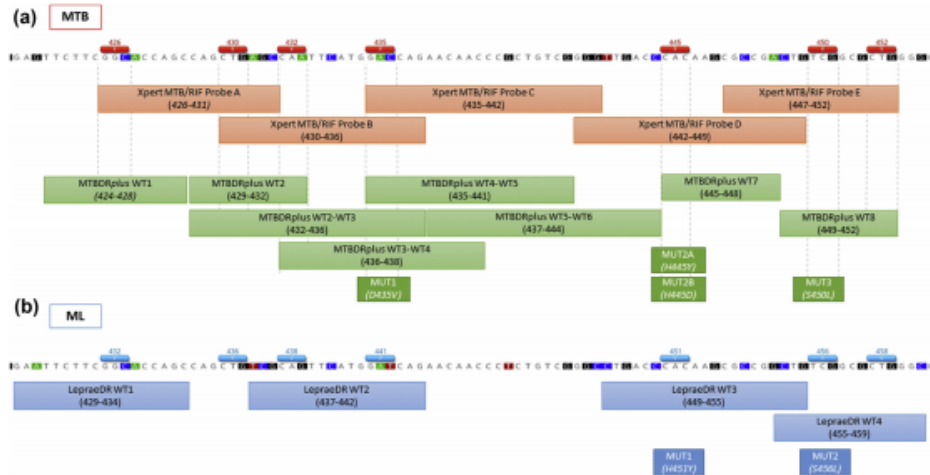


Fig. 2. (a) Alignment of *Mycobacterium tuberculosis* complex rifampicin-resistance-determining region (RRDR) sequence with the targets of Xpert MTB/RIF and the MTBDRplus V2.0 commercial assays. The red bars represent the location of the most common codons associated with rifampicin resistance conferring mutations. The orange bars indicate the regions covered by the five probes of the Xpert MTB/RIF assay. The green bars represent the regions covered by the eight wild-type (WT) and four mutation bands of the *GonoRx* MTBDRplus V2.0 assay. (b) Alignment of *Mycobacterium leprae* RRDR sequence with the targets of the LepreDR commercial assay. The blue bars represent the location of the codons associated with rifampicin resistance conferring mutations, the four WT bands and the two mutation bands. Graph created using Genious, Biomatters, version 9.1.

the ability to sequence simultaneously several genes associated with antimicrobial resistance. The inherent principles of sequencing require the use of an annotation system based on a consensus sequence, and starting at the beginning of each gene. Therefore, using *E. coli* as a reference sequence for *M. tuberculosis* complex and *M. leprae* is not reasonable, and hampers the necessity to correctly and precisely annotate genetic sequences and identify drug resistance-conferring mutations.

One example of how the use of different annotation systems can lead to major misunderstandings is the recent report from Swaziland. The authors reported that approximately one-third of the rifampicin-resistant *M. tuberculosis* complex strains were associated with the Ile491Phe mutation, a mutation that was supposedly never reported before, at least based on the *M. tuberculosis* complex numbering system [29]. However, this mutation is equivalent to Ile572Phe in the *E. coli* numbering system, a mutation that was described in rifampicin-resistant *M. tuberculosis* complex strains from many countries [58–63]. Sanchez-Padilla *et al.* used the *M. tuberculosis* complex-based annotation system, despite the fact that this denomination is almost non-existent in the scientific literature.

Perspectives

The characterization of the *rpoB* gene in *M. tuberculosis* complex strains, including the precise localization of mutations conferring rifampicin resistance, is probably one of the most common molecular biology analyses performed for clinical purposes worldwide. Unfortunately, a standardized reporting format cannot be obtained because of the coexistence of different numbering systems used by commercial and non-commercial assays targeting this gene.

There are three main reasons why the *E. coli* and the device-specific numbering systems should be changed to one standardized system based on the sequence of the reference strain of each species, namely H37Rv (GenBank reference AL123456) for

M. tuberculosis complex, *M. leprae* (GenBank reference AL583923) for *M. leprae* and *M. kansasii* ATCC 12478 (GenBank reference CP006835) for *M. kansasii*. First, although the homology between *E. coli* and *M. tuberculosis* complex is relatively high in the RRDR, it is significantly lower for the rest of the *rpoB* gene. Therefore, although the correspondence between the *E. coli* and *M. tuberculosis* complex numbering systems is relatively easy within the hotspot region (the difference is 81 codons), such a 'simple conversion' does not work when looking at a resistance mutation located outside this specific region (the difference between the *M. tuberculosis* complex codon 170 and its equivalent in the *E. coli* genome is 24) [28,64]. Second, the sequencing method used to identify mutations is currently shifting from traditional Sanger sequencing methods to whole genome sequencing. As the genome of *M. tuberculosis* complex reference strain H37Rv is standard strain used worldwide [65], any variant numbering nomenclature should be based on this sequence [29,39,56]. Such revision has been previously proposed for the *gyrB* gene associated with fluoroquinolone resistance, for which four different reporting systems have been used, resulting in mutation location discrepancies [66]. Finally, many of the *rpoB* mutations reported in clinical strains so far have distinctive characteristics in conferring phenotypic resistance, including varying cross-resistance to rifabutin. Therefore, a precise nomenclature for the location of the mutations is essential from a laboratory diagnostic perspective, especially for the development of individualized treatment regimens [25,32].

Accordingly, the use of divergent numbering systems for *rpoB* mutations generates risks related to miscommunication and delays the transfer of scientific knowledge into clinical care.

The aim of this manuscript is to facilitate the shift towards this universal annotation system, including the necessary figures and tables allowing researchers, microbiologists and clinicians to easily 'translate' other annotation systems into one common language.

Transparency Declaration

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3.3.3. Detection of RIF resistance by commercial rapid molecular assays

Xpert MTB/RIF

The original design of the Xpert MTB/RIF test was based on the amplification of the rifampicin resistance determining region (RRDR) of the *rpoB* gene followed by the binding of five different fluorescent probes, as illustrated in the two figure below (122). In this test, the detection of the five different coloured probes confirms the presence of MTBC with a wild-type sequence of the RRDR. When a resistance mutation is present, it prevents the binding of the probe covering this position, and therefore the reaction will only reveal four of the five fluorescent signals (104).

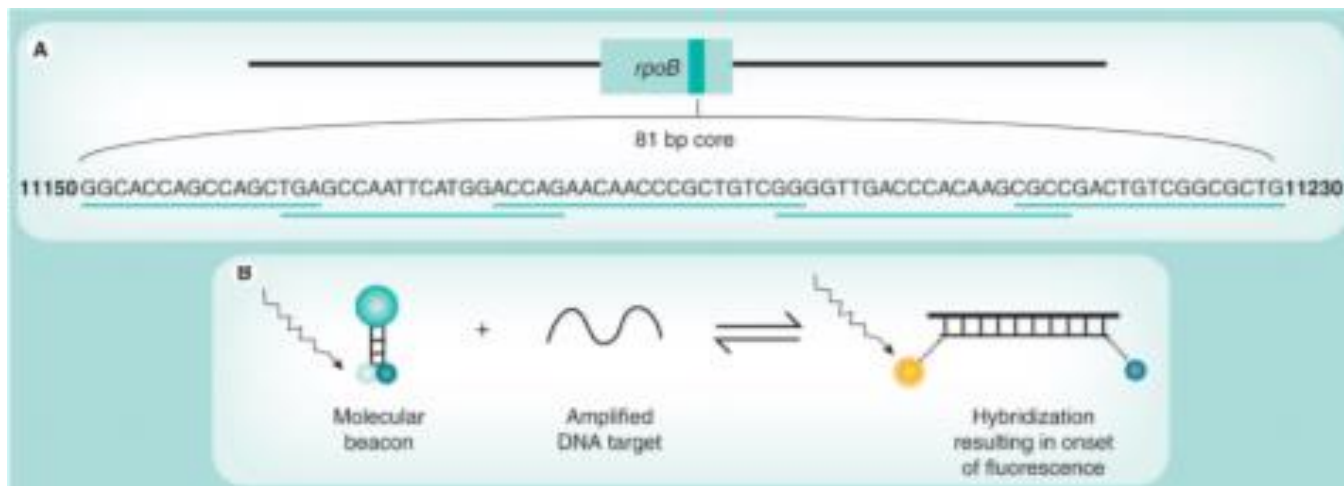


Figure 3.8: The *rpoB* gene core region target sequence and molecular beacon technology. (A) the *rpoB* gene and the localization of the complementary overlapping molecular beacon probes. (B) Following hybridization, conformational change in the probe leads to onset of fluorescence. (Source: Lawn, 2011)

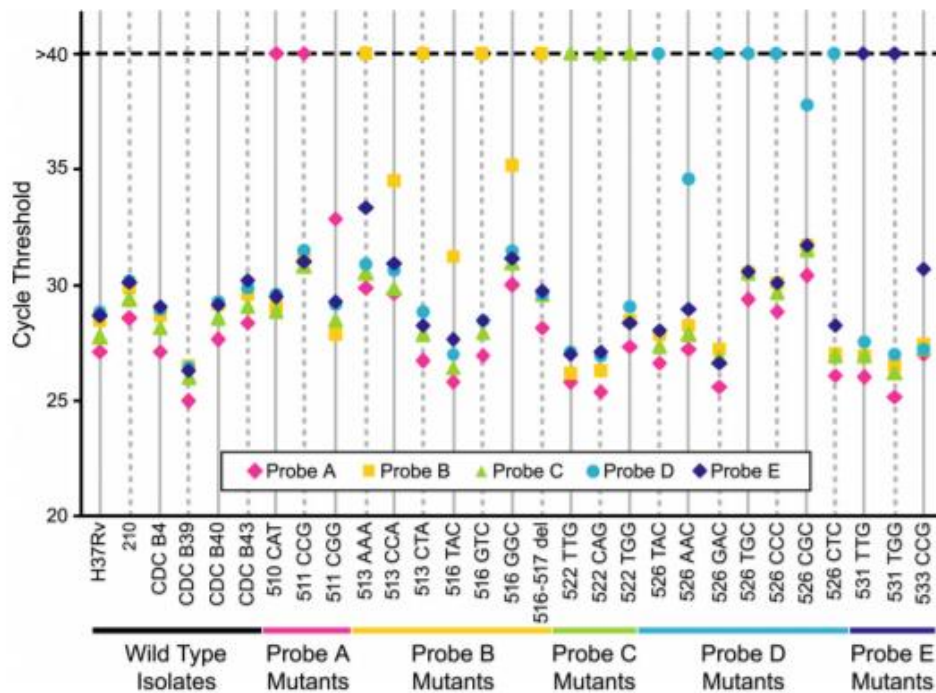


Figure 3.9: Xpert MTB/RIF individual probe results obtained from genomic *M. tuberculosis* DNA or artificial targets containing clinically relevant mutations mixed with TB-negative sputum. The results produced for each sample are indicated by a single vertical line on which the Cycle threshold (Ct) of each of the five molecular beacon probes is plotted. The numbering system used in this figure is the outdated *E. coli* system (Source: Helb et al, 2010)

LPA assays

The WHO has endorsed two commercial line probe assay (LPA) commercial tests for the detection of RIF and INH resistance (MTBDR*plus* V2.0, Hain Lifescience and GenoscholarTMNTM+MDRTB II, Nipro). In these assays, the detection of the PCR products is performed by hybridization to a number of specific probes linked to a paper strip. These assays only cover the 81bp region of the *rpoB* gene, and therefore miss the hard-to-detect mutation discussed above.

Xpert MTB/RIF Ultra

The Xpert MTB/RIF ultra test has not yet been released commercially and only partial validation results are available to date^{8,9} despite the fact that this test is planned to be released in 2017 and fully replace the previous version of the test. This assay only covers the 81bp region of the *rpoB* gene, and therefore misses the hard-to-detect mutation discussed above. There are two main differences compared to the previous generation of the test:

- First MTBC detection is based on the IS6110 and IS1081 sequences.
- Second, the detection of RIF resistance is no-longer performed with molecular beacon probes but now with more conventional probes with melting temperatures that vary depending on whether a mutation is present or not.

	codon	rpo1	rpo2	rpo3	rpo4
Wild type		69.3	73.1	75.3	66.8
L511P	511	63.2 (-6.1)	73.1	75.3	66.4
Q513K	513	65.5 (-3.8)	73.1	75.4	66.5
D516V	516	69.2	69.4 (-3.7)	75.2	66.6
DEL-516	516	69.4	66.7 (-6.4)	75.5	66.8
DEL-518	518	69.4	66 (-7.1)	75.4	66.4
S522L	522	69.3	70.1 (-3)	73.4	66.6
H526Y	526	69.2	73.5	72.8 (-2.5)	67.8
H526D	526	69.4	73.3	71.9 (-3.4)	67.5
H526D	526	69.2	73.2	71.8 (-3.5)	67.3
H526L	526	69.2	73.1	71.9 (-3.4)	66.5
R529K	529	69.2	72.8	74.2	63.9 (-2.9)
S531Q	531	69.3	73.1	71 (-4.3)	66.4
S531L	531	69.4	73.7	73 (-2.3)	73.6 (+6.8)
S531W	531	69.2	73.2	72.8 (-2.5)	70.5 (+3.7)
L533P	533	69.3	73.2	75.5	61.4 (-5.4)
L533R	533	69.4	73.2	75.5	61.4 (-5.4)

Table 3.1: Melting temperature (T_m) profile for a panel of RIF-resistant TB strains tested with the Xpert MTB/RIF Ultra assay (Cepheid, USA). The T_m is modified in the presence of an underlying mutations (Source: Alland D et al, 2015). The data presented suggest that some specific changes in the same may alter differently the T_m (e.g. in position 531). This level of precision may be later exploited to suggest or exclude transmission of TB among patients.

⁸ <http://www.croiconference.org/sessions/xpert-mtbrif-ultra-new-near-patient-tb-test-sensitivity-equal-culture>

⁹ <http://www.croiwebcasts.org/p/2017croi/croi33479>

3.3.4. Misdiagnosis of Rifampicin resistance by the Xpert MTB/RIF assay

From a theoretical point of view, the Xpert MTB/RIF assay presents two potential limitations with regard to its ability to accurately detect RIF resistance.

First, the test only covers the 81 bp rifampicin resistance determining region (RRDR) of the *rpoB* gene. Although most RIF resistance conferring mutations occur in the RRDR, at least two mutations located on codons 170 (formerly 146) and 491 (formerly 572) have been reported (123). As illustrated in the figure below, genotypic DST methods limited to the RRDR adequately detect mutations located in the RRDR (blue), but misdiagnose RIF resistance-conferring mutations when these occur outside this region (yellow box).

The Ile491Phe mutation has been reported prior to the large scale-up of rapid molecular tests, but at a low level. A scientific paper described that in 2009 and showed that 30% of MDR-TB strains from Swaziland had this mutation (124). Despite this situation, no systematic monitoring of this mutation was implemented at national or international levels.

The second limitation of Xpert MTB/RIF and other PCR-based assays compared to sequencing methods is that these methods will pick silent mutations the same way as mutations that alter the amino acid sequence and the protein structure. Such false resistance have been described due to silent mutation emerging at various positions in the RRDR of the *rpoB* gene (125-128).

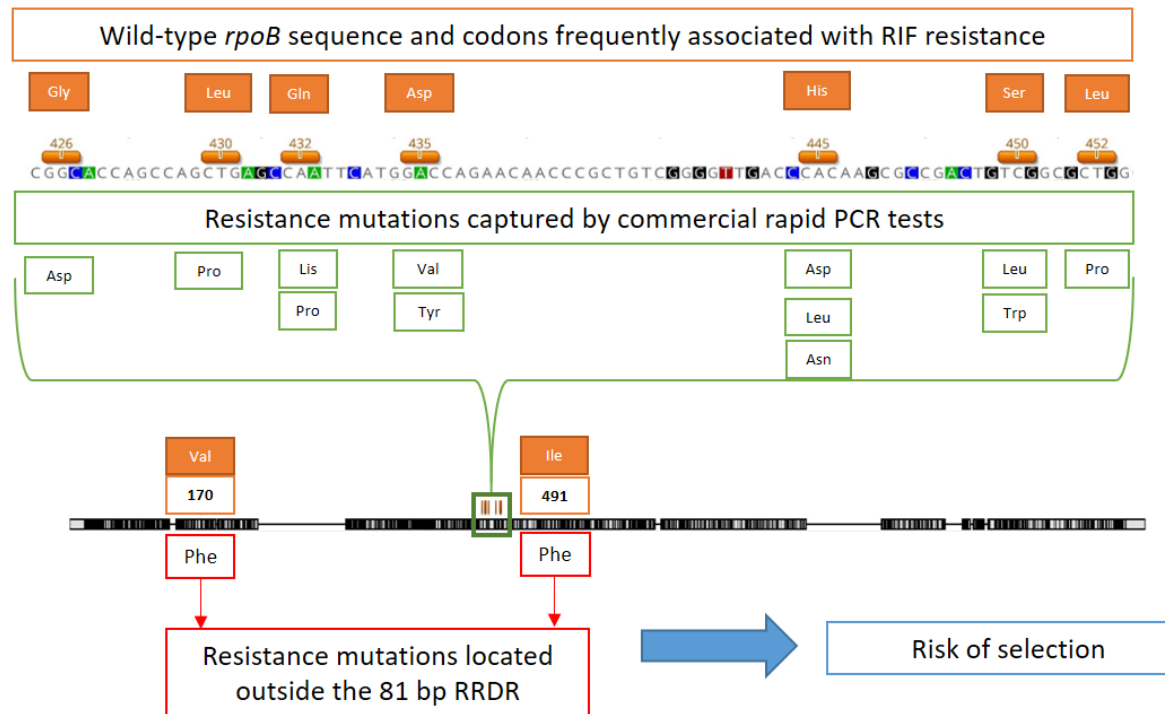


Figure 3.10: *rpoB* gene of MTBc and codons commonly associated with RIF resistance. Most RIF resistance conferring mutations occur in the rifampicin resistance determining region (RRDR). Genotypic DST methods limited to the RRDR adequately detect mutations located in this short region (green), but misdiagnose RIF resistance-conferring mutations when these occur outside the RRDR (red). The Ile491Phe mutation has been reported prior to the large scale-up of rapid molecular tests, but at a low level. A scientific paper described that in 2009, 30% of MDR-TB strains from Swaziland had this mutation. Despite this situation, no systematic monitoring of this mutation was implemented at national or international levels.

3.3.5. Development of a rapid PCR for the detection of the *rpoB* Ile491Phe RIF resistance mutation

The reference technique for detecting the Ile491Phe *rpoB* mutation is the sequencing of the *rpoB* gene in a larger segment than the 81bp region targeted by commercial assays. Both sanger sequencing (118) or high throughput sequencing methods (119) can be used. Alternatively, DST methods based on solid Loewenstein-Jensen cultures (129) can provide relatively good result.

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Research note

Novel rapid PCR for the detection of Ile491Phe *rpoB* mutation of *Mycobacterium tuberculosis*, a rifampicin-resistance-conferring mutation undetected by commercial assaysE. André^{1,2,*}, L. Goeminne¹, A. Colmant², P. Beckert^{3,4}, S. Niemann^{3,4}, M. Delmee^{1,2}¹ Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium² Service de Microbiologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium³ Molecular and Experimental Mycobacteriology Group, Research Centre Borstel, Borstel, Germany⁴ German Centre for Infection Research, Borstel Site, Borstel, Germany

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ABSTRACT

Objectives: Neither the liquid medium-based Bactec MGIT, nor commercial molecular assays such as the Xpert MTB/RIF and the MTBDRplus V2.0 assays are capable of detecting up to 30% of rifampicin-resistant *Mycobacterium tuberculosis* strains in Swaziland because of the large proportion of the *rpoB* Ile491Phe mutations. In other countries, the frequency of this mutation is thought to be low.

Methods: We designed a real-time multiplex allele-specific PCR assay to identify the *rpoB* Ile491Phe mutation responsible for these undetected resistant *M. tuberculosis* strains.

Results: The technique showed 100% similarity with *rpoB* sequencing on a panel of 78 strains from Swaziland.

Conclusions: We propose that the detection of the *rpoB* Ile491Phe *rpoB* mutation should complement commercial assays for the diagnosis of rifampicin-resistant *M. tuberculosis* in routine conditions, particularly in countries where this specific mutation is frequent. The technique proposed in this paper is adapted for most reference laboratories. **E. André, CMI 2016;1**

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Introduction

Multidrug-resistant tuberculosis (MDR-TB) is defined as disease caused by *Mycobacterium tuberculosis* strains resistant to the two most powerful first-line treatment regimen drugs, isoniazid and rifampicin. It has been estimated that 480 000 persons were diagnosed with MDR-TB in 2014 [1]. Only the rifampicin-resistance-conferring mutations located within the 81 bp so-called rifampicin-resistance-determining region of the *rpoB* gene are detected by commercial assays currently being rolled-out at global scale. A recent survey in Swaziland highlighted that up to

30% of MDR-TB presented a rifampicin-resistance mutation located outside the rifampicin-resistance-determining region [2]. The Ile491Phe mutation, previously identified as Ile572Phe in reports using the outdated *Escherichia coli* numbering system of the *rpoB* gene [3], has been previously reported in many countries [4–9] but generally represented between 1% and 5% of rifampicin-resistant strains. Furthermore, this resistance is not detected by the commercial liquid medium-based Bactec Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson, Franklin Lakes, NJ, USA) [9], and therefore only sequencing-based methods allow reliable detection of this mutation.

Materials and methods

We designed a multiplex allele-specific (MAS) PCR assay to detect the Ile491Phe rifampicin-resistance-conferring mutation in

* Corresponding author. E. André, 10, avenue Hippocrate, B-1200 Brussels, Belgium.

E-mail address: emmanuel.andre@uclouvain.be (E. André).

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rpoB using a one-step real-time PCR. In brief, MAS-PCR uses a combination of three primers, resulting in the amplification of allele-specific fragments distinguished by their size.

At the Research Centre Borstel, DNA isolation and *rpoB* gene sequencing of 78 *M. tuberculosis* strains from Swaziland [10] was performed [11]. DNA of these strains, among which 39 presented the *rpoB* Ile491Phe mutation and 12 harboured at least one other mutation within the *rpoB* gene, were sent to the Université catholique de Louvain where the MAS-PCR was performed. These tests were performed on the Light Cycler 96 (Roche, Mannheim, Germany), using the FastStart SYBR Green Master (ref. 04673484001, Roche) and 0.5 µM of each of the three MAS-PCR primers (Table 1). Final volume of the reaction was 25 µL, including 5 µL of DNA. Thirty-five cycles of amplification with annealing at 56°C were followed by a melting analysis of PCR products performed between 65°C and 97°C. The ramp was 0.1°C/second, with ten readings per °C. After classification of the 78 strains from Swaziland into mutant and wild-type, results were compared with previously generated sequencing data.

We further evaluated the limit of detection of the assay using different dilutions of DNA extracted from positive MGIT tubes on which we performed several dilutions (1, 1/50, 1/100, 1/500 and 1/1000).

Results

Applying the melting curve analysis following the MAS-PCR distinguished two populations of strains, each characterized by a specific melting temperature. Of the 78 strains, 39 showed a melting temperature ranging from 85.21°C to 85.56°C and 39 strains showed a melting temperature from 90.80°C to 91.06°C (Fig. 1). Subsequent correlation of strain classification by melting curve analysis with the previously obtained sequencing data of the *rpoB* gene showed that all 39 Ile491Phe (ATC > TTC) rifampicin-resistant mutant strains were grouped into one population clearly separated from the wild-type strains by a difference of at least 5.24°C in the melting temperature.

Table 1
Primers used for the *rpoB* Ile491Phe multiplex allele-specific PCR

Primer	Target	Sequence
<i>rpoB</i> _F	Wild-type and mutated strains	TGGAGTACGTGCCCTCGTC
<i>rpoB</i> _R	Wild-type and mutated strains	GTGGCCACCGACACCATCT
<i>rpoB</i> _I491_F	I491F mutated strains only	GGCCCAACATCGGTCTGA

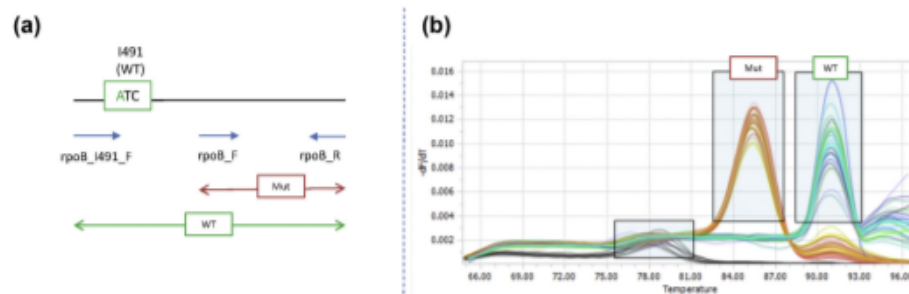


Fig. 1. (a) Design of the multiplex allele-specific PCR for the detection of the Ile491Phe *rpoB* rifampicin-resistance mutation. The *rpoB*_I491_F primer is specific to the wild-type profile, whereas the *rpoB*_F and *rpoB*_R primers are universal. (b) Results: the method allowed the correct identification of the *rpoB* Ile491Phe mutation among the 39/78 rifampicin-resistant strains.

We evaluated the potential interference linked with the presence of other *rpoB* mutations. In total, 12 strains presented other mutations, but none of these was associated with an erroneous result.

The assay performed well on the 78 DNA extracts from positive culture. We tested the assay on sequential dilutions of positive MGIT culture media. The test still showed correct results when the cultures were diluted 1/500 before DNA extraction. At these lowest dilutions, fluorescence microscopy performed on the positive culture was negative. The test was not designed to be performed directly on sputum and therefore was not evaluated as a direct diagnostic test.

This test was performed on a real-time PCR platform, which allows up to 96 samples to be investigated at the same time (or 32 samples if three PCRs are performed for each sample). Importantly, the technique does not require an electrophoresis gel step for the interpretation of the results, as we used melting curve analysis of the PCR products. Time-to-result is <3 h and the reagent cost is below US\$4.5 per test including reagents, man-power and equipment.

Conclusion

We propose a simple and rapid PCR method that may, at low-price, ensure reference laboratories to detect rifampicin-resistance mutations undetected by commercial molecular and phenotypic assays, including the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), MTBDRplus VER 2.0 (Hain Lifescience GmbH, Nehren, Germany) and MGIT assays. In countries like Swaziland where 30% of MDR-TB harbour the *rpoB* Ile491Phe mutation linked to a highly disseminated strain, the systematic identification of this mutation among patients with TB has become necessary. In other settings, the prevalence of the *rpoB* Ile491Phe rifampicin-resistance-mediating mutation should be assessed regularly with valid methods including the RT-PCR described here or sequencing methods.

Transparency declaration

The authors declare no conflict of interest. No external funding was received for this manuscript.

Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article can be found at <http://dx.doi.org/10.1016/j.cmi.2016.12.009>.

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3.3.6. Isoniazid-resistant TB treatment with first-line drugs

Difficult-to-detect RIF resistance mechanisms such as the Ile491Phe *rpoB* mutation may explain, in settings where MDR-TB is ruled-out based on a first-line RIF sensitive result, high rates of treatment failure among patients with reported “INH mono-resistance”. (15, 124, 130).

Isoniazid-resistant tuberculosis treatment with first-line drugs

We read with interest the systematic review and meta-analysis by Medea Gegia and colleagues¹ of use of first-line drugs for isoniazid-monoresistant tuberculosis. Among the main findings, this study revealed that 11% of new patients who received the first-line WHO standard regimen had treatment failure. On the basis of these observations, the authors suggest that standardised empirical treatment of new cases could be contributing substantially to the multidrug-resistant tuberculosis epidemic. However, the authors do not question the reliability of

In this context, assessment of the efficacy of first-line regimens among patients for whom whether they have isoniazid-monoresistant or undetected multidrug-resistant tuberculosis is of questionable value. In most studies used for this systematic review and meta-analysis, the frequency of negative clinical outcomes is similar to the expected level of detection of multidrug-resistant tuberculosis when suboptimal techniques are used. We would have appreciated this important question being addressed.

We declare no competing interests

***Emmanuel Andre, Anandi Martin**
emmanuel.andre@uclouvain.be

Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels B-1200, Belgium

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the initial bacteriological diagnosis that was made for these patients, which supported the clinical decision to initiate standardised first-line treatment.

Most studies referenced in the systematic review and meta-analysis used phenotypic drug susceptibility testing methods, which fail to detect a substantial proportion of rifampicin-resistant strains.² Of the 52 papers cited, only two used molecular methods for establishment of rifampicin resistance. None of the studies included sequencing of the *rpoB* gene and all were published between 1968 and 2014.

Only since 2014 have reports originating from different regions of the world highlighted the fact that several specific mutations were regularly undetected and thus responsible for missed opportunities to diagnose multidrug-resistant tuberculosis.³⁻⁵ To date, the most alarming figures originated from Swaziland, where a third of patients with multidrug-resistant tuberculosis had the *rpoB* Ile491Phe mutation,⁶ which is hardly detected by phenotypic drug susceptibility testing methods and missed by commercial molecular assays.

- 1 Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. *Lancet Infect Dis* 2016; published online Nov 16. [http://dx.doi.org/10.1016/S1473-3099\(16\)30407-8](http://dx.doi.org/10.1016/S1473-3099(16)30407-8).
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- 3 Andres S, Hillemann D, Rusch-Gerdes S, Richter E. Occurrence of *rpoB* mutations in isoniazid-resistant but rifampin-susceptible *Mycobacterium tuberculosis* isolates from Germany. *Antimicrob Agents Chemother* 2014; **58**: 590–92.
- 4 Ahmad S, Mokaddas E, Al-Mutairi N, Eldeen HS, Mohammadi S. Discordance across phenotypic and molecular methods for drug susceptibility testing of drug-resistant *Mycobacterium tuberculosis* isolates in a low TB incidence country. *PLoS One* 2016; **11**: e0153563.
- 5 Shah NS, Grace Lin SY, Barry PM, Cheng YN, Schecter G, Desmond E. Clinical impact on tuberculosis treatment outcomes of discordance between molecular and growth-based assays for rifampin resistance, California 2003–2013. *Open Forum Infect Dis* 2016; **3**: ofw150.
- 6 Sanchez-Padilla E, Merker M, Beckert P, et al. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. *N Engl J Med* 2015; **372**: 1181–82.

3.3.7. Multiple outbreaks of MDR-TB associated with a hard-to-detect RIF resistance mutations

Emergence of low-level *in vitro* RIF resistance mutations is a natural phenomenon that preceded the introduction of commercial phenotypic (MGIT) drug susceptibility testing methods. Nevertheless, scale-up of the MGIT SIRE assay (which occurred mainly in middle- and high-income countries) probably had a limited impact on the selection of strains harbouring the so-called “disputed mutations” that cause low-level *in vitro* RIF resistance. This is because these mutations usually co-exist with INH resistance and the MGIT SIRE test allowed this associated INH resistance to be detected (131). Consequently, the MGIT SIRE test often caused treatment for the patients and their infected contacts to include more drugs, potentially improving the prognosis and limiting dissemination of the resistant strain (Figure 3.11A).

When instead of the MGIT SIRE test that tests for resistance to four drugs, GeneXpert (that only tests for resistance to RIF) is used then the selection mechanism associated with the emergence of the Ile491Phe *rpoB* mutation is different. This mutation is very rare (<2%), but could be amplified in a setting with very high levels of TB was associated with a massive utilization of the Xpert MTB/RIF assay. As this test is used to operationally rule-out MDR-TB, monitoring of the emergence of INH resistance was not performed. As a result, these strains have a major epidemic potential, as patients and their infected contacts with these strains are very likely to receive inappropriate treatment (132) (Figure 3.11B).

Strains harbouring the Ile491Phe mutation have currently been reported in many countries, and some of them have demonstrated outbreaks with MDR-TB strains harbouring this mutation: Swaziland (outbreak reported since 2009 (124)), South-Africa (two outbreaks revealed below); Mozambique (personal communication from Stefan Niemann) and UK (personal communication from Tim Walker: outbreak in Birmingham). These findings highlight the importance of monitoring the emergence of strains harbouring this mutation, particularly in settings where RIF resistance and MDR-TB are operationally ruled-out based using laboratory methods that miss the Ile491Phe *rpoB* mutation.

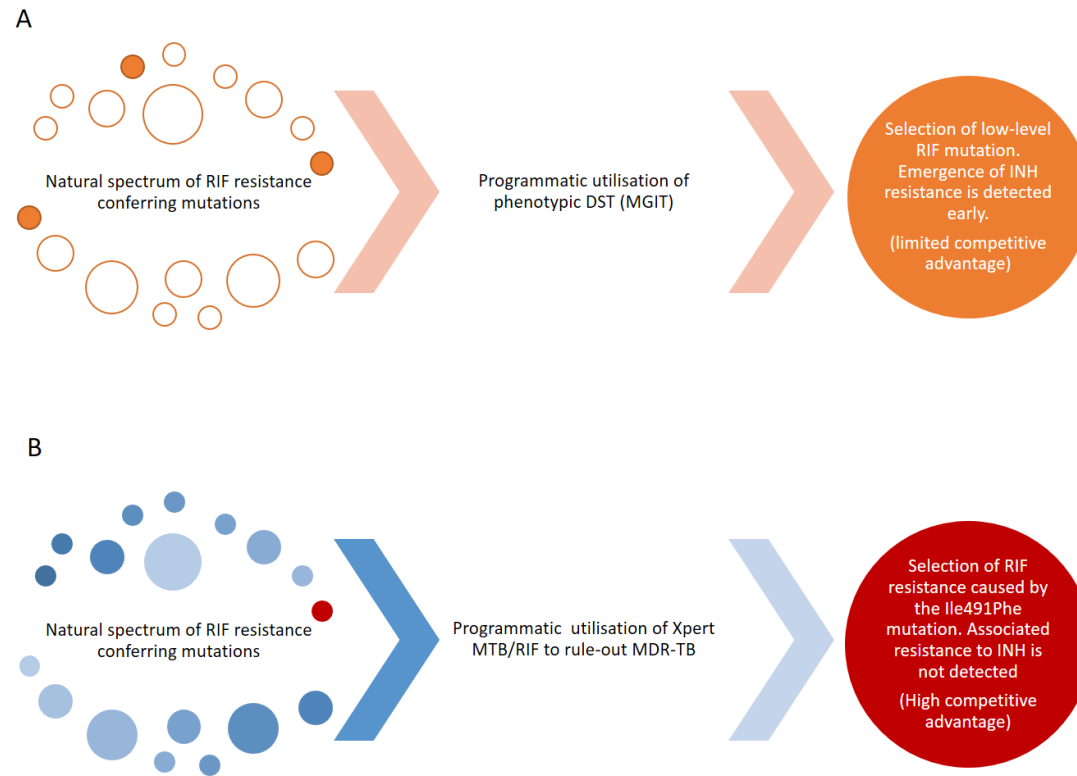


Figure 3.11: Mechanism of selection by MGIT-based DST (A) and Xpert MTB/RIF (B) of strains harbouring undetected RIF-resistance mechanisms. The fact that MDR-TB and INH resistance are ruled-out based on a “RIF sensitive” test result contributes to the increased epidemic potential of these strains.

3.3.8. Missed opportunities for diagnosing MDR-TB in South-Africa

We used the technique described above to screen for the *rpoB* Ile491Phe mutation (133) among 277 samples originating from 3 provinces in South-Africa.

All these strains had previously been reported as RIF S, INH R in South-Africa, using the current algorithm, which includes Xpert MTB/RIF testing, LPA and phenotypic DST by (MGIT, BD).

Valid PCR results were obtained for 259 samples (93,5%), and we found 37 (15%; CI 95%: 10.6 – 19.2%) samples harbouring the *rpoB* Ile491Phe RIF-resistance mutation. In all 37 cases, RIF resistance was confirmed by Sanger sequencing.

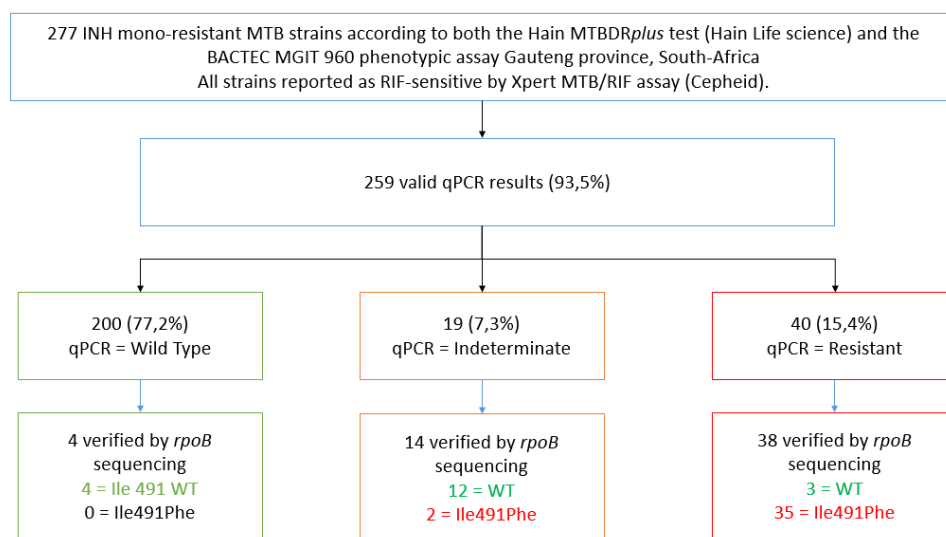


Figure 3.12: Screening results for 277 MTB strains reported as INH mono-resistant using current programmatic algorithms in South-Africa. A total of 37 strains were found to harbour the Ile491Phe *rpoB* mutation.

At the time of writing (July 2017), we had performed the NGS Deeplex MycTB (Genoscreen, France) for 54 samples originating from Swaziland (n=17, strains from the 2009 national drug resistance survey, kindly provided by Borstel lab in Germany) and all strains from South-Africa for which the Ile491Phe PCR was positive or indeterminate (n=37). The results of these tests are available in the annex section.

As expected, all 17 strains from Swaziland harboured the same spoligotype (SIT 34) and resistance profile (resistance to RIF, INH, EMB, PZA and SM)

Among the 37 south-African strains:

- 8 originated from the Tshwane municipality in the Gauteng province
 - 5 presented exactly the same spoligotype and resistance profile as the strains from Swaziland (SIT 34)
 - 2 strains presented a different spoligotype (SIT 92) and the same resistance profile (resistance to RIF, INH, EMB, PZA and SM)
 - 1 strain was un-typable (low coverage)
- 2 originated from the Thembisile municipality in the Mpumalanga province
 - 1 presented exactly the same spoligotype and resistance profile as the strains from Swaziland (SIT 34)
 - 1 strain presented the SIT 92 profile (resistance to RIF, INH, EMB, PZA and SM)
- 27 originated from the the North-West province (21 from Rutsenburg, 1 from Ngobi and 5 from Moses-Kotane municipalities respectively)
 - 24 presented exactly the same spoligotype and resistance profile as the strains from Swaziland (SIT 34)
 - 1 strain presented the SIT 92 profile (resistance to RIF, INH, EMB, PZA and SM)
 - 2 strains were un-typable (low coverage and mixed populations)



Figure 3.13: Origin of 37 MDR-TB strains previously reported as INH mono-resistant by current diagnostic algorithms and presenting the *rpoB* Ile491Phe mutation.

Rutsenburg, a municipality from which the majority of the strains originated although being the most distant from Swaziland, is a rapidly growing city that has been the centre of international attention in 2012, due to the Marikana strikes and killing of miners. It hosts the world's largest platinum mine. There are probably important migration movements between this city and Swaziland due to the industrial activity.

Interestingly, we found in this preliminary evaluation that the Ile491Phe mutation was present in only a 15 out of 42 of clinics (36%; 95% CI: 24– 54), but that when it was present, there was an average of 2.4 cases found (from one to eight cases reported in the same clinic). This is consistent with horizontal transmission of these drug-resistant strains, possibly occurring in these clinics. In all 18/18 patients with the *rpoB* Ile491Phe mutation, this mutation was also associated with high-level INH resistance caused by *katG* mutations. In contrast, among patients with confirmed INH mono-resistance, only 8/20 patients had this high-level resistance ($p=0.0001$).

In figure 3.14, we propose a model for explaining how these two clusters of MDR-TB have emerged and successfully spread in 3 distinct provinces of South-Africa. We believe that the massive utilization of Xpert to rule-out MDR-TB and the high HIV burden among these patients have contributed to this selection.

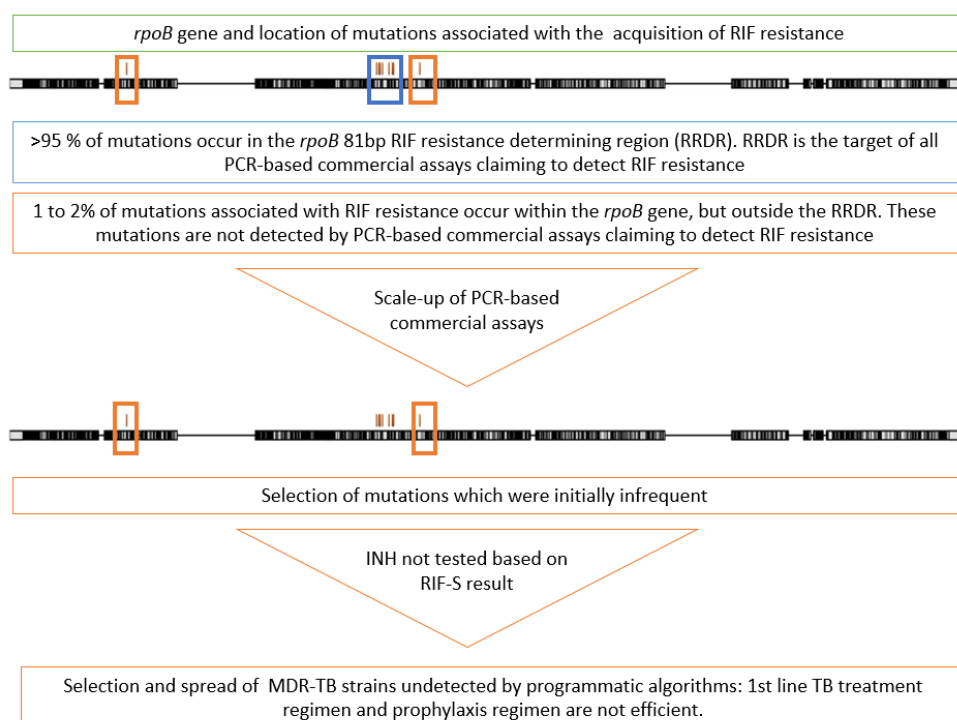


Figure 3.14: Hypothetical explanation for the emergence of *rpoB* Ile491Phe-associated MDR-TB outbreaks in South-Africa.

3.3.9. Principle of next-generation sequencing

High-throughput sequencing methods allow much deeper genomic, transcriptomic or microbiota analysis. There are several technologies available, and their common principle is that they produce up to millions of sequences that are then re-assembled using dedicated software.

This technology has decreased dramatically the cost of sequencing, and different applications have emerged and are being introduced as routine diagnostic tests in the field of TB, in particular whole genome sequencing (WGS)¹⁰ and multiplex Next Generation Sequencing (NGS)-based commercial kits¹¹.

These new technologies allow the simultaneous detection of various genes associated with resistance. In the future, in order to avoid the selection of rare variants and provide the most efficient treatment to each patients, NGS-based diagnostic methods will need to be implemented in programmatic conditions. Automation of the complex pre-analytical steps required and the implementation of data analysis pipelines remain the main challenges today.

¹⁰ <http://www.ntgmc.nhs.uk/2017/03/29/whole-genome-sequencing-to-diagnose-tb/>

¹¹ http://www.stoptb.org/wg/new_diagnostics/assets/documents/NDWG-Meeting-2016_Session2_03_Supply_DeepLex-MycTB-Test.pdf

Chapter 4: Logistical challenges associated with the introduction of rapid molecular tests in low resource settings

4.1. Context

With the support of a TB REACH grant, we had the opportunity to implement 10 GeneXpert machines in the South-Kivu province of the Democratic Republic of Congo (DRC) from 2012. These were the first Xpert machines to be introduced in the country, and these were installed in the major cities of the province, some of them being only accessible by 4x4 or by plane (figure 4.1).



Figure 4.1: Installing GeneXpert machines in 10 different cities of the South-Kivu province required to ship these automates across the province. In many cases, there were no roads allowing travelling from Bukavu to these cities. (Photo: Crossing a river on the way from Bukavu to Baraka with a GeneXpert machine and Xpert MTB/RIF cartridges in the back of the car)

4.2. Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries

Together with eight other countries (Bangladesh, Cambodia, Kenya, Malawi, Moldova, Mozambique, Nepal and Pakistan), we performed an evaluation of our early experience (134). In these countries, GeneXpert was used to increase the sensitivity of laboratory testing among people suspected of having TB rather than to detect drug resistance. The pre-test criteria varied among countries: some countries utilized the Xpert MTB/RIF test as a primary screening tool among high-risk groups, while others such as our project used the test as a secondary tool among people suspected of having TB but whose smear microscopy result was negative. One important issue that we studied was the proportion of failed tests. It appeared that our project in DRC had the greatest proportion of failed tests. With 16.3% of tests providing no result, we were concerned that these high rates would have consequences in terms of costs or may imply the inability of the local staff (or their trainers) to correctly use this new technology.

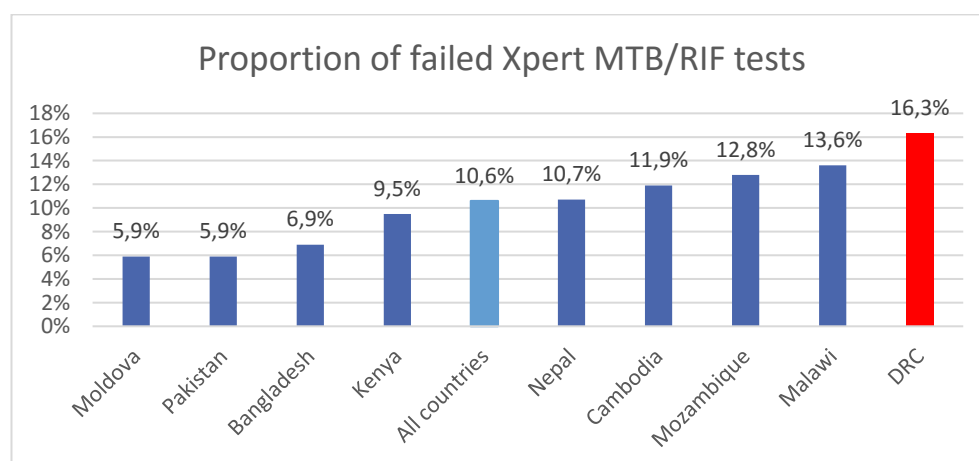


Figure 4.2: Proportion of failed Xpert MTB/RIF tests among early users of the technology from nine different countries (Modified from Creswell et al, 2015)

The full study is available online (open access)

RESEARCH ARTICLE

Open Access

Results from early programmatic implementation of Xpert MTB/RIF testing in nine countries

Jacob Creswell^{1*}, Andrew J Codlin², Emmanuel Andre³, Mark A Micek^{4,5}, Ahmed Bedru⁶, E Jane Carter⁷, Rajendra-Prasad Yadav⁸, Andrei Mosneaga⁹, Bishwa Rai¹⁰, Sayera Banu¹¹, Miranda Brouwer¹², Lucie Blok¹³, Suvanand Sahu¹ and Lucica Ditiu¹

Abstract

Background: The Xpert MTB/RIF assay has garnered significant interest as a sensitive and rapid diagnostic tool to improve detection of sensitive and drug resistant tuberculosis. However, most existing literature has described the performance of MTB/RIF testing only in study conditions; little information is available on its use in routine case finding. TB REACH is a multi-country initiative focusing on innovative ways to improve case notification.

Methods: We selected a convenience sample of nine TB REACH projects for inclusion to cover a range of implementers, regions and approaches. Standard quarterly reports and machine data from the first 12 months of MTB/RIF implementation in each project were utilized to analyze patient yields, rifampicin resistance, and failed tests. Data was collected from September 2011 to March 2013. A questionnaire was implemented and semi-structured interviews with project staff were conducted to gather information on user experiences and challenges.

Results: All projects used MTB/RIF testing for people with suspected TB, as opposed to testing for drug resistance among already diagnosed patients. The projects placed 65 machines (196 modules) in a variety of facilities and employed numerous case-finding strategies and testing algorithms. The projects consumed 47,973 MTB/RIF tests. Of valid tests, 7,195 (16.8%) were positive for MTB. A total of 982 rifampicin resistant results were found (13.6% of positive tests). Of all tests conducted, 10.6% failed. The need for continuous power supply was noted by all projects and most used locally procured solutions. There was considerable heterogeneity in how results were reported and recorded, reflecting the lack of standardized guidance in some countries.

Conclusions: The findings of this study begin to fill the gaps among guidelines, research findings, and real-world implementation of MTB/RIF testing. Testing with Xpert MTB/RIF detected a large number of people with TB that routine services failed to detect. The study demonstrates the versatility and impact of the technology, but also outlines various surmountable barriers to implementation. The study is not representative of all early implementer experiences with MTB/RIF testing but rather provides an overview of the shared issues as well as the many different approaches to programmatic MTB/RIF implementation.

Keywords: New diagnostics, Xpert MTB/RIF, Tuberculosis, Case detection, Innovation

* Correspondence: creswellj@who.int

¹Stop TB Partnership, Secretariat, Geneva, Switzerland

Full list of author information is available at the end of the article



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Background

Each year an estimated 8.6 million people worldwide develop tuberculosis disease (TB), yet fewer than 6 million cases are reported and treated [1]. In addition, of the more than 300,000 estimated multidrug-resistant TB cases among all notified cases, less than a third were reported in 2012 [1]. One of the impediments to improving case detection and early initiation of treatment for both drug-sensitive and-resistant TB is the limited availability of sensitive and rapid TB diagnostics.

In December 2010, the World Health Organization (WHO) endorsed the use of the Xpert MTB/RIF assay (MTB/RIF) for the detection of TB and rifampicin resistance [2]. The assay uses a closed cartridge that simply requires insertion into a GeneXpert machine, with similar infection control laboratory interventions as those for smear microscopy [3]. These characteristics allow placement of the system at lower level health facilities (e.g. district or sub-district facilities), which should theoretically improve access to testing. A multi-country demonstration study documented high levels of sensitivity and specificity to detect *Mycobacterium tuberculosis* bacteria and rifampicin resistance [4], while a recent Cochrane systematic review supported the initial demonstration study's findings [5]. WHO recommends using the MTB/RIF assay as a primary diagnostic test for persons suspected of having a DR-TB or in settings with high HIV prevalence and, where resources permit, as a second test for those with an abnormal chest x-ray (CXR) and/or negative smear microscopy results [2].

Globally, there has been significant interest in using the MTB/RIF test across a variety of settings, with over 2.3 million Xpert® MTB/RIF cartridges procured in the public sector through March 2013 [6]. Various studies have documented use of the MTB/RIF test in people with signs and symptoms of TB, on clinical specimens [7-11], and for the detection of drug resistance [12,13]. Several commentaries, cost-effectiveness studies and models have presented potential uses for and likely impacts of this assay [14-21]. However, there is a dearth of published literature produced by implementers of the technology in programmatic settings. As National TB Programs (NTPs) and their partners scale up the use of MTB/RIF tests for case detection, there is a need to share results from implementation in programmatic settings, especially since there is evidence that research studies and product inserts often provide a more optimistic view of test performance, as compared to real-world implementation [22].

TB REACH, a global initiative of the Stop TB Partnership [23], has supported many partners with GeneXpert technology through its competitive grant making process. We present results from nine TB REACH interventions, review the main challenges experienced and formulate recommendations for other early implementers.

Methods

During the period from September 2011 to March 2013, a total of 30 TB REACH grants across 24 countries designated funds to procure GeneXpert machines and the Xpert MTB/RIF assay. Of these, a convenience sample of nine projects was selected for inclusion in this study with the aim of covering a range of implementers, regions and approaches. Standard quarterly reports from projects were utilized to analyze MTB/RIF testing data for patient yields, rifampicin resistance patterns and failed tests. The proprietary files were directly exported from the GeneXpert Dx software to verify data in quarterly reports. The data presented reflect the first twelve months of MTB/RIF assay implementation for each project. The start date of each project was different; however all data were collected between October 2011 and March 2013.

All projects completed an electronic questionnaire covering procurement and deployment experiences, diagnostic algorithms, training requirements, and recording and reporting issues. The questionnaire was followed up by semi-structured interviews with staff from each project either in person or over the phone. Interviews with the manufacturer were also conducted, addressing aspects of procurement. Questionnaire and interview data were analyzed for major themes across different aspects of MTB/RIF assay implementation.

Records utilized were from standard NTP data collected in the course of routine programmatic implementation with no personal identifiers. Therefore ethical review for this analysis was not sought. Individual projects in The Democratic Republic of Congo (DRC), Kenya, Pakistan, Bangladesh and Mozambique had waivers for their projects. The remaining projects in Cambodia, Malawi and Nepal were being implemented by or on behalf of NTPs as part of routine operations and did not seek ethical review.

Results

Placement and utilization

The projects were often the first implementers of the MTB/RIF test for TB case detection in their respective countries. Table 1 provides an overview of the selected nine TB REACH projects including location and case finding strategy. Placements included public and private hospitals and lower primary care facilities, private diagnostic laboratories, HIV centres, prisons, reference laboratories and mobile units. The projects were able to run the machines at district hospitals and at lower levels of care although in only a few situations were peripheral microscopy centres included, mostly because of throughput concerns. Additional files 1,2,3,4,5,6,7,8,9 show pictures of a number of the places where MTB/RIF was used. A number of interventions initially projected low MTB/RIF testing numbers due to the high cost of assay

Table 1 Overview of early Xpert MTB/RIF test placement and TB case finding strategy in nine countries

Country	WHO Country classifications	Placement	Number of GeneXpert machines		Case finding approach
			2-Module	4-Module	
Bangladesh	HBC, HDR	Two urban private diagnostic laboratories	2		Systematic screening of all attendees
Cambodia	HBC	Both machines used at one-day mobile camps set up at primary care facilities		2	Active with promotion of testing days in community
DR Congo	HBC, HDR, HTH	One primary care center, one sub-district hospital, five district hospitals and one provincial laboratory.	7	1	Primarily passive but with employed community groups to refer people with suspected TB
Kenya	HBC, HTH	Two district hospitals and one health center. All had ART delivery		3	Mixed, employed community cough workers to augment numbers of people tested
Malawi	HTH	One central hospital, four district hospitals, one health center in a district with no district hospital, one mission hospital and one community hospital.		8	Passive
Moldova	HDR	17 in public facilities at lowest level for TB diagnosis, two in regional AIDS centers, two in the national reference laboratory, two in the regional reference laboratory, and two in the penitentiary sector	16	9	Passive
Mozambique	HBC, HTH	Four district hospitals		4	Passive
Nepal	HBC	Four regional hospitals (two public and two private), one district hospital, two primary health centers, one private referral center, and one reference laboratory	3	6	Primarily passive with some awareness raising/and educational activities to improve numbers of people tested
Pakistan	HBC, HDR	Four urban private diagnostic laboratories	4		Systematic screening of all attendees

HBC = High Burden Country, HDR = High MDR-TB Burden Country, HTH = High TB/HIV Burden Country.

cartridges and the fact that the test was a new technology. In these instances, less expensive and lower-capacity 2-module machines were procured rather than standard 4-module units (see Table 2 for recommendations).

The projects consumed on average 979 tests per four-module machine per year (range 675 in Moldova-1849 in Cambodia). Theoretically a 4-module machine could run 4,000 tests a year (four tests per module/day, and 250 working days a year). However, several factors impacting utilization were identified: the number of hours laboratory technicians were available to work (frequently only 4–5 hours per day), available power supply during the day, the number of people with suspected TB attending the facility, and the selectivity of the testing algorithm and case finding approach. For example, Cambodia only tested 4 days a week due to mobile clinic logistics and travel.

Algorithms and case finding approaches

A wide variety of diagnostic algorithms incorporating MTB/RIF testing were implemented (Table 3). Several projects employed multiple algorithms, though the majority used the MTB/RIF assay as a second test for patients with negative smear microscopy results. Some projects used active case finding strategies, others used a passive approach. Funding requirements of TB REACH focused all MTB/RIF testing on patients with suspected TB, as opposed to already-diagnosed patients suspected

of having DR-TB. In practice however, a number of projects reported use of a small number of MTB/RIF tests as drug sensitivity test (DST) in specific cases. All projects tested pulmonary samples only, and while some projects accepted samples from children, very few children were tested due to difficulties in getting quality sputum samples.

The majority of projects used some sort of referral system to increase the numbers of people tested (Table 1). None of the projects systematically provided the MTB/RIF assay as a primary diagnostic test for people with HIV. Although many sites had ART clinics in their facilities, the majority of people tested were not from HIV centres, and the HIV status of those being tested with the MTB/RIF assay was often unknown.

Time to diagnosis

Table 3 presents an overview of the time needed for a patient to receive results of an Xpert MTB/RIF test. In four of the projects the intent was to provide results the same day and initiate treatment. This was generally implemented except when the number of samples exceeded machine throughput. Other projects used algorithms and/or referral systems that improved the standard turnaround time for bacteriological tests, but were not the same day. Turnaround times ranged from same day to over a week when a referral system was in place and/or

Table 2 Challenges and recommendations for programmatic implementation of Xpert MTB/RIF testing

Implementation area	Challenges	Recommendations
Machine utilization	Overestimation of testing numbers and underutilization of machine capacity.	Conduct a needs assessment that includes the current number of people with suspected TB tested at the facility, the need for a referral system and the impact of the proposed testing algorithm on testing numbers. Two-module machines may be a less expensive alternative in many settings.
Testing algorithms	Lack of national guidance on testing algorithms and use of questionable testing strategies.	To improve yield and reduce cartridge use, consider screening with CXR. Consider elimination of smear microscopy as a first test to reduce delay, loss to follow-up, and avoid repeat testing of most individuals due to large proportion of smear negative results.
Time to diagnosis	Sputum transport systems and testing algorithms can prolong delay between sputum submission, results and treatment initiation.	Xpert MTB/RIF testing sites should be located as close to the patient as possible to allow for rapid treatment initiation keeping in mind throughput and electrical power limitations. Referral networks can help utilization rates but can also be costly to maintain.
Procurement	Short shelf life of cartridges, use of machine beyond date of needed calibration, unanticipated extra costs of shipping and customs clearance, local procurement agent not always helpful.	Stagger cartridge shipments to avoid stock outs and expiry. Plan for module calibration by ordering test kits early. Plan for extra costs associated with shipment and customs clearance.
Training	Staff rotation and new practices around request forms, specimen transport and clinical decisions for rifampicin resistant results.	Testing can be easily done by well-trained lay workers to support laboratory staff. The manufacturer has conducted web trainings via videoconference and webinars and has released a web-based training video which some projects used as an adjunct tool for facilitation of trainings.
Infrastructure and power supply	UPS (15 minutes) Cepheid offers is inadequate in many settings. Most laboratories need some infrastructure improvements to allow proper testing.	A standard 800-2000VA inverter and a 12 V/120-200 AH battery provided power for over 6 hours to one four-module GeneXpert machine and a laptop (200 wattage required). As a general rule, a 10AH battery will be able to power a requirement of 100 watts per hour, but more AH are needed when the discharge is over a short period of time (less than 20 hours). These can be procured locally.
Failed tests	Differences between types of failed tests are unclear and available data not always used. High failed test rates in certain projects.	Error results are coded by the machine and should be documented. The .gpx files can provide this information and the types, reason, locations, and technicians associated with the error should be tracked. No result test results are often caused by a power failure. Invalid results are not caused by testing a specimen of insufficient volume or one which contains saliva the way poor sputum quality is defined in a NTP. Rather, they seem to be caused by other problems with sputum. Emphasizing correct sputum collection techniques, including mouth rinsing to remove food or particles which could inhibit the assay, may help reduce Invalid results, as well as improve yield.
Drug resistance	Some confusion about clinical decisions after receiving rifampicin resistant and indeterminate results. Trepidation over Xpert MTB/RIF use overburdening DR-TB programs.	Patients with indeterminate results have TB, but rifampicin resistance cannot be confirmed due to a very low burden of TB bacilli in the specimen. Unless there is documented DR-TB risk, first-line treatment can be started. Follow-up of these patients is warranted. The majority of drug-resistant cases in almost all countries will be found among new cases, requiring testing of people with suspected TB rather than patients already in TB treatment. Patients with rifampicin resistance will be detected in greater numbers and with greater speed than under current conditions, and significant resources and coordination will be required.

Table 2 Challenges and recommendations for programmatic implementation of Xpert MTB/RIF testing (Continued)

Recording and reporting	Supplied GeneXpert Dx software is not appropriate for analyzing patient cohorts, many times failed tests are recorded on paper with a generic error 'result', underreporting of errors, lack of clear guidance from national programs on recording and reporting of cases identified by Xpert MTB/RIF.	Dissemination and uptake of WHO guidelines on recording and reporting should be adopted by countries and their NTPs. Automated reporting mechanisms can improve both the timeliness and accuracy of reporting as well as assist in supply chain management.
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the sample was transported from a remote facility. At sites that used referral systems, most testing was conducted on people presenting at the facilities rather than on sputum transported through referral systems. This diagnostic timeline still appeared to represent a shorter time to treatment for those with smear negative but MTB-positive results compared to initiative treatment as a smear negative case, but it was difficult to measure. In Moldova however, where parallel smear microscopy was conducted for all people with suspected TB, among those with MTB-positive results, only 56% were positive

by smear microscopy. This led to immediate treatment for a large proportion of people who otherwise would have had to wait for CXR and/or culture results before treatment could begin.

In Cambodia and for hospitalized patients in Malawi, MTB/RIF tests were performed on the same day that sputum was submitted but, due to the number of individuals indicated for testing and laboratory work load, some had to wait to receive their results until the following day. In Pakistan, Bangladesh, Mozambique and Kenya, smear microscopy and, if indicated, MTB/RIF

Table 3 Overview of early Xpert MTB/RIF testing approaches in nine countries

Country	Xpert MTB/RIF testing strategy	Point-of-care (POC) or referral for treatment	Laboratory process for requesting Xpert test	Time from initial screen to Xpert + result	Time from submission of sputum for Xpert MTB/RIF test to result
Bangladesh	A - Direct to Xpert for all with history of previous TB treatment B - Xpert following SS- results and TB Suggestive CXR	POC	Automatic in laboratory	0-1 day	Same day
Cambodia	Xpert following positive verbal and/or CXR screens	POC	Automatic in laboratory	0-1 day	0-1 day
DR Congo	Xpert following SS- results	Both, using a hub and spoke transport system to augment testing numbers	After follow up tests and review	7-10 days	1 day
Kenya	Xpert following SS- results	Both, using a hub and spoke transport system to augment testing numbers	Automatic in laboratory for those with negative smear results	1-4 days	1 day
Malawi	A - Direct to Xpert for all hospitalized patients with suspected TB B Xpert following SS- results	Both, using a hub and spoke transport system to augment testing numbers	Automatic in laboratory for those with negative smear results	1 day for walk in patients and up to 10 days for referral patients	0-3 days
Moldova	Direct to Xpert; parallel smear microscopy	POC at most facilities; referral at AIDS Centers	Automatic in laboratory	0-1 days	0-1 days
Mozambique	Xpert following SS- results	Referral	Automatic in laboratory for those with negative smear results	Up to 7 days for referral patients	2 days
Nepal	Xpert following SS- results and a suggestive CXR	Both, using a hub and spoke transport system to augment testing numbers	After follow up tests and review	1 day for walk in patients and up to 10 days for referral patients	1-2 days
Pakistan	A - Direct to Xpert for all with history of previous TB treatment B - Xpert following SS- results and TB Suggestive CXR	POC	Automatic in laboratory	0-1 day	Same day

testing, were performed on a single sputum specimen submitted by each presumptive TB patient, shortening the time from sputum collection to MTB/RIF results. The DRC project reported a wide variance of times to diagnosis depending on the length of time it took to transport samples from peripheral microscopy laboratories. Malawi used a referral network including community sputum collection points and microscopy centres with two deliveries per week to augment the number of people tested. Mozambique also used a hub system, although systems to transport and process referred samples were difficult to maintain over time. Measures taken to reduce turn-around-time included using the same specimen to perform smear microscopy and the MTB/RIF test, training lab technicians to automatically conduct the MTB/RIF tests following smear-negative results (as opposed to waiting for a referral form), and paying for extra human resource support for laboratory staff.

Preparation and installation requirements

Procurement and service

For all projects, procurement was done through the Global Drug Facility, with cartridges shipped in two or three batches to compensate for the short shelf life of the product in 2012 (8–12 months). The median lead time (order placement until delivery to port of entry) for ordering machines and test cartridges was 40 days (range 12–62). Shipping costs added to the test cost and ranged from 0.33 USD to 1.44 USD per test.

Following the cartridge price reduction in July 2012 from USD 16.68 to USD 9.98 [24], a number of shipments were delayed due to a surge in orders from countries that had postponed ordering to benefit from the price reduction. This resulted in a backlog of orders that could not be met by Cepheid's production capacity at that time. In addition, a manufacturing problem lowered production capacity in the Winter and Spring of 2012–13 [25]. As of July 2013, the cartridge shortage issue had been resolved.

Cepheid has agreements with local service providers in different countries which are intended to assist in procurement, service and repair needs. The experience of projects with these providers was quite varied and some local providers were found to be severely inadequate. As increasing Xpert MTB/RIF testing occurs, the demand for competent in-country services will also increase. Identifying competent and responsive local providers will be necessary for scale up efforts.

Training/human resource issues

Many projects mentioned that when placing machines in more peripheral sites, training in how to use a computer was more difficult than training for the test procedure itself. Several also reported that the lack of multi-language settings for the GeneXpert Dx software was a challenge,

particularly in settings where the Roman alphabet is not used. Implementers employed a range of people to manage GeneXpert equipment, including lay workers, TB staff, general laboratory staff and new project hires. Prior laboratory experience is not required to competently use the assay (Table 2). Pakistan, which employed lay individuals to perform testing, had one of the lowest failed test rates of the projects (Table 4). All projects reported high levels of laboratory staff satisfaction with the machine, its ease of use, the ability to complete other tasks while testing, and rapid test results.

The programmatic aspects of implementation, including clinical decisions on testing, completion of MTB/RIF request forms, transportation of specimens, and registering results and treatment decisions, especially in cases of rifampicin resistant results, required the most training.

Infrastructure and power supply

In most cases, local infrastructure improvements to address issues of climate control, work space security, ventilation, and dust control were required to set up GeneXpert equipment. Procurement needs included air conditioners or heaters, fans, safety equipment, and in a couple of instances, refrigerators for stored samples. The main improvement required was for uninterrupted power supply to complete each test. Although the manufacturer offers an uninterrupted power supply (UPS) device for each GeneXpert machine, additional protection is often needed as said UPS was developed for high-income settings with relatively stable power supplies. In Cambodia, a generator was used for machines in mobile clinics (Additional files 8,9). Many projects used locally sourced power solutions including inverters and large backup batteries at costs comparable to or lower than the UPS supplied by the manufacturer (300–900 USD). See Table 2 for recommendations on UPS specifications and Additional files 5,6,7,8 for images. The laptop's built-in battery drains the UPS less quickly than a desktop and may result in fewer failed tests; however, laptops may be more vulnerable to theft. The four-module desktop machine costs 17,000 USD (500 USD less than a laptop unit).

Test results

Overall results

Over the first 12 months of implementation the nine projects consumed 47,973 MTB/RIF tests (range 1,428–11,472). MTB/RIF testing resulted in a positive test for 15.0% (7,195) of all cartridges used and for 16.8% of all valid tests (excluding failed tests). Positivity among valid results varied between 10.1% and 23.6% (Table 3). Due to the wide variety of diagnostic algorithms used by the different projects, it is difficult to directly compare yields across sites. The yield of TB cases at sites utilizing CXR as a screening test and/or using Xpert MTB/RIF as the first

Table 4 Result data from first 12 months of Xpert MTB/RIF implementation in nine countries

Xpert site	Tests	MTB Positive		Rifampicin				Failed Tests		Type of failed test			MTB + w/o failed tests	
		N	%	Sensitive		Indeterminate		Resistant		Error	Invalid		No result	%
				n	%	n	%	n	%		n	%	n	%
Bangladesh	1428	286	20.0%	249	87.1%	11	3.8%	26	9.1%	85	10.1%	10	4	21.5%
Cambodia	3697	768	20.8%	741	96.5%	22	2.9%	5	0.7%	169	38.5%	264	6	23.6%
DR Congo	6348	567	8.9%	498	87.8%	17	3.0%	30	5.3%	868	84.1%	107	57	10.7%
Kenya	2803	258	9.2%	238	92.2%	11	4.3%	9	3.5%	240	90.2%	23	3	10.2%
Malawi	6258	632	10.1%	602	93.5%	9	1.4%	21	3.3%	853	13.6%	265	42	11.7%
Moldova	11472	1965	17.1%	1227	62.4%	48	2.4%	690	35.1%	387	61.7%	105	181	18.2%
Mozambique	6823	910	13.3%	826	90.8%	18	2.0%	66	7.3%	521	59.8%	216	134	15.3%
Nepal	6943	1376	19.8%	1257	91.4%	14	1.0%	105	7.6%	409	55.0%	148	187	22.2%
Pakistan	2201	433	19.7%	390	90.1%	13	3.0%	30	6.9%	77	59.2%	37	16	20.9%
All Countries	47973	7195	15.0%	6028	83.8%	163	2.3%	982	13.6%	3302	64.7%	1175	630	16.8%
South Africa*	1180669	171792	14.6%	155811	90.7%	2443	1.4%	12266	7.1%					15.0%

*Reference 26. Data for South Africa is presented as a comparison after 25 months of implementation.

test (Bangladesh, Cambodia, Moldova, Pakistan) was 50-70% higher than sites which went straight from smear microscopy to the MTB/RIF test (Kenya, Mozambique, DRC). The overall positivity in these 9 projects was similar to yields from the South Africa's rollout of Xpert MTB/RIF (15%) [26]. Among all MTB-positive results, 2.3% (163) were RIF indeterminate. Unless there was some DR-TB risk, first-line treatment was started on patients with RIF-indeterminate results and a repeat MTB/RIF test was not performed.

Failed tests

The overall failed test rate was 10.6% (range 5.9% in Moldova and Pakistan to 16.3% in the DRC). When data was pulled directly from the GeneXpert machine as opposed to the laboratory registers, a number of unreported failed tests were discovered. It appeared that technicians may have re-tested specimens until a positive or negative result was achieved, and reported only the final result. More than one quarter of failed tests were directly related to power issues, either through *No Result* outcomes or 2127 *Errors* but varied greatly by country.

A common test-related error was that of signal loss associated with an earlier version of MTB/RIF cartridges (G3 vs G4). Signal loss errors made up 44% of all errors in Nepal and 28% in DRC, but only 11% in Pakistan (data not shown) and appeared to be related to specific batches of G3 cartridges rather than a general problem. In Kenya, *Error* rates were much higher among G3 tests (12.9% vs 5.2%), but in other countries there were a higher proportion of *Error* results among the G4 cartridges. *Error* results made up the majority of the failed tests (64.7%), however in some sites *No Result* and *Invalid* findings contributed significantly to the failed test results. See Table 2 for descriptions and recommendations concerning failed tests. We were unable to accurately track the exact rates of retesting among the projects as laboratories attempted to use the same sputum sample, but with referred samples follow up was often difficult and this information was not well documented.

The overall module failure rate in the projects was 42% (77/182), but in Moldova only 7% (5 of 68) modules needed replacement while in Mozambique, Nepal and Malawi more than half needed to be replaced. In Bangladesh and Pakistan all modules needed to be replaced. This was not just calibrated modules but overall failure. Many module problems seemed to be clustered suggesting that other implementation issues, such as irregular power supply and currents, dust build-up, overheating, and staff quality control may have contributed to the failures.

Rifampicin resistance

Among all valid tests, 2.3% of results showed rifampicin resistance, (0.9% excluding Moldova). Overall, 13.6%

(n = 982) of MTB-positive results were resistant to rifampicin, with Moldova accounting for 69.5% of those cases and Nepal accounting for 10.6%. Excluding Moldova, 5.6% of MTB-positive results were resistant to rifampicin. Projects referred the patients to the MDR-TB treatment sites but did not generally provide active follow-up as TB REACH does not provide any funding for drug resistance treatment or support. Complete data on follow-up culture and DST were not available for many of the projects as almost all samples were sent to distant referral laboratories. Projects in Mozambique, DRC, Malawi and Nepal noted important barriers in getting DST results due to distance, and overworked laboratories. Most noted significant loss to follow-up of patients having to travel long distances though it was not quantified. Algorithms for treatment of people with a rifampicin resistant result varied. In settings where there are high levels of DR-TB, such as Moldova, any patient with a rifampicin resistant result was placed on a standardized second line treatment regimen immediately, reducing the treatment delay significantly over the previous system of consolidating samples and follow-up DST. In settings where drug resistance was less prevalent, confirmatory DST was requested before patients started second line regimens. Most patients who had a prior treatment history were able to initiate second line treatment immediately. Patients with identified rifampicin resistance through MTB/RIF testing would most likely not have been diagnosed with DR-TB in the absence of such testing until they had failed treatment or relapsed, as the projects targeted people with no history of TB and with limited access to conventional DST methods.

Recording and reporting

All of these nine implementers were among the first to use MTB/RIF testing in their country and/or were the first to use it in a programmatic setting. When NTP reporting guidelines for MTB/RIF testing existed, they were often for diagnostic algorithms focused on detecting drug resistance, and were inadequate for these case-finding interventions. The projects in Cambodia and DRC were run by national and provincial TB programs respectively and had the ability to set recording guidelines. Cambodia records all MTB-positive results as smear-positive, while in DRC and Mozambique they are recorded as smear-negative. In other countries, reporting procedures had to be discussed with the NTP and the lack of global guidance on the issue at the time of testing created some confusion. In Pakistan, MTB-positive patients were at first reported as smear-positive, but following changes in NTP guidelines, they were subsequently reported as smear-negative. In Nepal, patients detected on the MTB/RIF assay were reported as smear-negative, but when patients had MTB-positive results with no CXR result, treatment was initially delayed due to confusion around guidelines. Malawi had no

national guidelines for reporting MTB/RIF results, leading to variable practices among districts until implementing partners developed standard guidelines.

Projects took advantage of the fact that all machines are coupled with a computer to develop electronic registers, but there was general agreement that the pre-installed GeneXpert Dx software is not useful for capturing patient cohort information due to test data being individually processed with limited collating and exporting options. All projects used different locally developed databases to track tests and patients. Most projects enforced strict prohibitions on the use of external drives with the computers due to virus and malware threats. Data were downloaded from the machines via blank CDs or abstracted through automated reporting systems. DRC, Kenya, Pakistan and Bangladesh employed systems that provided immediate reporting of test results to the providers and referral systems over a GSM network.

Discussion and conclusions

The findings of this study begin to fill the gaps among guidelines, research findings, and real-world implementation of MTB/RIF testing, where NTPs and their partners work to test people with suspected TB and drug resistance. The projects documented here had a wide range of experiences, approaches and results, demonstrating the versatility of Xpert MTB/RIF, but also highlighting a number of barriers to implementation and possible solutions. As others have pointed out, the technology has great potential, but also has important limitations. This study begins to address some of the concerns delineated through implementation experiences in the field. These projects have showed that with proper support for procurement, infrastructure and supervision, Xpert MTB/RIF can be deployed at or very close to the point of care in many settings initially considered difficult because of the hurdles to overcome [26]. Our findings generally coincide with other early assessments of the challenges and benefits of Xpert MTB/RIF implementation where many barriers can be surmounted if proper systems are in place and a coordinated country approach is taken with the best interests of the patient in mind [27].

Xpert MTB/RIF testing detected a large number of people with TB that routine services failed to detect. Data from South Africa shows similar results for MTB-positive yield but much lower error rates [28] although many of the projects described here placed the machines in different types of facilities, including mobile units and primary care facilities in challenging settings. When available, the use of CXR as a highly sensitive screening tool should be considered before testing with Xpert MTB/RIF, to save cartridges and increase yield [2,29]. Placing smear microscopy ahead of the MTB/RIF test in the diagnostic algorithm often resulted in lower yields

compared with specimens tested directly on the MTB/RIF assay, as smear-positive patients were then excluded. However, the underlying prevalence of TB in the screened population, as well as the quality of smear microscopy services [30], play important roles in influencing yields among smear-negative specimens. Xpert MTB/RIF testing on smear-negative individuals, the general approach used here, expends significantly more resources and time, as all individuals are tested using smear microscopy and 80-90% of them are re-tested with Xpert MTB/RIF. New WHO guidance recommends that Xpert MTB/RIF replace smear microscopy when resources allow [31]. All of the projects described here had external funding for Xpert MTB/RIF testing and so it is curious that only a few used it to replace smear, but this was many times limited by national policy.

Tracking the types, codes, reason, locations, and technicians associated with failed tests is important for users to improve performance. There are generally three possible types of failed tests for MTB/RIF: human, power supply and test-related. Human errors generally arose from inadequate sputum collection or specimen preparation and can be tracked by their numeric code [32]. Automated reporting mechanisms can improve both the timeliness and accuracy of reporting errors and all results as well assist in supply chain management [33]. Some projects had very high rates of *Invalid* results which seem to be caused by problems with the quality of the sputum sample and proper mouth cleansing techniques. Better coaching from staff may be able to help in these instances. While the failed test rates are much higher than in South Africa, they are similar to some other larger early implementer multi-country initiatives [34]. Most of the projects placed the machines at lower level facilities which presents more of a challenge for implementation and supervision. An early experience in South Africa using mobile testing had comparable failed test results, although most were power supply related. [Personal Communication Lies Page-Shipp] *No result* outcomes are often linked to power supply problems. In environments with irregular and unpredictable power, a backup power supply with an inverter should be capable of supporting the GeneXpert machine and computer for at least 2 hours in order to finish any test begun before a power failure (see Table 2). When GeneXpert machines are placed in facilities with other equipment needing power supply, generators are likely to be available and backup power may only be needed until the generator can be started. Ensuring timely calibration of the GeneXpert machines will also reduce errors. If the calibration is done after the module warranty expires and the module must be replaced, a new module or an extended warranty must be purchased. Modules can still fail outside of calibration and extended warranties are available and should be purchased to protect the users.

As Xpert MTB/RIF provides both TB and rifampicin resistance results, it is important to weigh the benefit of early detection of drug resistance against the concern that large increases in DR-TB detection would overwhelm advanced diagnostic facilities and nascent drug-resistant TB treatment programs. Focusing MTB/RIF testing solely on DR-TB suspects is unlikely to produce large yields as the numbers of failures and retreatment cases at a district level is quite small in most settings. Furthermore, the majority of DR-TB cases will be found among new cases [35], requiring testing of people with suspected TB rather than patients already receiving TB treatment if meaningful MDR-TB treatment scale up is to take place. The application of algorithms focused on people with suspected TB will generally result in large increases in drug sensitive cases detected and a lower proportion of rifampicin resistant results. Nevertheless, DR-TB patients will be detected in greater numbers and with greater speed than under current conditions, and significant resources and coordination will be required for treatment, especially if MTB/RIF testing is to be done in areas without DR-TB treatment capacity as was the case in many of these projects. These projects were limited given funding was not provided for DR-TB treatment and active follow-up of rifampicin resistant patients was generally not conducted and data is limited. However, in many countries, overall donor funding for DR-TB treatment remains unused as many people with DR-TB have not been diagnosed; so opportunities for expansion exist. Given the concern about slow expansion of MDR-TB treatment globally, South Africa has clearly shown that huge gains in detection and notification are possible in a short period of time using Xpert MTB/RIF as the primary diagnostic tool [1].

Apart from testing approaches and results, there were a number of other implementation issues that merit discussion. Although many point of care testing strategies focus on finger prick or dipstick testing, a recent review discussed the positioning of diagnostic tests in the health system and argued that many tests could be considered point of care, including smear microscopy and MTB/RIF, if the test and treat cycle could be completed in the same clinical encounter (or the same day) [36]. However, testing algorithms and work-flow practices often gets in the way of providing quick results to the patient and ensuring same-day TB treatment and this was seen in a number of projects [36]. While Xpert MTB/RIF testing can provide highly sensitive results quickly in a number of different settings, the health system must provide a supportive environment; tightly controlling the machine and testing in high level laboratories may sometimes be achieved to the detriment of the patient. A recent study has made a strong argument for the use of MTB/RIF as a point-of-care test showing a reduction in delay, empiric treatment, and the ability of nurses to implement the test in primary care settings [37].

Decisions on machine location should be given careful consideration with respect to expected numbers of people tested and the need for referral systems in addition to power supply. Although many peripheral health facilities do not have the infrastructure to handle complicated testing [38], the main limitations we identified for decentralized MTB/RIF testing - algorithms, power, temperature and numbers of tests expected - can all be overcome with proper support, although in some remote areas supervision is difficult and seemed to complicate performance.

Training staff to use GeneXpert equipment was generally described as straightforward, supporting views of other early implementers [39]. However, the need for retraining and supervision cannot be overlooked as staff turnover is a constant reality. As part of training, ensuring solidified national testing and treatment guidelines are in place is critical. Country adoption of the WHO policy guidance around testing algorithms [2] as well as recent WHO new case and outcome definitions incorporating MTB/RIF and other WHO recommended tests [40], will greatly help standardize the process. All people with MTB/RIF positive results should be recorded as bacteriologically positive cases. The adoption of WHO recommended strategies and reporting systems will reduce the inconsistencies that the early implementers faced, though it must be acknowledged that field adoption of new guidance often takes time. Delays in customs clearing, in country transportation and other preparatory activities, such as training, will shorten the effective usage time for the cartridges. Staggering orders can help avoid expiry of cartridges, which have a short shelf life.

The technology has the possibility of being quite disruptive to national testing and treatment policy, and to recording and reporting forms and training. With donors including PEPFAR and The Global Fund beginning to fund the expansion of MTB/RIF testing in many countries, lessons learned from pilot projects that can be applied to a large expansion of testing must be disseminated. One of the main areas of concern about MTB/RIF testing is the additional cost to country diagnostic budgets which these projects did not have to address as the work was grant funded, therefore this experience cannot directly address or inform those concerns. Just as a few countries fund their own second line treatment programs, we feel that the benefits of improved diagnostics for TB and drug-resistant TB provide ample justification for long term support of improved diagnostics from donors. This study is not representative of all early implementer experiences with MTB/RIF testing but rather provides an overview of the shared issues across countries and of many different approaches to MTB/RIF implementation on a programmatic basis. Further programmatic studies are needed on issues such as case notification, time to diagnosis, cost

effectiveness of MTB/RIF use in case finding programs and use at the most peripheral of health units (point of care testing) - all issues that our work was not designed to answer.

The experiences of these early implementers show that a variety of MTB/RIF testing approaches can be successfully employed across many different settings. This early information can be usefully applied in the on-going global scale up of MTB/RIF. The MTB/RIF test has limitations, but also provides an excellent opportunity to improve the way TB and drug-resistant TB are diagnosed on a wide scale.

Additional files

Additional file 1: Xpert equipment in USAID AMPATH Mycobacteriology Reference Laboratory, Moi Teaching and Referral Hospital, Eldoret, Kenya.

Additional file 2: Busia District Hospital, USAID AMPATH Reference lab, Busia, Kenya.

Additional file 3: Working in a private laboratory in Dhaka, Bangladesh.

Additional file 4: Training for Xpert MTB/RIF in Nepal.

Additional file 5: Two linked 4-module Xpert machines with backup power supply in Zomba Hospital, Malawi.

Additional file 6: CIDASA primary health center in Bukavu, South Kivu Province, DR Congo.

Additional file 7: Shabunda Reference Hospital, South Kivu Province, DR Congo.

Additional file 8: Cambodia mobile clinics showing GeneXpert machine connected to a generator.

Additional file 9: Cambodia mobile clinics showing CXR screening powered by a generator in truck outside window.

Competing interests

Jacob Creswell and Suvannand Sahu are members of the TB REACH secretariat and Lucica Ditiu is the Executive Secretary of the Stop TB Partnership, but were not involved in the initial projects design or the decision to fund any of the projects.

Authors' contributions

JC, AJC, EA, SS and LD conceived of the study design. AJC, EA, MM, AB, EJC, RPY, AM, and BR supervised the testing and data collection in countries. LB and MB were responsible for monitoring and evaluating data collection. JC, AJC and MB drafted the manuscript. All authors interpreted the data and reviewed the manuscript. All authors read and approved the final manuscript.

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Author details

¹Stop TB Partnership, Secretariat, Geneva, Switzerland. ²Interactive Research and Development, Karachi, Pakistan. ³Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain (UCL), Brussels, Belgium. ⁴Health Alliance International, University of Washington, Seattle, WA, USA. ⁵University of Wisconsin, Madison, WI, USA. ⁶Project HOPE, Mulanje, Malawi. ⁷Warren Alpert School of Medicine at Brown

University, Providence, RI, USA. ⁸Stop TB Unit, WHO, Phnom Penh, Cambodia. ⁹Center for Health Policies and Studies, Chisinau, Republic of Moldova.

¹⁰International Organization for Migration, Kathmandu, Nepal. ¹¹Tuberculosis & Leprosy Research Group; Centre for Communicable Diseases; International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh. ¹²PHTB Consult, Tilburg, Netherlands. ¹³NT Development Policy & Practice, Royal Tropical Institute, Amsterdam, Netherlands.

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4.3. Understanding the cause of high rates of invalid results

We performed a systematic root-cause analysis of the different possible reasons for having high rates of invalid results. This analysis included the assessment of the different elements generally associated with analytical issues in clinical laboratories including laboratory environment, technical staff, equipment and reagents.

4.3.1. Electrical supply

Electrical supply in the South-Kivu province is irregular in all major cities, and frequently absent in small cities or villages. Therefore, most hospitals are equipped with fuel-based generators. This alternative has a high cost for these under-resourced settings. These generators regularly run out of fuel and are turned on only for critical medical emergencies.

Running an Xpert MTB/RIF assay on a GeneXpert instrument requires two hours of uninterrupted power supply. Among the ten hospitals, only two, both supported by Médecins Sans Frontières (MSF) had an uninterrupted electrical supply.

Eight hospitals only had irregular power supply. The solution implemented for these laboratories was to accumulate electrical energy in car batteries during the hours where wired electrical supply was available, so that these batteries could take-over when necessary (figure 4.3). A normal UPS system would only allow 20 minutes of autonomy.



Figure 4.3: Illustration of the electrical installation required for using the GeneXpert in the Shabunda hospital. From the left to the right: battery of the solar panel installation, charger-converter, truck battery, stabilizer, UPS, laptop with internal battery and GeneXpert).

4.3.2. Equipment quality

We analysed data from eight GeneXpert machines (33 analytical modules) from the South-Kivu province between 2012 and 2014.

We observed a high level of variability concerning the proportion of failed tests per analytical module. Overall, 10/33 (30%) modules had an error rate higher than 10%.

Most of the deficient modules were not under-utilized compared to normally-functioning modules. This reflects the fact that neither laboratory technicians nor the GeneXpert system are able to identify under-performing equipment.

The error rate was not associated with the GeneXpert instrument or the laboratory staff: for the same machine located in the same lab, different modules showed profoundly different error rates.

When looking at the performance of each module over time, we found that the deficient modules actually presented a high proportion of failed test from the first month of utilization, and that this proportion continuously increased over time. In comparison, the modules functioning “normally” during the first month continued to perform well over the time. This observation supports the hypothesis that not all modules distributed by Cepheid are of equal quality.

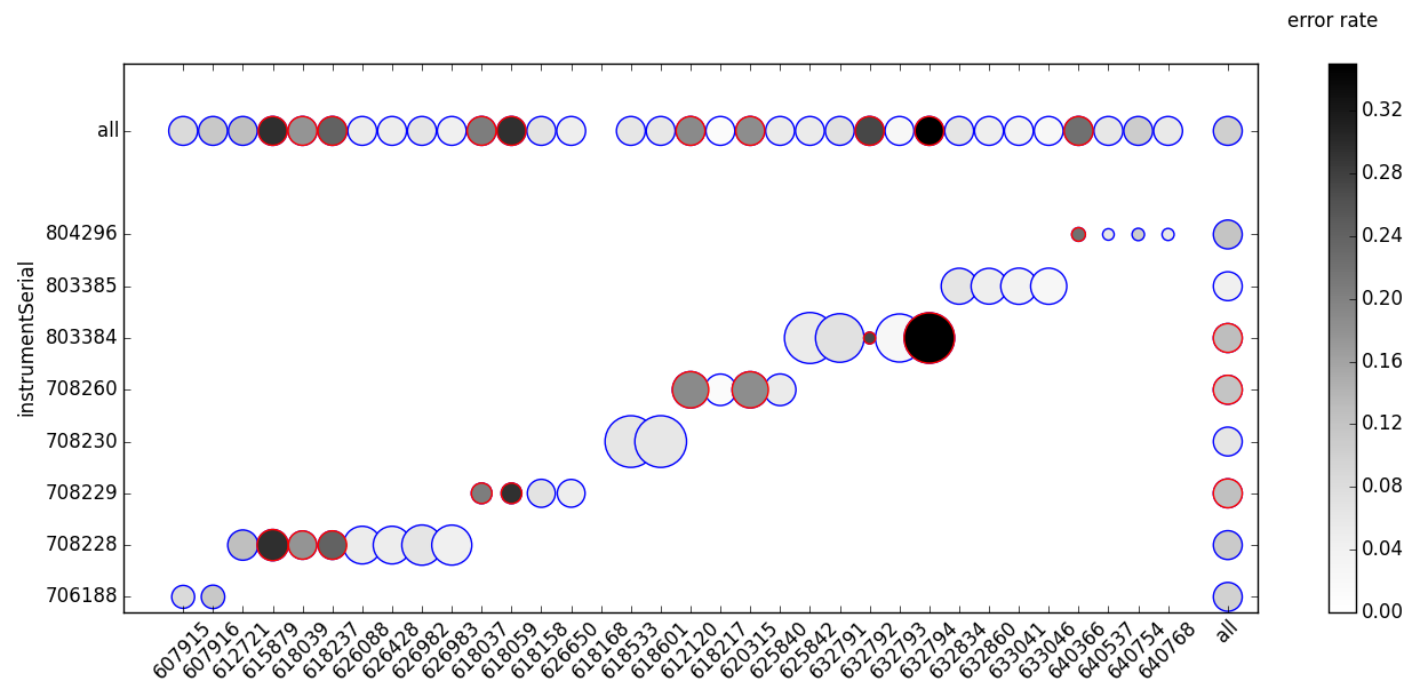


Figure 4.4: Utilisation and error rate of GeneXpert instruments (vertical axis) and their respective analytical modules (horizontal axis) in the South-Kivu province. The size of the circles represent the number of tests performed, and the colour represents the proportion of failed tests (error rate). This figure illustrates that high error rates do not prevent a massive utilization of failing analytical modules (e.g. instrument 803384, module 632793). Furthermore, it appears that failing analytical modules appear in nearly all laboratories. (Source: E. Renard and E. André, 2015)

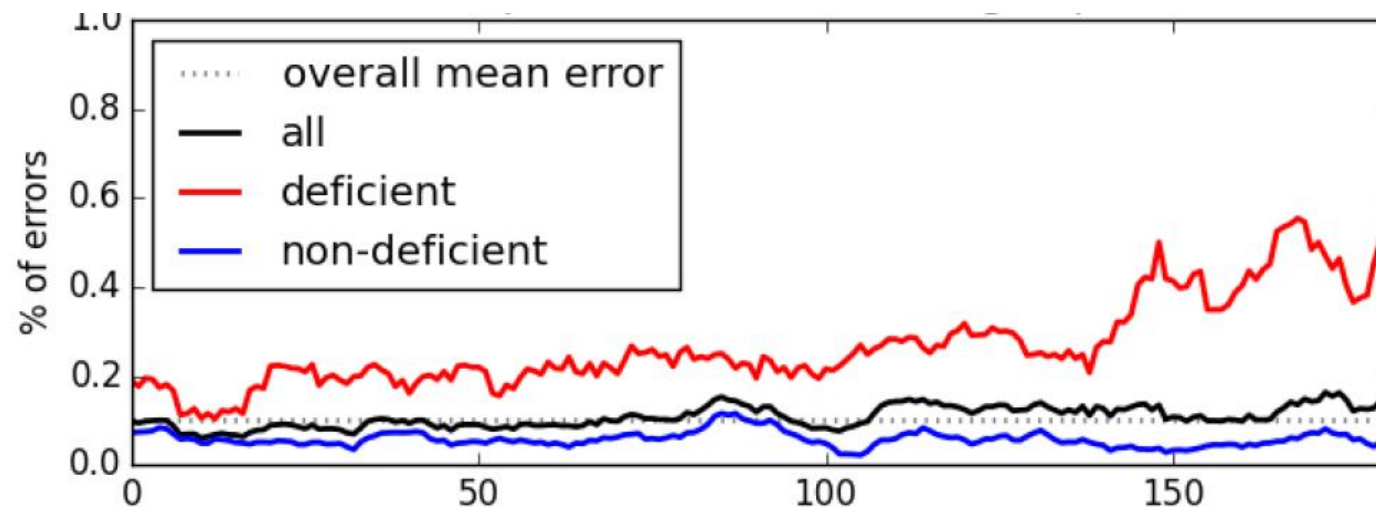


Figure 4.5: Evolution of the proportion of failed tests between the modules that have later been identified as deficient due to a high mean error rate in the retrospective analysis (red) and those with a mean error rate < 10% (blue). Number of days of utilization are represented in the horizontal axis. It appears that deficient modules have already present signs of higher error rates from the first month of utilization.

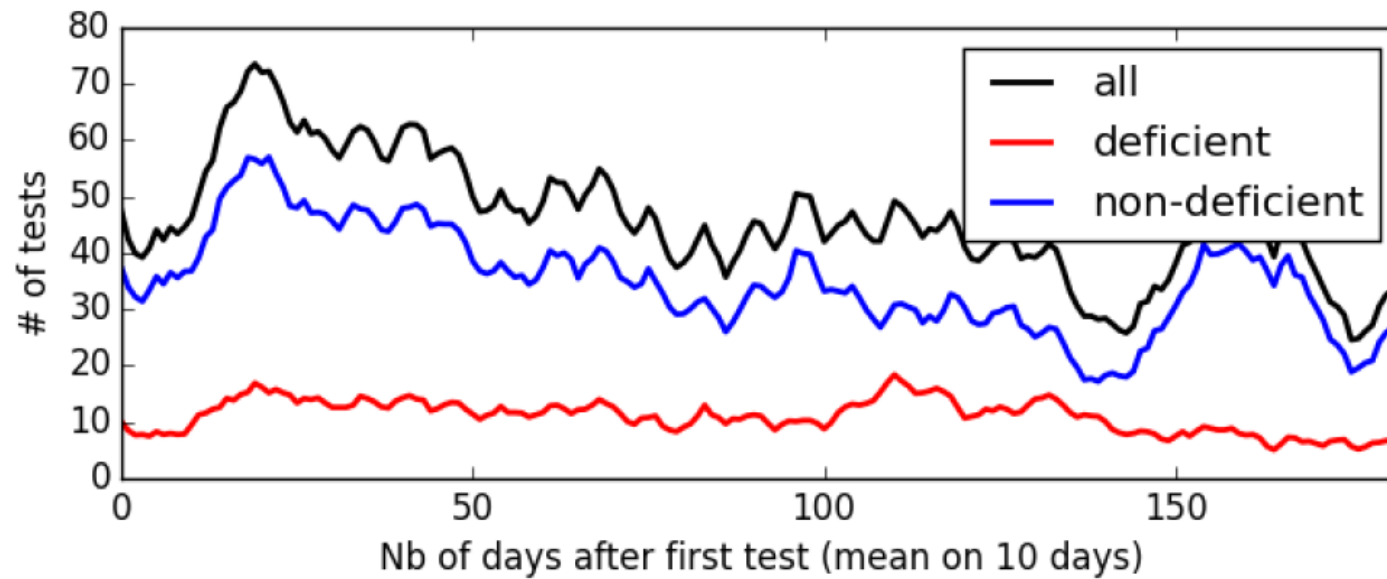


Figure 4.6: Evolution of the utilisation of modules. Deficient modules are represented in red and those with a mean error rate $<10\%$ are represented in blue. Number of days of utilization are represented in the horizontal axis. It appears that it end-users can continue using deficient modules for more than 6 months without self-reporting a technical issue.

4.3.3. Quality of reagents

Cepheid recommends storing the reagents in a temperature range between 4 and 25 °C. It is a major challenge for low-resource countries facing tropical climatic conditions to control these storage conditions throughout the transportation process from the border to the remote health facilities. Acknowledging this fact, we performed an analysis of the quality of the Xpert reagents.

Stability of reagents

We analysed data from 11.492 Xpert MTB/RIF tests (9 different reagent lots). We first looked at the stability of the internal control (named SPC) of the Xpert MTB/RIF tests for which a valid test result was available (positive or negative). We hypothesised that if the reagents were stored in suboptimal conditions that would affect their performances, the cycle threshold (Ct) of the internal control would vary significantly. We found that the distribution of the SPC Ct among these tests was normal, reflecting an overall very good stability of the reagents despite the fact that these were exposed to uncontrolled storage conditions.

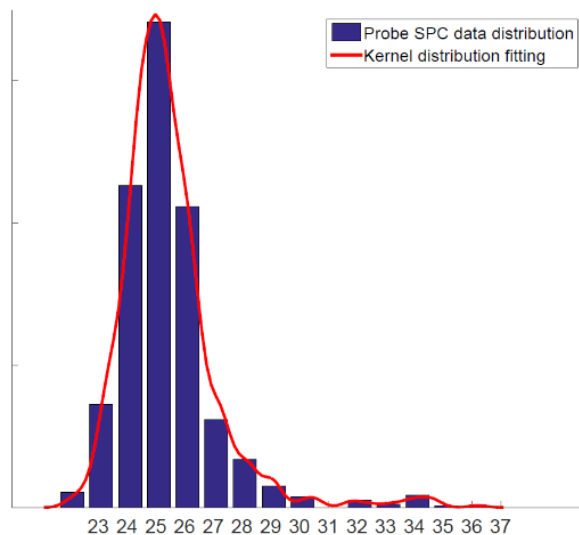


Figure 4.7: Distribution of Cycle threshold (Ct) values obtained for the internal SPC control of the Xpert MTB/RIF valid tests performed in the South-Kivu province. (Source: C. Vande Kerckhove and E. André, 2015). The normal distribution observed suggests a satisfactory stability of reagents despite un-controlled storage conditions.

Impact of reagents lots on error rates

We further looked at the proportion of failed tests per reagent lot number and found a significant variation concerning the mean error rate per reagent lots.

Among the nine reagents lots used in the South-Kivu province, the mean percentage of failed tests seemed to be partially associated with the reagent lot used. One lot was clearly an outlier, with 50% of the tests failing. In total, 692 tests were performed with this deficient lot, illustrating the fact that laboratory technicians or the GeneXpert system are not able to autonomously detect such issue.

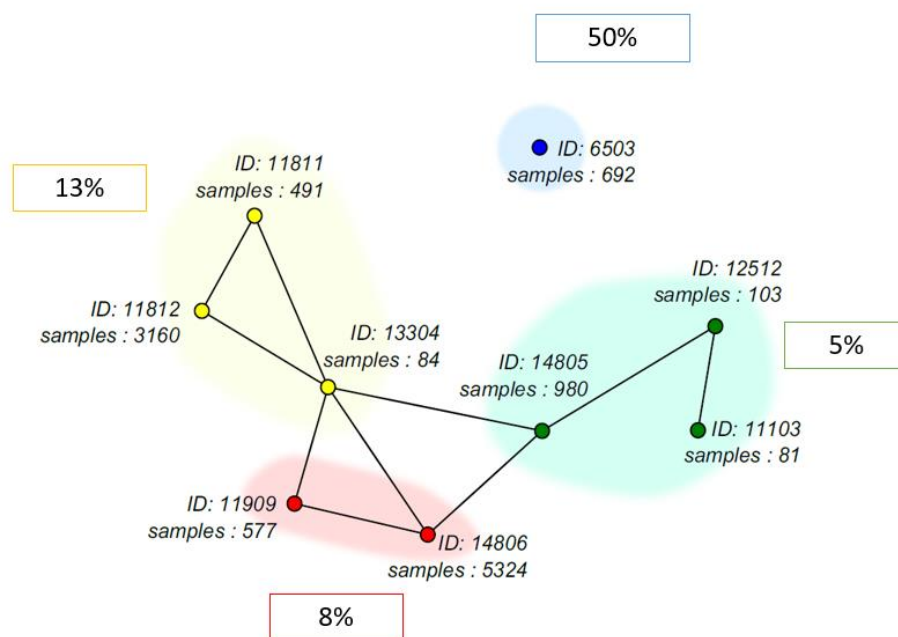


Figure 4.8: Mean percentage of failed tests per Xpert MTB/RIF reagent lots and number of samples analysed with cartridges of each lot.

4.3.4. Evolution of the number and proportion of invalid Xpert MTB/RIF test results (2012-2014)

Despite continuous efforts to decrease the proportion of invalid results, we could not achieve significant improvements during the two first years after implementation. The main reason for this is that the majority of invalid results were associated with failing equipment and reagents.

4.4. Sample transportation for molecular testing in centralized laboratories

Unlike culture-based techniques, molecular techniques such as PCR or sequencing do not require living bacteria. Therefore, conservation procedures of sputa can be simplified.

We validated a method that allows the sample to be preserved for 30 days by mixing the sputum with 96° ethanol. This method allowed increased referral of samples from remote areas to GeneXpert laboratories (see conference paper 1 in annex section).

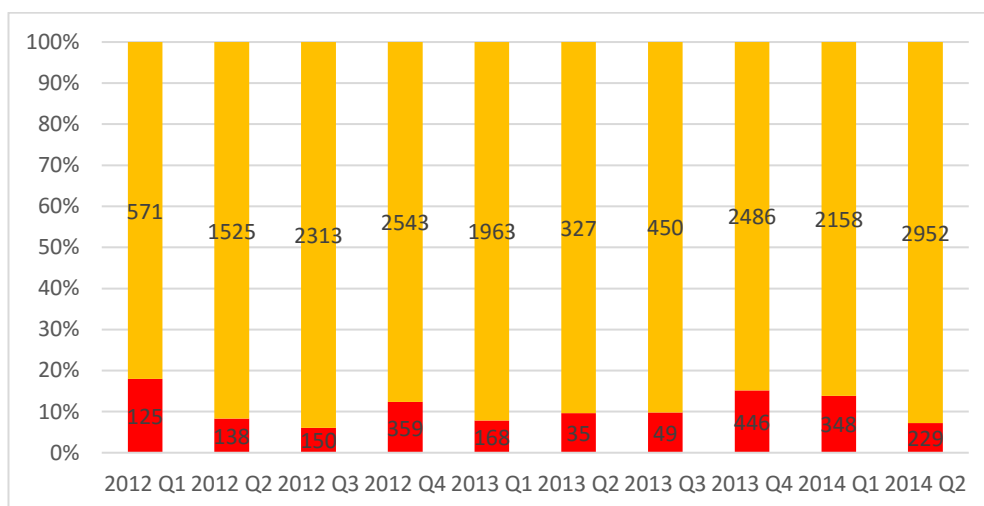


Figure 4.9: Evolution of the number and proportion of valid (orange) and invalid (red) Xpert MTB/RIF test results over de time in the South-Kivu province of DRC. In total, 2.047/19.335 (10,6%) tests were not associated with a valid result. Most of these invalid tests were associated with the utilization of failing analytical modules of the Genexpert automates.

4.5. Impact of Xpert MTB/RIF on TB and RR-TB detection

4.5.1. Positivity

In our experience Xpert MTB/RIF was used as a follow-up test for patients with TB-associated symptoms who had an initial negative smear microscopy result.

Between Q1 2012 and Q4 2014, we performed 17.288 Xpert MTB/RIF tests. Among these, 1.232 (7,1%) samples were positive for TB.

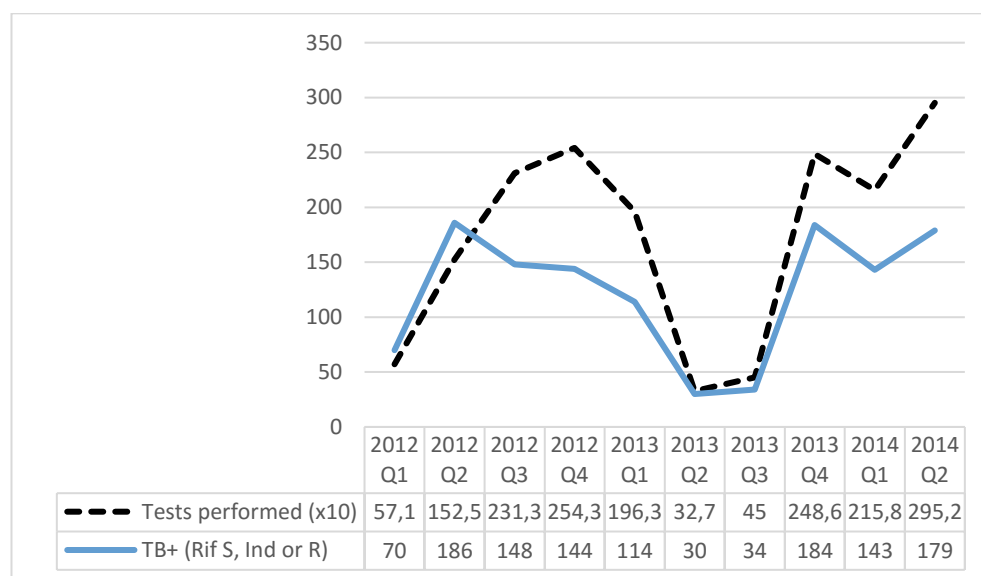


Figure 4.10: Utilization and yield of Xpert MTB/RIF in the South-Kivu province of DRC for the detection of TB among patients suspected of having TB despite a first negative smear result

The trends in positivity rate show a high stability following the initial months of utilisation.

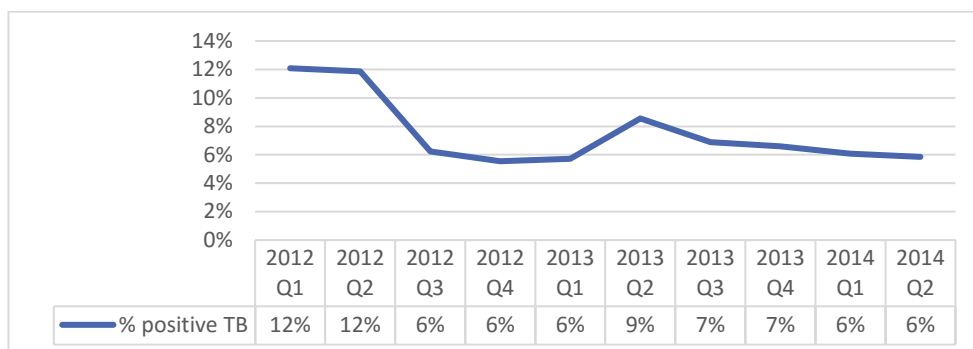


Figure 4.11: Trends in positivity rate of Xpert MTB/RIF tests performed in the South-Kivu province of DRC among patients suspected of having TB despite a first negative smear result.

4.5.2. RIF-resistance

Concerning the proportion of RIF resistant TB cases, the trend was relatively stable over time. On average, 8.9% of patients with a positive TB result had RIF resistance detected. In this analysis, we could not estimate the amount of retreatment cases tested.

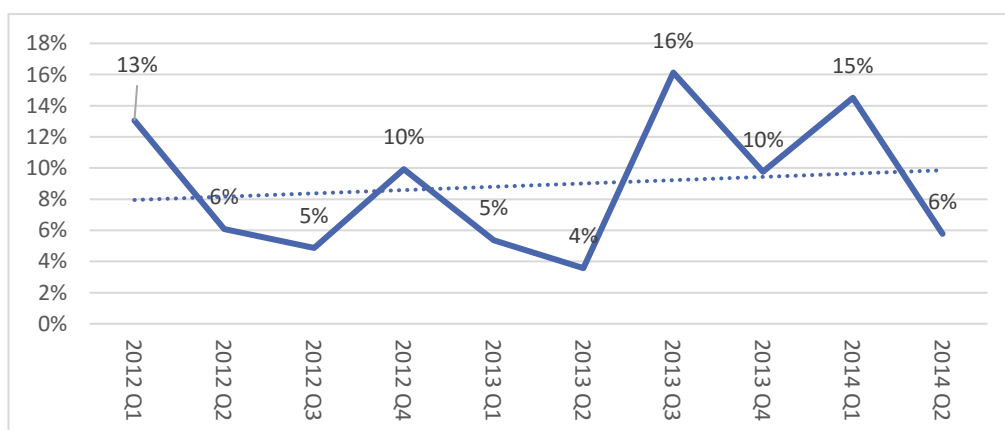


Figure 4.12: Percentage of RIF-resistant TB samples in the South-Kivu province of DRC

4.6. Confirmation of RIF-resistant TB

4.6.1. Challenges

In DRC, the National Reference Laboratory (NRL) in Kinshasa performs drug susceptibility testing (DST) for the whole country. Before the introduction of GeneXpert, DST was performed only among retreatment cases, and the number of samples received was limited by the logistical constraints associated with the transport of samples from the different provinces of the country. In 2014, only 442 TB cases in DRC were confirmed with MDR-TB, which represents only 6% of the estimated total number of MDR-TB.

Following the introduction of GeneXpert, our intervention considerably increased the number of RIF-resistant cases diagnosed. In such circumstances, the WHO recommends that a confirmatory test be performed, either phenotypic DST or LPA¹².

In our early experience associated with the implementation of GeneXpert, it was a challenge to confirm resistance and obtain a consistent DST result. Among the difficulties, timely transport of samples in adequate conditions, cost of sample transportation, high culture contamination rates and delayed transmission of results by the NRL were among the major difficulties encountered. Transportation of RIF-resistant samples generally required to transport the fresh sample from the Genexpert lab to the provincial TB coordination office (this can take several days), followed by air-transport of the sample in CPC to Kinshasa (there are only 1-2 flights per week). Figure 4.13.

In the South-Kivu province, after two years of GeneXpert utilization, only 3 out of 31 patients with Rif resistant TB had a DST result available. All three confirmed MDR-TB. (Source: CPLT Sud-Kivu, 19 of September 2014).

¹² http://apps.who.int/iris/bitstream/10665/112469/1/9789241506700_eng.pdf?ua=1



Figure 4.13: In the South-Kivu province, samples detected as RIF-resistant TB have to be transported from the peripheral health facilities to Kinshasa. After arriving in Bukavu, samples are transferred to the national reference laboratory located at over 2,000 km in Kinshasa. The process of sample transportation generally takes several weeks. (Photo: Jean-Paul Chirambiza, biologist at the provincial TB program in Bukavu, holding a package with RIF-resistant samples to be sent to Kinshasa)

4.6.2. Implementation of a Hain MTBDR*plus* laboratory in the South-Kivu province

The Democratic Republic of Congo is among the countries with the highest burden of TB and multi-drug resistant TB (MDR-TB). Despite this alarming situation, drug susceptibility testing (DST) is still performed only in the National Reference Laboratory of Kinshasa. The South-Kivu province, which is in the other extremity of the country, has identified 183 new patients with rifampicin resistant TB (RR-TB) following the introduction of Xpert MTB/RIF in 2012. Among these, results of DST only confirmed eight cases (4,4%) of multi-drug resistant TB (MDR-TB). For the rest of the patients, results were unavailable or did not confirm MDR-TB.

This observation was the main reason for implementing a molecular laboratory platform and starting performing LPA tests (MTBDR*plus*, Hain Lifescience, Germany) in Bukavu.

From the fourth quarter of 2012, we implemented a LPA test (MTBDR*plus*, Hain Lifescience) in Bukavu, the capital city of the South-Kivu province. This was the first time that such technology was introduced outside the capital of the country.

This new laboratory allowed MDR-TB to be confirmed among 28 additional patients (+350%), constituting all 22 new MDR-TB cases since 2014. Out of the 54 results which presented RIF resistance on the LPA test, 26 (48%) did not show any isoniazid (INH) resistance mutation on the *katG* or *inhA* genes. In this limited sample, we did not find discordant results.

This strategy was confronted with major logistical issues: the unstable electric power supply deeply affected the conservation of reagents, samples and DNA extracts that need to be stored at -20°C. Furthermore, we were confronted with a very limited uptake from the NTP concerning this technique, and reagents could never be procured through the public system.

This experience demonstrated the feasibility and utility of introducing LPA confirmatory testing at the provincial level in DRC. This high level of RIF mono-resistance found using this test was not confirmed by national results obtained with next-generation sequencing methods and presented in section 4.8 (10% of RIF-R samples tested). This may suggest that the LPA test performance in detecting INH-resistance may have been affected by poor sample and reagent storage conditions.

As a result, due to the limited level of infrastructure, we do not think that the implementation of this technology has significantly influenced the clinical management of people infected with drug-resistant tuberculosis.

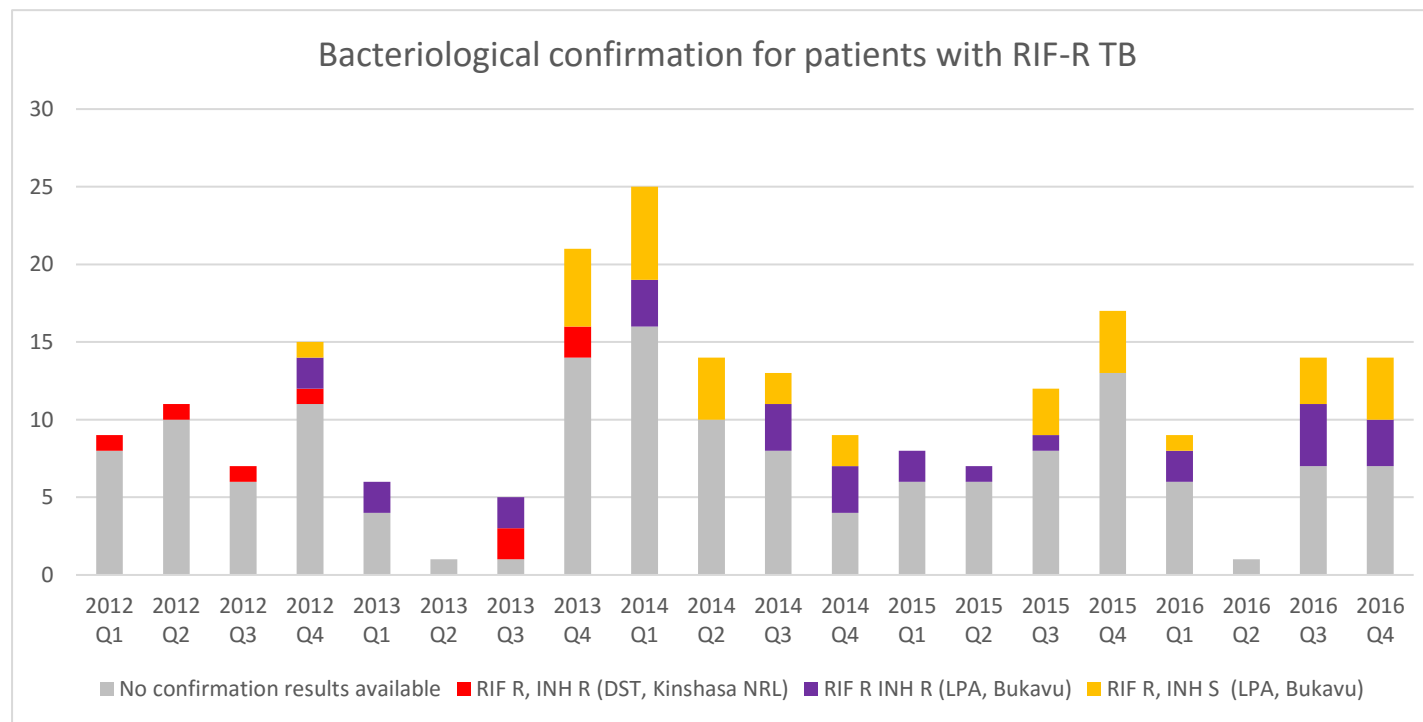


Figure 4.14: Bacteriological confirmation of RIF-R cases detected by Xpert MTB/RIF in the South-Kivu province of DRC between 2012 and 2016. The implementation of an LPA laboratory at the Provincial level allowed a 350% increase in the number of cases confirmed with MDR-TB over the evaluation period.

4.7. Xpert connectivity

The necessity for remote monitoring of the activity of GeneXpert automates installed in peripheral laboratories was initially linked to the necessity to monitor the utilisation of GeneXpert in remote areas. In the South-Kivu province, most laboratories were located in cities distant from Bukavu city, and regular on-site supervision was very difficult: it required travel for several days through sometimes insecure regions, and was associated with high costs.

Interactive Research and Development (IRD, Pakistan) initially developed with the support of TB REACH an open-source IT solution (XpertSMS) which allows the Xpert MTB/RIF results from the GeneXpert platform to be captured and transformed to a format that can be exported. We held an initial technical workshop in Uganda with the IRD IT team in order to explore the possibility of introducing this technology in the South-Kivu province.

It appeared that this technology presented two limitations for deployment in our setting. First, it relied on internet or 3G connectivity for transferring the data, but these were not available in most laboratories where GeneXpert was introduced. Second, we wanted to link the test results with patient data in order to better capture the identification, demographics and medical history of the patients undergoing an Xpert test.

We therefore developed a complementary module called GenXchange which allowed the patient data to be captured and transfer the information using SMS technology. SMS further allows to send a receipt confirmation.

This solution was successfully implemented at the provincial level and was subsequently adopted by the National TB program of DRC to connect all the GeneXpert in the country.

GenXchange 2.0 © Pays : CONGO DRC Laboratoire : KATANGA CENTRE

Le test GeneXpert est terminé.

Le résultat est **MTB DETECTED** **Rif Resistance DETECTED**

Merci de compléter les informations ci-dessous.

Prescripteur :

Opérateur GeneXpert:

Site de la maladie:

Identification du patient:

Nom : Prénom : Sexe : ☐ Femme ☐ Homme

Date de Naissance :

Ville ou Localité: Commune ou Quartier :

Téléphone Patient : +243

Raison du test :

Autres Informations :

Statut VIH: Contact TB-MDR: Diabète :

Figure 4.15: Data capture proposed by the GenXchange software (2013 version) following a positive Xpert MTB/RIF test results. This screen automatically pops-up on the screen of the GeneXpert computer when a test is finalized. Following the capture of this information, the data are transferred to a central database together with the raw Xpert MTB/RIF test results.

4.8. Utilization of molecular diagnostic tests for the first national drug resistance survey in DRC

4.8.1. Context

The DRC had never performed a drug resistance survey at the national level until 2016. The only published survey was limited to Kinshasa and was performed in 2007 (135). Since several years, the NTP, the WHO and their technical partners were confronted by the technical difficulties associated with the organisation of such survey in a country with very limited laboratory infrastructure and nearly no sample transportation system.

Such surveys require that samples collected in randomly selected health facilities around the country (30 in the present study) would be adequately collected, transported and tested. Although a budget was available to overcome these issues, the fear of the study coordinators was that the quality of the laboratory results would be insufficient to provide valid information in regard to the prevalence of drug-resistant TB in DRC.

We provided technical support for organizing the collection, transportation and testing of the samples (figure 4.16). The originality of the proposed protocol relied on the proposition that one sample would be stored in ethanol in order to stabilize the sample and allow the realization of complementary molecular testing in case the cultures or DST performed at the NRL and the supra-national reference laboratory (SRL) were inconclusive due to culture contamination or discrepancies.

We performed NGS analysis (Deeplex MycTB, Genoscreen) on the samples from this survey at the Saint-Luc hospital (UCL, Belgium) laboratory.

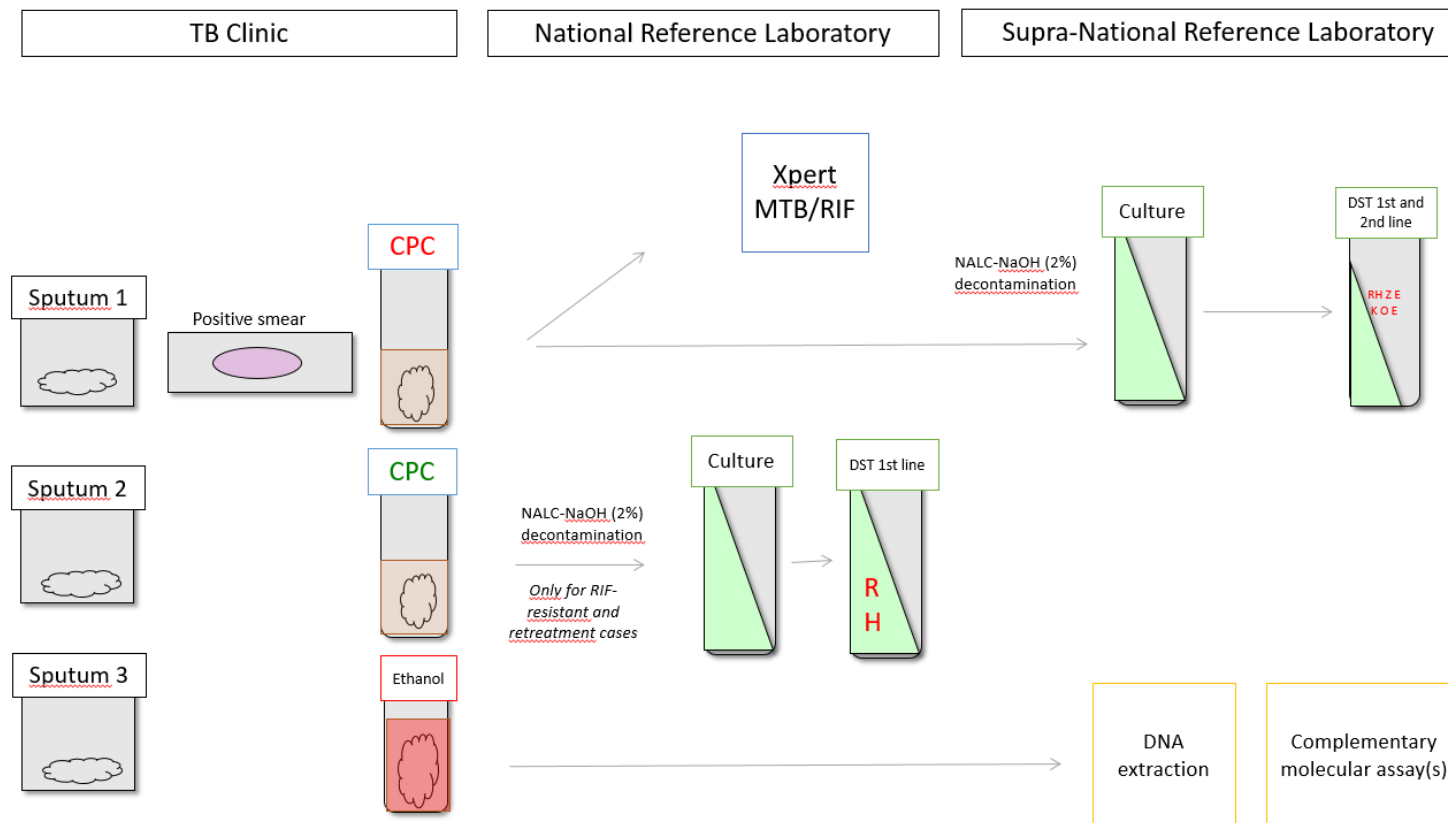


Figure 4.16: Proposed sputum collection procedure for the national drug resistance survey to overcome the technical challenges. This system allowed solving most barriers preventing this study to be operationalized.

4.8.2. Preliminary results of the Deeplex MycTB assay (Genoscreen, France)

The NGS Deeplex MycTB assay (Genoscreen, France) is probably the commercial TB diagnostic test that will provoke the most disruptive impact on patient care and disease surveillance in the near future.

The test can be performed directly from sputum samples, including from smear negative samples (figure 1; source: Genoscreen).

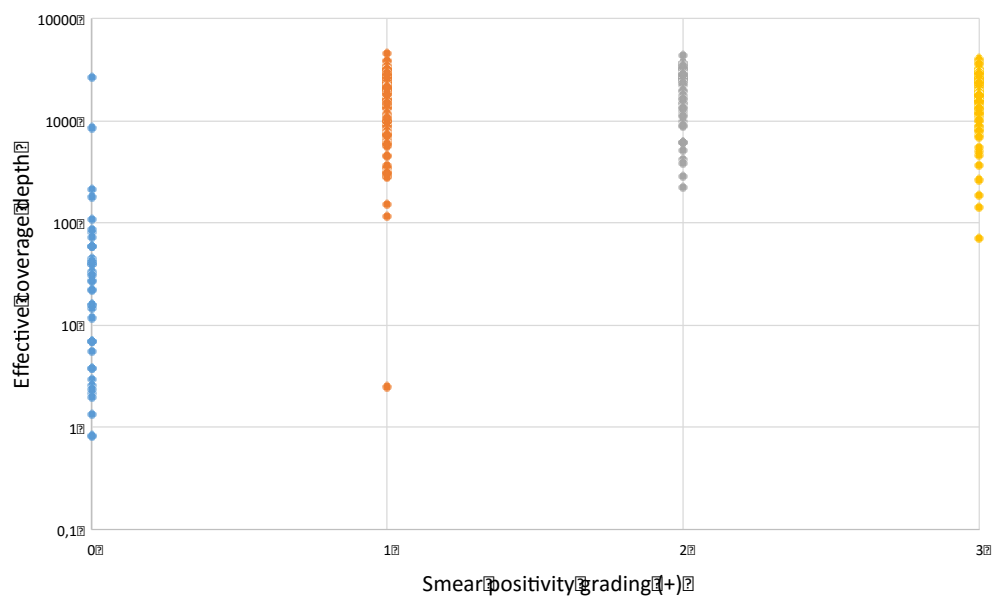


Figure 4.17: Performance of the Deeplex MycTB assay based on the microscopic examination grading. (n=300), for Xpert MTB/RIF positive samples. Source: Genoscreen

In total, the multiplex assay covers 21 targets including drug resistance genes, identification genes and typing genes (figure 2: Source: Genoscreen):

- Drug resistance (first line, second line and third line drugs).
 - The test relies on sequencing, meaning that the risk of false resistance due to silent mutations is decreased compared to PCR-based assays
 - The test widely covers the target genes, including in regions not covered by commercial PCR-based assays. Therefore, the risk of missing “atypical” resistance mutations is minimal
 - The test can detect minority populations (limit of 2%)
- Typing information:
 - Precise identification, including if presence of non-TB mycobacteria
 - Spoligotyping
 - Subtle genetic variations (“single nucleotide polymorphisms”)

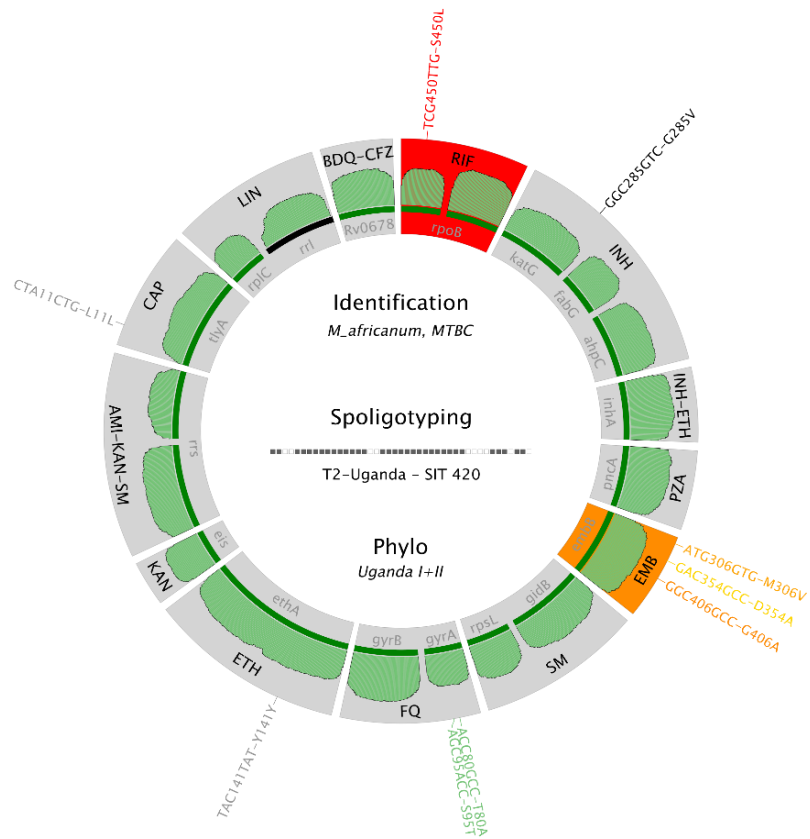


Figure 4.18: Overview of information generated for each test by the Deeplex MycTB assay (Genoscreen, France)

4.8.3. Material tested

The samples tested in for this study are from sputum samples, presumably positive for *Mycobacterium tuberculosis* complex (MTBC) based on a positive smear microscopy result performed in local health facilities of the DRC. These samples have been conserved for several months before DNA extraction. DNA extraction was performed at ITM (Antwerp, Belgium).

Considering this specific context, we expected that a proportion of samples have no MTBC at all (false positive results from microscopy), or an insufficient quality to generate the expected test results. The proportion of failed tests was around 10% among the preliminary samples sent to Genoscreen.

In our experience, we could provide a valid result for 92,5% of the samples. Among these, *Mycobacterium tuberculosis* DNA was not detected by the assay for 42 samples (6,2% of valid results).

4.8.4. Number of tests performed

The number of NGS tests to be performed in such a short timing was a major challenge for our clinical laboratory. It takes 3-4 days of full-time technical work to perform approximately 90 tests. The NGS analysis itself takes approximately 18 hours per lot of 45 samples.

We could test all DNA extracts received on time. Including the tests that needed to be repeated and the internal controls performed, we performed a total of 921 tests. Some re-tests or additional tests may need to be performed in the future following the demand of the study.

We received a total of 671 DNA extracts, for which we could obtain a valid result for 92,5% (n=621). The results presented below cannot be interpreted from an epidemiological point of view as these represent only a part of the samples collected

4.8.6. Mixed populations

We further analysed the homogeneity of the NGS sequences obtained in order to determine the frequency of hetero-resistance, meaning the simultaneous presence of a wild-type and a resistant genetic profile in the clinical sample (figure 4.18).

We found that hetero-resistance was present for all drug resistance found. This was particularly the case for PZA (14/15 strains). The significance of these findings still need to be clarified.

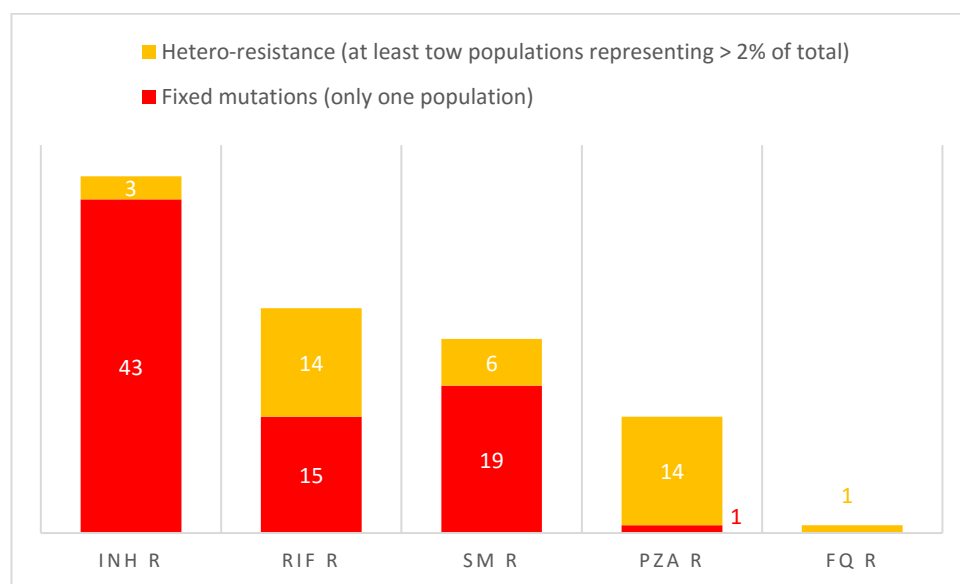


Figure 4.20: Frequency of hetero-resistance among samples presenting a resistance mutation. The results were obtained with the Deeplex MycTB assay (Genoscreen, France).

4.8.7. Association of drug resistance

Rifampicin (RIF)

Among the samples tested, 29/621 (4,7%) presented a resistance mutation to **RIF**.

- **12/29 (41,4%) of samples with RIF resistance were MDR-TB**
 - 1/29 (3,4%) was associated with INH, PZA, EMB and SM resistance mutations
 - 3/29 (10,3%) were associated with INH, EMB and SM resistance mutations
 - 4/29 (13,8%) were associated with INH and EMB resistance mutations
 - 1/29 (3,4%) was associated with INH and SM resistance mutations
 - 3/29 (10,3%) were associated with INH resistance only
- **17/29 (58,6%) of samples with RIF resistance were not MDR-TB**
 - 13/29 (44,8%) were associated with PZA resistance only
 - 1/29 (3,4%) was associated with EMB resistance only
 - 3/29 (10,3%) were not associated with any resistance mutation

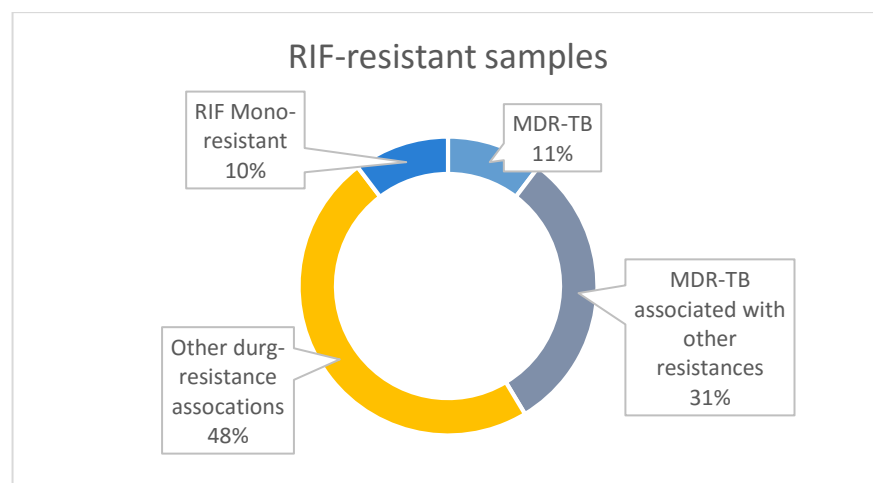


Figure 4.21: Drug resistance profile of the 29 RIF-resistant samples tested for the drug resistance survey in DRC

Isoniazid (INH)

Among the samples tested, 46/621 (7,4%) presented a resistance mutation to **INH**. Among these, 35 (76,1%) samples had a mutation in the *katG* gene, and 11 (23,9%) samples had a resistance due to a variation in the *fabG1-inhA* region.

- **12/46 (26,1%) of samples with INH resistance were MDR-TB**
 - 1/46 was associated with RIF, PZA, EMB and SM resistance
 - 3/46 were associated with RIF, EMB and SM resistance
 - 4/46 were associated with RIF and EMB resistance
 - 1/46 were associated with RIF and SM resistance
 - 3/46 were associated with RIF resistance only
- **34/46 (73,9%) of samples with INH resistance were not MDR-TB**
 - 8/46 (17,4%) were associated with SM resistance only
 - 26/46 (56,5%) were not associated with any resistance mutation

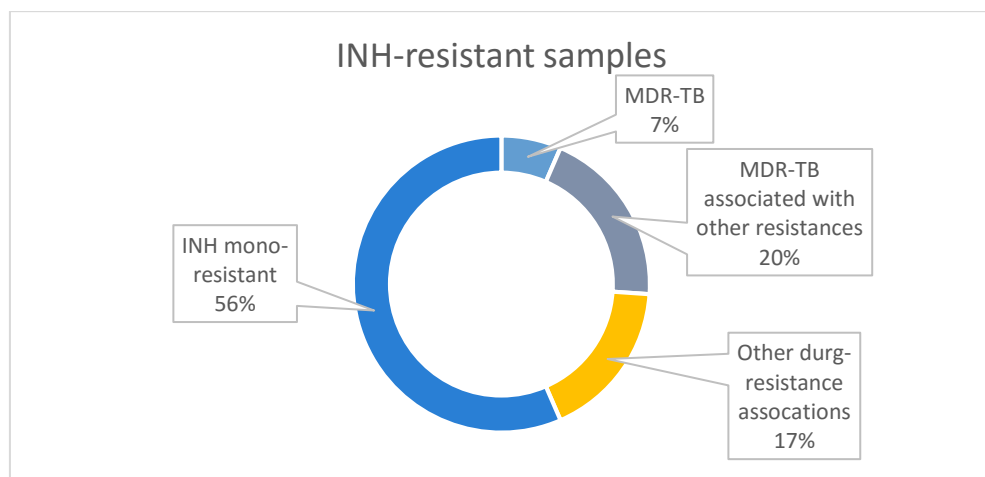


Figure 4.22: Drug resistance profile of the 46 INH-resistant samples tested for the drug resistance survey in DRC

Pyrazinamide (PZA)

Among the samples tested, 15/621 (2,4%) presented a resistance mutation to **PZA**.

- 1/15 (6,7%) was associated with RIF, INH, EMB and SM resistance mutations
- 13/15 (86,7%) were associated with RIF resistance mutations only
- 1/15 (6,7%) was not associated with any resistance mutation

Ethambutol (EMB)

Among the samples tested, 11/621 (1,8%) presented a resistance mutation to **EMB**.

- 1/11 (9,1%) was associated with RIF, INH, PZA and SM resistance
- 3/11 (27,2%) were associated with RIF, INH and SM resistance
- 4/11 (36,4%) were associated with RIF and INH resistance
- 1/11 (9,1%) was associated with RIF resistance only
- 2/11 (18,2%) were not associated with any other resistance

Streptomycin (SM)

Among the samples tested, 25/621 (9,6%) presented a resistance mutation to **SM**.

- 1/25 was associated with RIF, INH, PZA and EMB resistance
- 3/25 were associated with RIF, INH and EMB resistance
- 8/25 were associated with INH resistance only
- 12/25 were not associated with any drug resistance

Fluoroquinolones (FQ)

Among the samples tested, 1/621 (0,2%) presented a resistance mutation to **FQ**.

This sample did not present any other drug resistance associated.

Linezolid (LZD)

The test could not detect any described genetic variation associated with LZD resistance (*rrl* and *rplC* genes). 32/574 samples presented uncharacterized mutations in these genes.

Bedaquiline (BDQ)

The test could not detect any described genetic variation associated with BDQ resistance (rv0678 / *mmpR* gene). 10/574 samples presented uncharacterized mutations, and 3 samples lacked the rv0678 gene, suggesting possible resistance to BDQ.

It is to be noted that the test does not cover the *atpE* and *pepQ* (rv2535c), which are less frequently associated with BDQ resistance *in vivo* (*atpE*) or *in vitro* (*pepQ*).

Chapter 5: Laboratory connectivity

5.1. Mining of data – a new research focus

The success of TB elimination strategies will depend on the ability to tailor generic tools to the context of local settings. Local particularities should be revealed at the stage of planning interventions rather than at the result analysis stage. Although important efforts are still to be made to improve the collection of programmatic information and contextual data, the most important step will be to use this information for action (136).

Following the scale-up of GeneXpert instruments in low-resource countries, a series of third-party stakeholders developed connectivity solutions allowing to automatically collecting and transfer test results in central servers. These solutions, namely XpertSMS (IRD, Pakistan), GxAlert (Abt associates, USA) and GenXchange (UCLouvain, Belgium), allowed many countries to better follow the utilization of Xpert MTB/RIF tests. These were presented at the Global Laboratory Initiative (GLI) meetings held by the WHO in 2014¹³ and 2015¹⁴ (137). It is only later that Cepheid provided built-in connectivity solutions.

In low-resource countries, there was minimal laboratory connectivity and we coordinated the elaboration of a large consensus statement highlighting the potential of connectivity not only for monitoring of utilization, but also as a tool which permits enhanced laboratory quality management, patient follow-up and disease surveillance at the international level (138-142).

¹³ 6 th Global Laboratory Initiative (GLI) Partners Meeting: Modern TB Diagnostic Services: Optimizing the Old with the New :

http://www.stoptb.org/wg/gli/assets/documents/6th%20GLI%20meeting%20AGENDA-for%20WEBSITE%20Update_9July2014.pdf

¹⁴GLI-GDI Joint partner Forum for strengthening and aligning TB diagnosis and treatment: <http://megaslides.es/doc/3193633/2015--gli-gdi-joint-partners%E2%80%9999-meeting-for-strengthening-a...>

This statement concluded:

“In the future, all diagnostic technologies should be interconnected, allowing data generated by laboratories to be merged in a common repository while safeguarding patient confidentiality. The TB community could use such a repository to monitor progress and identify problems and potential solutions at both patient and global levels. Data pooling will open up opportunities to comprehend the rapid evolution of drug-resistance mutations, which will aid in selecting cost-effective treatment schemes and improving patient management. With the many solutions it can provide, data pooling at an international level is an absolute priority, as it will accelerate progress in critical sectors, including patient care, epidemiological surveillance and operational response. As an international health emergency, the TB epidemic requires optimal international collaboration and unambiguous political commitment for intensifying data-sharing efforts.”

Together with the technical evolution of the connectivity systems, this vision possibly contributed to the endorsement by the WHO of connectivity solutions^{15,16}.

5.2. Connectivity of diagnostic technologies: improving surveillance and accelerating TB elimination

The full study is available online (open access)
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¹⁵ GLI Practical Guide to TB Laboratory Strengthening (March 2017) :

https://www.ghdonline.org/uploads/GLI_practical_guide.pdf

¹⁶ GLI quick guide to TB diagnostics connectivity solutions (October 2016):

http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf

Connectivity of diagnostic technologies: improving surveillance and accelerating tuberculosis elimination

E. Andre,^{***} C. Isaacs,⁵ D. Affolabi,[¶] R. Alagna,^{**} D. Brockmann,^{††††} B. C. de Jong,⁵⁵
E. Cambau,^{†††††} G. Churchyard,^{***} T. Cohen,^{†††} M. Delmee,^{††} J.-C. Delvenne,^{†††††} M. Farhat,^{††††}
A. Habib,^{†††} P. Holme,^{†††††} S. Keshavjee,^{††††} A. Khan,^{††††} P. Lightfoot,⁵ D. Moore,^{†††††}
Y. Moreno,^{††††††††} Y. Mundade,^{†††††} M. Pai,^{†††††} S. Patel,^{†††††} A. U. Nyaruhirira,^{††††††††}
L. E. C. Rocha,^{†††††††††} J. Takle,^{†††††††} A. Trébuq,^{†††††††} J. Creswell,^{††††††††} C. Boehme⁵

^{*}Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels; [†]Service de Microbiologie, Département de Biologie Clinique, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [‡]European Society for Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Mycobacterial Infections (ESGMYC), ESCMID, Basel; [§]Foundation for Innovative New Diagnostics, Geneva, Switzerland; [¶]Faculty of Health Sciences, Abomey-Calavi University, Cotonou; ^{||}National Tuberculosis Programme, Cotonou, Benin; ^{**}TB Supranational Reference Laboratory, Istituto di Ricovero e Cura a Carattere Scientifico San Raffaele Scientific Institute, Milan, Italy; ^{††}Institute for Theoretical Biology, Department of Biology, Humboldt University of Berlin, Berlin; ^{†††}Epidemiological Modelling of Infectious Diseases, Robert Koch Institute, Berlin, Germany; ^{††††}Unit of Mycobacteriology, Department of Biomedical Sciences, Institute of Tropical Medicine, Belgium; ^{†††††}Université Paris Diderot, Institut National de la Santé et de la Recherche Médicale, Unité mixte de recherche 1137, Infection, Antimicrobiens, Modélisation, Evolution, Paris; ^{‡‡}Bactériologie, Assistance Publique-Hôpitaux de Paris, Hôpital Lariboisière, Paris, France; ^{§§}Aurum Institute, Johannesburg, South Africa; ^{|||}Yale School of Public Health, Yale University, New Haven, Connecticut, USA; ^{||||}Institute of Information and Communication Technologies, Electronics and Applied Mathematics, and ^{|||||}Centre for Operations Research and Econometrics, Université Catholique de Louvain, Belgium; ^{|||||}Massachusetts General Hospital, Boston, Massachusetts, USA; ^{|||||}Interactive Health Solutions, Karachi, Pakistan; ^{|||||}Sungkyunkwan University, Seoul, South Korea; ^{|||||}Harvard Medical School Center for Global Health Delivery, Dubai, United Arab Emirates; ^{|||||}Interactive Research and Development, Karachi, Pakistan; ^{|||||}TB Centre, London School of Hygiene & Tropical Medicine, London, UK; ^{|||||}Institute for Biocomputation and Physics of Complex Systems (BIFI), and ^{|||||}Department of Theoretical Physics, Faculty of Sciences, University of Zaragoza, Zaragoza, Spain; ^{|||||}UNITAID, Geneva, Switzerland; ^{|||||}McGill International TB Centre & McGill Global Health Programs, McGill University, Montreal, Quebec, Canada; ^{|||||}University Hospital Southampton NHS Foundation Trust, Southampton, UK; ^{|||||}Management Sciences for Health, Gauteng, South Africa; ^{|||||}Karolinska Institutet, Stockholm, Sweden; ^{|||||}Université de Namur, Namur, Belgium; ^{|||||}Global Connectivity LLC, Somerville, Massachusetts, USA; ^{|||||}International Union Against Tuberculosis and Lung Disease, France; ^{|||||}Stop TB Partnership, Geneva, Switzerland

SUMMARY

In regard to tuberculosis (TB) and other major global epidemics, the use of new diagnostic tests is increasing dramatically, including in resource-limited countries. Although there has never been as much digital information generated, this data source has not been exploited to its full potential. In this opinion paper, we discuss lessons learned from the global scale-up of these laboratory devices and the pathway to tapping the potential of laboratory-generated information in the field of TB by using connectivity. Responding to the demand for connectivity, innovative third-party players have proposed solutions that have been widely adopted by field users of the Xpert® MTB/RIF assay. The experience associated with the utilisation of these systems, which facilitate the monitoring of wide laboratory networks, stressed the need for a more global and comprehensive approach to diagnostic connectivity. In addition to

facilitating the reporting of test results, the mobility of digital information allows the sharing of information generated in programme settings. When they become easily accessible, these data can be used to improve patient care, disease surveillance and drug discovery. They should therefore be considered as a public health good. We list several examples of concrete initiatives that should allow data sources to be combined to improve the understanding of the epidemic, support the operational response and, finally, accelerate TB elimination. With the many opportunities that the pooling of data associated with the TB epidemic can provide, pooling of this information at an international level has become an absolute priority.

KEY WORDS: laboratory; connectivity; tuberculosis; surveillance; data

Correspondence to: Emmanuel André, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, 10 avenue Hippocrate Brussels 1200, Belgium. e-mail: emmanuel.andre@uclouvain.be; emmanuel.andre@outlook.be

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IN THE PAST DECADE, the use of new diagnostic tests has increased dramatically in the laboratories of developing countries and, more recently, in decentralised point-of-care facilities. Self-contained molecular diagnostic devices have been successfully deployed to detect tuberculosis (TB) (e.g., Xpert® MTB/RIF; Cepheid, Sunnyvale, CA, USA¹) or monitor treatment for the human immunodeficiency virus (HIV) (e.g., Alere Pima™ CD4, Alere, Waltham, MA, USA²) in very basic clinical facilities. Despite the accumulating evidence that these tools can be successfully used in the most challenging environments^{3,4} and the establishment of distribution and funding channels that should theoretically allow any country to access and scale up these new technologies, the majority of patients that could benefit from these technical evolutions still do not have access to them. It is clear that the introduction of an improved TB diagnostic tool is not sufficient to assure improved outcomes for patients, as the details of implementation within existing health delivery systems have a critical influence on impact.⁵

We suggest that the introduction of new tools such as Xpert offers an important opportunity to better understand, monitor and improve such delivery systems to assure greatest impact. If the scale-up of novel diagnostic devices can be accompanied by the simultaneous introduction of up-to-date quality indicators and technical connectivity solutions, the vast amount of data generated by this new generation of automates could both simplify and potentiate the global response to the TB epidemic.

On a national and global level, as the quantity of information produced following the introduction of new-generation laboratory instruments was not anticipated, no plans were in place on how to manage the information flow or orient it in such a way that it could generate an evolution in the organisation of the epidemic response. In the absence of adequate laboratory information technology infrastructure, complemented by standardised reporting solutions for screening activities and treatment follow-up, many low-resource countries have continued to use slow, error-prone paper-based recording systems. In such systems, editing and transmission of paper reports cause inherent delays and contribute to the cost, complexity and relative inaccuracy of data interpretation.

Diagnostic e-health solutions have the potential to help overcome some of these problems and maximise the patient and public health impact following the introduction of a particular technology. The combination of this unprecedented evolution of the laboratory landscape and the potential of e-health could be leveraged to generate the revolution in national and global health delivery systems that is needed to achieve TB elimination. Pragmatically, this requires device connectivity, whereby secure testing

data and results are automatically sent to repositories, translated into useful information and channelled to appropriate parties. Although device connectivity within other industries has been routine for some time, within the health care community it is still largely in its infancy.⁶

In this paper, we discuss lessons learned from the global scale-up of the first generation of easy-to-connect diagnostic tools⁷ and the pathway to tapping the potential of connectivity in the field of TB diagnostics.⁸

EXPERIENCE FROM FIRST-GENERATION CONNECTED DIAGNOSTICS: THE EXAMPLE OF XPERT

During the last decade, several diagnostic companies, such as Cepheid Inc and Alere Inc, have begun developing a new generation of tests essential to fight diseases of poverty such as TB and HIV, with significant support from public and philanthropic funders, including the National Institutes of Health (Bethesda, MD, USA) and the Bill & Melinda Gates Foundation (Seattle, WA, USA).

The Xpert assay, which is run on the GeneXpert platform, was the first truly game-changing test to come out of this research, and it has since been widely distributed in health facilities with limited human and infrastructure resources. The coverage of Xpert varies considerably between countries, with some countries still having only a limited number of machines based in reference laboratories, and others, such as South Africa, that realised the advantages of implementing this novel platform as a first-line test fairly rapidly.⁹ In the last 5 years, more than 13 million Xpert tests have been procured worldwide. When GeneXpert was rolled out in 2010, the instrument had no built-in connectivity outside basic standards, and the TB community did not have the software tools to connect to GeneXpert machines and use the data being generated to its full capacity. Valuable information housed in the hard drives of local computers was thus never used to inform surveillance efforts or health care providers, and was largely lost.

In the light of this issue, national TB programmes (NTPs) called for tools to reduce loss to follow-up and improve device and laboratory management, including a better ability to maintain cartridge supplies and local redistribution, and evaluate and fulfil the training needs of device operators and laboratory technicians. Likewise, NTPs voiced a need for connectivity systems that could relieve the high overhead costs of data aggregation and analysis, which hamstringing the process of collecting raw data and turning it into useful information.

In 2012, responding to this critical gap in the implementation landscape, innovative third-party players developed connectivity solutions. GxAlert

(ABT, Cambridge, MA, USA, and SysmOne, Horsforth, UK), XpertSMS (Interactive Research and Development, Karachi, Pakistan, and TB REACH, Geneva, Switzerland) and GenXchange (Université Catholique de Louvain, Louvain, Belgium, and the NTP, Kinshasa, Democratic Republic of Congo) were devised to respond to the needs of low-resource countries, where internet is often unavailable or unreliable and laboratory information systems or electronic medical records are not widely used. These tools offered immediate solutions and, in response to national requests, hundreds of local laboratories have since become interconnected on implementing these systems. The scaling of these connectivity solutions has been taken back by dedicated companies (Global Connectivity, Somerville, MA, USA. <http://www.globalconnectivity.co/>; and Savics, Brussels, Belgium. <http://www.savics.org/>).

Cepheid, the manufacturer of GeneXpert, also worked to enable remote monitoring of their devices in response to expressed national needs and requests from the TB community. Like many developers, Cepheid lacked comprehensive information about what use-cases needed to be supported, and for ethical and regulatory reasons they prioritised data security and confidentiality. As a result, the company launched an initial software tool that was a step forward but was unable to fulfil all NTP needs.

In response, an alliance of key implementation partners, such as USAID (Washington DC, USA), MSF (Paris, France), Clinton Health Access Initiative (Boston, MA, USA) and Foundation for Innovative New Diagnostics (FIND) (Geneva, Switzerland) and donors, such as UNITAID (Geneva, Switzerland) and the Global Fund (Geneva, Switzerland), was formed, led by the World Health Organization (WHO), to work with Cepheid in ensuring secure, open access to critical data and finding a broader, holistic approach to connectivity and data management. An immediate solution was found, and both Cepheid and the alliance remain interested in the creation of a non-proprietary, long-term connectivity platform or a series of integrated and inter-operational platforms. This highlights how the global TB community can collectively define priority needs and work with manufacturers to negotiate and realise solutions for accessing and utilising key data.

Another important lesson from the implementation of first-generation connected diagnostics is the importance of a well-tailored delivery pathway for connectivity software that supports sustainable uptake in a given country. For example, Alere, the manufacturer of Pima™ CD4, devised a country-based public-private partnership model to ensure appropriate training and support for their connectivity software. Without this support and engagement of key stakeholders, many countries would have struggled to make use of the influx of data. While the tool

itself has limited wider applicability because of the proprietary nature of the software, the partnership model offers a valuable example of how non-proprietary, interoperable systems could be disseminated and nurtured in the future.

CONNECTIVITY OF DIAGNOSTICS: A SHARED RESPONSIBILITY AND PUBLIC HEALTH NECESSITY

The WHO and research funding agencies have been advocating for, and implementing, data-sharing policies for some time. While these efforts have increased access to synthesised research data, efforts to make NTP data available are in their infancy. The use of new-generation diagnostic platforms has triggered thinking about the potential utility of real-time analysis of national data, and how diagnostic connectivity could further improve epidemiological surveillance and guide targeted public health responses. Accelerated TB elimination, for example, as called for in the WHO End TB strategy,¹⁰ can only be realised if case detection, individual patient management and epidemiological surveillance are intensified simultaneously, and if these efforts are closely monitored and validated. Data generated by Xpert testing can be used both to improve patient management and treatment efforts and to provide important population-level information on average infectiousness as a predictor for TB burden¹¹ and spread of new mutations. This requires optimised programmatic data management, pooling, sharing, analysis and use. To realise improvements in surveillance and public health demands, the information generated by diagnostic technologies in programme conditions should be easily accessible and usable for national programmes. Ultimately, data access, enabled by diagnostic connectivity, should be seen as a public health good. Countries, international organisations, test developers and civil society organisations have a collective responsibility to work together to ensure sustainable use of information and communications technology to improve health care. In doing so, important questions regarding ethical obligations and data ownership and stakeholder interests, such as market competitiveness, need to be acknowledged and addressed. International collaborative efforts must furthermore address the issue of personal unique identifiers in a context of continuous human migration and data mobility.

THE WAY FORWARD: REALISING THE POTENTIAL OF CONNECTED DIAGNOSTICS

Built-in connectivity has become an evident prerequisite for upcoming diagnostic platforms.¹² Tests that until recently were un-connectable, such as rapid diagnostic tests for, for example, HIV and malaria,

can now be connected to digital readers, with collection of results, storage and transfer (e.g., Fio Corp, Toronto, ON, Canada).

In the field of TB diagnostics, a wide range of complementary laboratory tests are used. This includes rapid diagnostic tests and more conventional approaches such as microscopy, culture, drug susceptibility testing and sequencing.¹³ Inter-connecting these diagnostic devices and further integrating this information with clinical indicators is the upcoming challenge for the TB community.

The Connected Diagnostics Initiative (CDx), coordinated by FIND, is an example of a potential solution for accelerating the connectivity and interoperability of diagnostic devices. CDx is providing an open-source software platform that allows centralised aggregation of data from diagnostics, regardless of the manufacturer. For this new effort to succeed, wide buy-in from implementers, policy makers and developers will be essential. In parallel, FIND is working with the WHO towards guidelines for standardised result reporting for diagnostic devices and assisting developers to be compliant with these standards. These efforts go hand in hand with further deployment of local laboratory information systems and electronic medical records.¹⁴

Alongside this initiative, various groups are creating global databases with the intention of enhancing research and development applications for data. For example, genTB (Harvard University, Cambridge, MA, USA) is an open-source platform that allows for the pooling, analysis and visualisation of genetic, epidemiological and clinical data. A global partnership, including the WHO, the US Centers for Disease Control and Prevention (Atlanta, GA, USA), the Center for Policy Analysis on Trade and Health (San Francisco, CA, USA), Stop TB (Geneva, Switzerland), the National Institute of Allergy and Infectious Diseases (Bethesda, MD, USA) and FIND, has been established to develop a data platform (ReSeqTB) to store, curate and provide access to globally representative TB data that can inform the development of new diagnostics, facilitate clinical decisions and improve surveillance of drug resistance. While opportunities for sharing information at an international scale must be promoted, countries must also be provided with technical solutions that can support them in efficiently managing with whom, and for what purposes, national data are shared, and to ensure that these database efforts ultimately benefit patients.

Consensus is forming around the central role that connected diagnostics and digitisation can play in tackling health systems weaknesses and diseases of poverty. However, the global health community must also address the complex question of how new tools and practices can be implemented effectively in health systems. Substantial programmatic changes will be

required in the countries to absorb the innovation of connectivity and capture its benefits. This demands a holistic approach to cultivating effective development and adoption of new diagnostic tools. In this context, laboratory connectivity may also serve the need for more efficient post-marketing surveillance of newly rolled out diagnostics, for both national stakeholders and their global partners. As the amount of information collected will rapidly increase beyond our conventional capacities of analysis, the global health community will also need to initiate and intensify innovative collaboration to exploit the data collected using big data analysis and self-learning algorithms. Managing, visualising and analysing big data creates challenges beyond the capacities of standard statistical methods, and thus generates an increasing demand for data science and multidisciplinary efforts.

CONCLUSION

Our common goal of TB elimination is longer a dream: it is an achievable objective, with clear milestones.^{15,16} The elimination effort will require strengthened collaboration between information technology and big data specialists, social medicine and private companies.⁶

In the future, all diagnostic technologies should be interconnected, allowing data generated by laboratories to be merged in a common repository while safeguarding patient confidentiality. The TB community could use such a repository to monitor progress and identify problems and potential solutions at both patient and global levels. Data pooling will open up opportunities to comprehend the rapid evolution of drug-resistant mutations, which will aid in selecting cost-effective treatment schemes and improving patient management. With the many solutions it can provide, data pooling at an international level is an absolute priority, as it will accelerate progress in critical sectors, including patient care, epidemiological surveillance and operational response. As an international health emergency, the TB epidemic requires optimal international collaboration and unambiguous political commitment for intensifying data-sharing efforts.

Conflicts of interest: none declared.

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5.3. Quality Management System

In chapter five, we demonstrated how sub-optimal equipment and reagents could alter the impact of diagnostic tools. We also showed how computer-analysis of large database of test results could identify technical issues that undetected at the laboratory level.

The international standards for laboratories require to monitor not only the quality of equipment and stock management, but also to follow the skills of the staff and to perform regular quality controls. All these elements require a considerable expertise that is not available in peripheral laboratories. In the future, connectivity systems should include these services.

As an illustration, we could validate the DataToCare© (Savics, Belgium) connectivity system for reporting external quality controls in Rwanda. In the future, coordinating bodies will be able to send quality control samples to a multitude of laboratories around the world, and these will be able to report and compare their performance through the connectivity solutions.

Connectivity could further be used a mean to understand discrepant results reported from different laboratory methods. As an example, one would expect that when a RIF-resistant sample receives a confirmatory DST, the latter would report a RIF resistant results. In case the DST reports a RIF sensitive result, there are at least three different possible explanations: first, the laboratory has not tested the right sample. Second, the reference laboratory has had an undetected technical problem. Third, the strain presented a low-level resistance mutation that cannot be detected by conventional DST methods. To exclude the third explanation, one could analyse the raw rapid diagnostic test results. Considering that only a few well-documented mutations are responsible for low-level *in vitro* resistance (Leu430Pro, Asp435Tyr, His445Leu and His445Asn), one could check if the raw test data are compatible with such possibility.

- Leu430Pro can be suggested when
 - Xpert MTB/RIF detects RIF resistance based on a negative Probe A
 - Hain MTBDRplus detects RIF resistance based on a negative WT 2 and/or WT3 bands
 - Nipro detects RIF resistance based on a negative S1 band
- Asp435Tyr can be suggested when
 - Xpert MTB/RIF detects RIF resistance based on a delayed Probe B

- Hain MTBDRplus detects RIF resistance based on a negative WT 3 and WT4 bands, in the absence of a MUT1 band
- Nipro detects RIF resistance based on a negative S2 band, in the absence of a R2 band
- His445Leu and His445Asn can be suggested when
 - Hain MTBDRplus detects RIF resistance based on a negative WT7, in the absence of a MUT2B band,
 - Nipro detects RIF resistance based on a negative S4 band, in the absence of a R4b or R4a band
- Leu452Pro can be suggested when
 - Xpert MTB/RIF detects RIF resistance based on a delayed Probe E
 - Hain MTBDRplus detects RIF resistance based on a negative WT8, in the absence of a MUT3 band,
 - Nipro detects RIF resistance based on a negative S5 band, in the absence of a R5 band

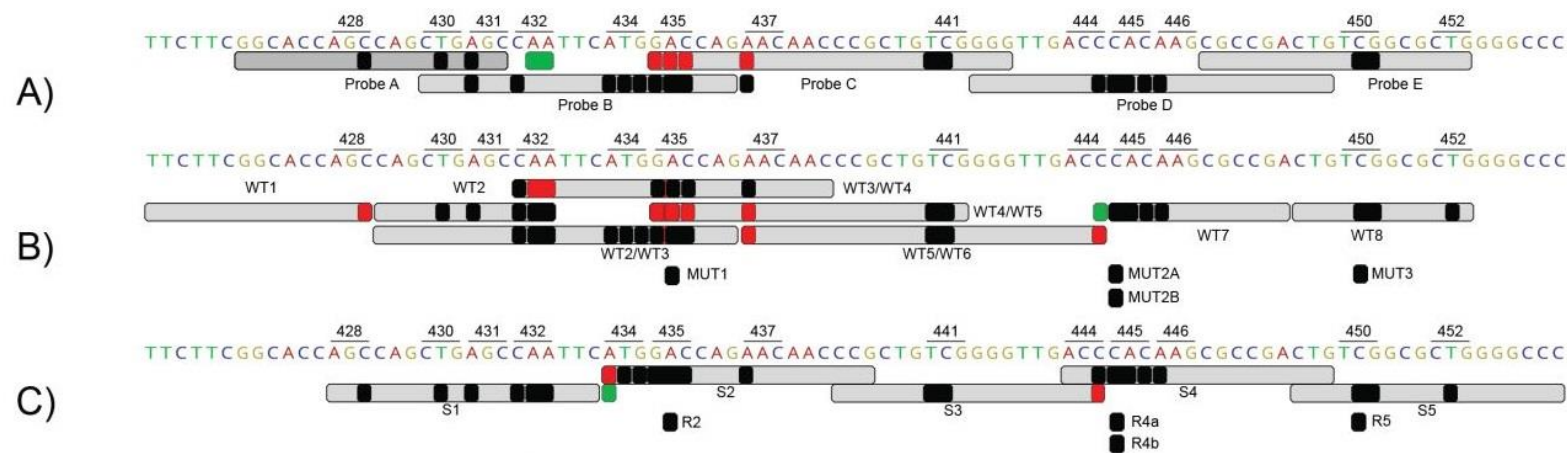


Figure 5.1: Overview of RIF resistance mutations captured using (A) Xpert MTB/RIF, (B) MTBDRplus and (C) Genoscholar TMNTM+MDRTB II. This representation should allow reference laboratories and automated analysis software to enhance the possibility to track RIF-resistant TB transmission using raw data generated by rapid molecular tests. (Source: Kamela Ng, 2017. Unpublished)

5.4. Electronic medical record systems

Early laboratory connectivity solutions were initially limited to the GeneXpert technology and the field of TB. Rapidly, WHO and the end-users of these solutions highlighted the necessity to extend the scope of test results captured to LPA, culture and DST results, but also to other disease. Until recently, the main technical issue for extending the scope of connectivity solutions was their inability to develop or use unique patient identification systems (143).

We recently validated in Rwanda the DataToCare (Savics, Belgium) connectivity system for integrating different biological and clinical information into a patient-specific electronic medical record system. In the future, this technology should allow palliating to the absence of medical record systems and facilitating the remote monitoring of cohorts and patients by experts.

Further field validation will be required to verify the usability and impact of such innovation (144, 145).

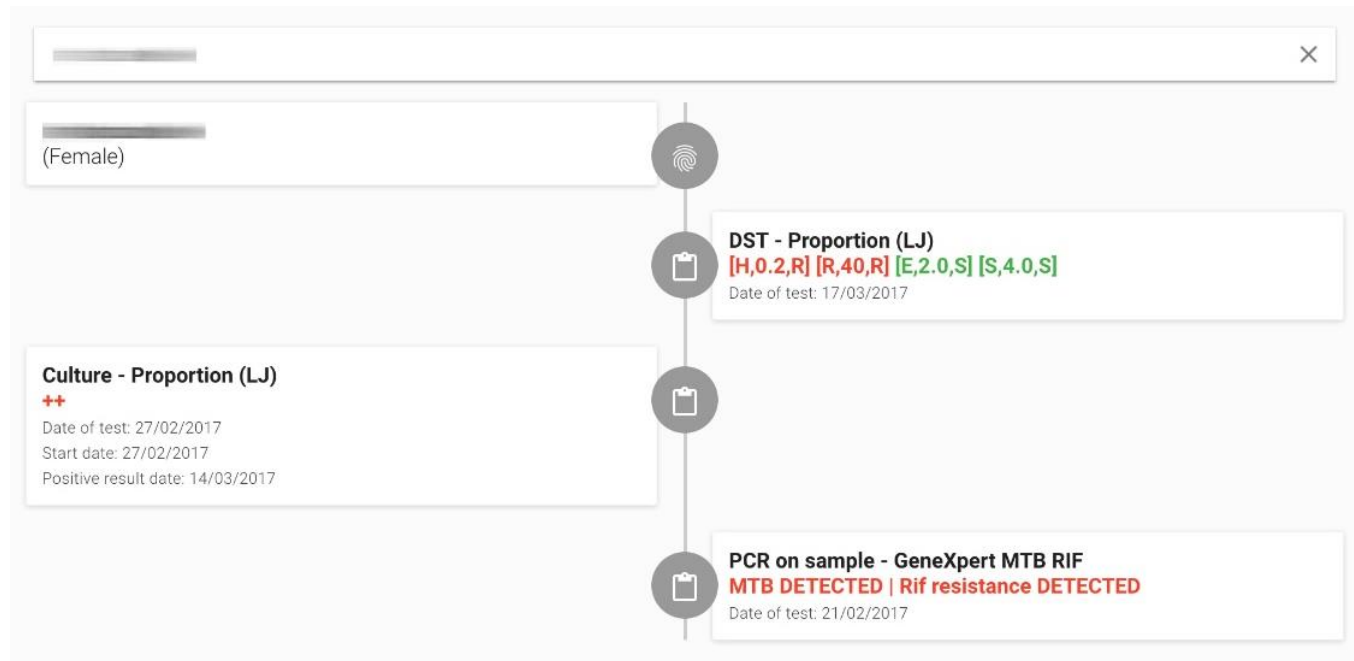


Figure 5.2: Illustration of the DataToCare (Savics, Belgium) Electronic Medical Record system. Different tests performed at different times in different laboratories can be automatically merged into a same electronic medical record system. The concentration of all medical records in national servers should allow experts to monitor patients who escape the “normal” process of diagnosis and follow-up or who require adapted therapy.

5.5. Disease surveillance

Traditionally, disease surveillance consists of monitoring the evolution of epidemic and the emergence of clusters of transmission. In the chapters above, we understood that the surveillance of the TB epidemic should be much more sophisticated if surveillance is to be utilized as a mean to rapidly implement appropriate and targeted interventions.

5.5.1. Collection and utilization of laboratory test results

Nowadays, diagnostic test results are collected instantaneously from a multitude of laboratories around the world. The number of positive tests and the positivity rate can therefore be mapped in real-time and reflect both the burden of disease and the efforts implemented to actively screen TB in each setting.

Since several years, the connectivity tools are able to collect additional information communicated by the clinicians when prescribing a laboratory test. This information includes the reason for testing (diagnostic or treatment follow-up), the type of sample (pulmonary or extra-pulmonary), HIV coinfection or the duration of symptoms prior to diagnosis. To date, this information has been anecdotally exploited as a mean to monitor the determinants of the TB and drug-resistant TB epidemic in each particular setting.

Today, connectivity solutions such as DataToCare® have become more robust and can serve additional applications such as treatment follow-up and treatment outcome. We believe that these applications will be widely adopted by clinicians and will allow to extensively monitoring the quality of medical care, adoption of innovations⁽¹⁴⁶⁾ and determinants of the TB epidemic.

5.5.2. Monitoring of transmission

General principle

Transmission is the major vector of drug resistant TB. Preventing transmission requires appropriate infection control measures and laboratory methods allowing to suspect or, at the contrary exclude that two patients are infected with the same strain. The degree of genetic similarity between different strains can be determined by different methods.

Most genetic variations occur in non-coding segments of the bacterial DNA, as these do not alter the viability of the bacteria. Spoligotyping, MIRU-VNTR or IS6110-RFLP methods are based on this principle (103, 147). More recently, whole genome sequencing (WGS) followed by analysis of single nucleotide polymorphism (SNP) has been used as a promising tool (148). WGS further captures variants associated to drug-resistance, being a considerable advantage compared to other typing methods that do not provide information directly useful for patient management. NGS-based methods including WGS and commercial solutions (e.g. Deeplex MycTB, Genoscreen, France) are currently being evaluated for large-scale deployment. Recently, the UK has implemented the utilisation of WGS for all TB cases.

If these complex test results can be collected, transferred and analysed through standardized pipelines such as the ones already available for detecting drug resistance variants (e.g. ReSeqTB), these sequences could be used to reconstruct in real-time the dynamic of TB transmission in a population. Using appropriate SNP analysis, not only clusters will be identified but also potentially the direction of transmission between patients. This would offer game-changing applications for better understanding the epidemic and supporting the elimination of TB at the international level.

Utilisation of rapid diagnostic tests for disease surveillance

Commercial rapid diagnostic tests (RDTs) detect drug resistance by revealing the occurrence of resistance-associated mutations in targeted genes. In some genes such as *katG* (associated with high level INH resistance), resistance-associated mutations only occur in specific positions of the genome. For other genes such as *rpoB* (121), *pncA* (149) or *gidB* (150, 151), resistance occurs following the appearance of mutations on various positions within the gene, and diversity could therefore be used as a surrogate marker of transmission.

We evaluated the potential of different WHO-endorsed RDTs for potential application as surveillance tools for rifampicin resistant TB transmission. 108 non-clustered RR-TB strains representing 35 different *rpoB* mutations, including 32 located within the 81 bp hotspot, were selected from the Belgian Coordinated Collections of Microorganisms hosted at the ITM. These strains were subjected to Xpert MTB/RIF (Cepheid), MTBDR_{plus}v2.0 (Hain), and GenoscholarTMNTM+MDRTB II (Nipro).

Raw data of each RDT were evaluated to assess their ability to discriminate different mutant sequence profiles. Sufficient discriminatory power, reproducibility, and concordance of RDTs support the validity of potential use of RDT raw data as a first line tool for suspecting RR-TB transmission. For definitive confirmation of transmission, complementary testing with well-validated methods will remain necessary due to the inherent imperfect discriminatory power of these techniques.

Anticipated advantages of new generation RDTs

The Xpert MTB/RIF Ultra (Cepheid, USA) was initially announced to be released in 2017. This new tests could offer additional potential in regard to disease surveillance because the melt-curve technology¹⁷ used in this assay allows improving the precision in regard to the nucleotide variations (cfr 4.3.3). The validation studies published to date¹⁸ suggest that the melting temperature of each probe are differently altered depending on the mutation. This could increase the discriminatory power when compared to the previous Xpert MTB/RIF generation of tests.

The Xpert XDR (Cepheid, USA) and the different LPA assays are detecting mutations in the *rpoB*, *katG*, *inhA*, *pncA* and other genes associated with second line drug resistance mechanisms. These comprehensive resistance profiles could be used as a reflect of genetic variations and therefore as a first-line molecular epidemiology tool allowing to suspect transmission of TB between different patients (152).

¹⁷ https://www.finddx.org/wp-content/uploads/2016/10/9th-Symposium-2016-01_Denkinger.pdf

¹⁸ <http://www.croiconference.org/sessions/xpert-mtbrif-ultra-new-near-patient-tb-test-sensitivity-equal-culture>

Critical discussion

In 2015, more than 10 million people worldwide developed active TB disease. Nearly 2 million people died, more than HIV, Malaria and Ebola put together.

Four main characteristics can explain why the international public-health response has failed to eradicate the disease or efficiently control the epidemic for over 50 years.

- Globally, one third of people with active TB remain undiagnosed. In some areas of Africa, this proportion climbs to 75%.
- Most of the people affected by TB are poor and live in poor communities, under-served by high-quality medical care
- Each year, nearly half a million people are made ill by multidrug-resistant TB. Only a very small minority of these people will receive appropriate diagnosis and treatment
- The response to the TB epidemic is largely insufficient due to the lack of political commitment and funding, but also due to the absence of efficient tools to guide and monitor large scale interventions.

High conservatism of the medical community

Our experience working with National TB Programs illustrates how complex (but feasible) it can be in some cases to implement innovative interventions, even when these are supported by clear guidelines and dedicated funding. During our experience, we observed a high conservatism and, in some cases acute financial interest of people having higher responsibilities within the health system.

In this aspect, the effectiveness of a National TB Program organization can be highly dependent on the people who lead the system, and this has a direct impact on the health of people affected by TB.

Role of international stakeholders

Although international stakeholders, including the funders and technical partners should not aim to replace the national dynamic when it is present, our impression is that these should act more responsibly and actively when clear programmatic failures appear and are not resolved with time and external funding.

In such “blockage” situation, particularly when domestic funding level is very low, illustrating low political commitment, the role of international stakeholders should be to push ambitious and “mentality-changing” interventions. Unfortunately, in some cases, we also found that some technical advisors hired by international donors or organizations fail to provoke the real change demanded by the funders and contribute to a steady state of failing systems.

Mining, TB and corruption

It is well known that mining is a major factor contributing to TB in Africa.

In the case of DRC, one of the world’s richest countries with regard to mining resources, it is revolting to see how financial resources have been diverted towards enrichment of the ruling people and maintenance of a corrupted system rather than serving the interest of populations. It is even more revolting to see that the country utilizes less than two million USD of its domestic resources¹⁹ per year while the extent of corruption in the mining sector alone is estimated to be over 250 million USD per year²⁰ (figure 6.1).

¹⁹

https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=CD&outtype=html

²⁰ <http://www.bbc.com/news/world-africa-40680795>

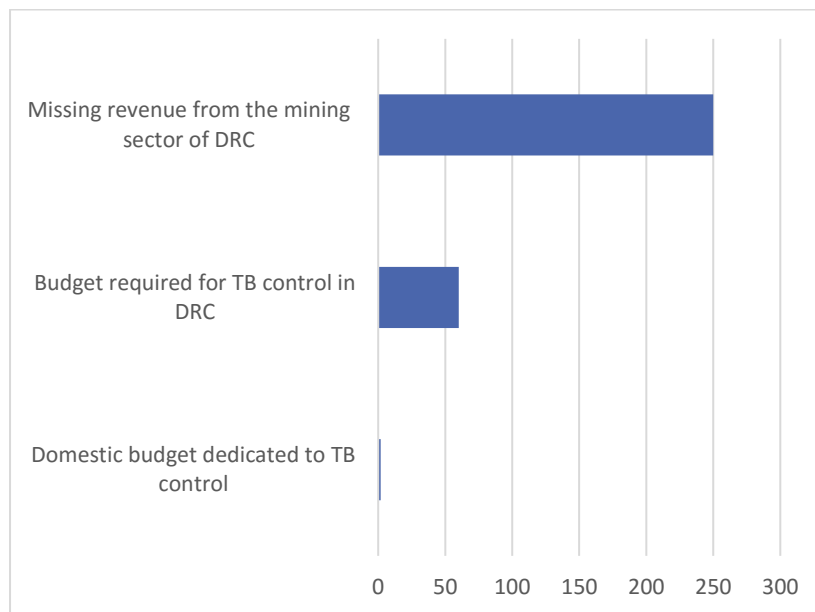


Figure 6.1: Extent of corruption in the mining sector of DRC and budget required for TB control (Year 2015, million USD per year).

The change will come from the TB affected communities

Our experience largely discussed in this thesis showed how people directly affected by TB and their communities can take an active responsibility in the fight against the epidemic. In this work, we demonstrated how they can organize themselves and reach highly impressive qualitative and quantitative results.

We further believe that, in communities and countries hardly affected by the TB epidemic, the civil society needs to take more responsibilities with regard to how the TB strategies are planned, budgeted and executed. Unfortunately, the capabilities of these people are still often underestimated or under-recognized by a TB community which is driven by the hierarchy of traditional health systems.

In many cases, doctors and nurses do not live in the communities where most of the TB-affected populations live. As a result, their understanding of the socio-economic repercussions is very limited (or absent), and their expectancies towards the patients (ie coming each day to the clinic to take the medications) can be unrealistic. When these health professionals will accept the important role that TB affected people can play, we believe that this will have a direct impact not only

on TB detection, but also on stigma, economic burden for patients and treatment outcome.

The impact of new technologies

The introduction of new diagnostic technologies such as the Xpert MTB/RIF test have not achieved the impact expected with regard to TB detection or TB control, apart from when this technology was well integrated with coordinated screening strategies. While this test has importantly increased the detection of RIF-resistant TB, its utility is limited by the fact that it does not provide more information for other first- or second-line drugs, particularly in a country like DRC where less than 50% of RIF resistant TB is associated with INH resistance. We further demonstrated in this work how the intensive use of this technology in South-Africa may have led to the selection of MDR-TB strains that are undetected by this test and other commercial solutions currently available.

During this work, we had the opportunity to test a new commercial NGS test (Deeplex MycTB, Genoscreen). Considering the results obtained, we believe that this test will constitute the next revolution for TB diagnostics. Nevertheless, the scale-up of this test (particularly in areas with high rates of drug resistance) will require improved automation.

In the future, TB control will be achieved when well-planned and coordinated screening strategies will be integrated with improved tools for people working in the communities and in the health centres (eg. Digital medical records, computed-assisted diagnosis, treatment decision support tools, ...). Connectivity solutions should allow real time collection information constantly generated by the field workers, and artificial intelligence methodologies should now be used to analyse and use this information so that it can have a direct impact on individual care and disease control.

A strategy for TB control in DRC?

There are many reasons that make us think that TB control can be achieved in DRC.

- First, TB is a curable disease and we showed in this document that drug resistance and adverse treatment outcomes are still not major problems in this country
- Second, the health system of DRC is already in place and is particularly well structured, providing health services close to the patient, while referral of complex patients or biological samples can be achieved
- Third, we could show that patients and communities could effectively complement the health system in areas and communities where it is absent.
- Fourth, we could show that adapted IT systems which can manage both medical and logistical information can be developed and scaled-up at national level.
- Last, we showed that the very poor laboratory system in place, relying on an outdated vision of mycobacteriology, could be replaced by innovative tools adapted for first-line diagnosis, tertiary hospitals and disease surveillance.

In this context, we are convinced that an effective commitment from the political and economical rulers of the country should allow disruptive change to be provoked. As this commitment will be attentive to the cost-effectiveness of its interventions, we expect that the misuse of resources by the health system will decrease to an acceptable level and increase the impact of the strategies. To date, NGOs supporting the health system do not currently have the human or technical capacities to palliate to the programmatic gaps or push new approaches, nor have the mandate to do so. We nevertheless believe that original collaborations with organizations capable of bringing to scale very large TB screening programs could achieve rapid results.

In the short term, we recommend that the mining sector of DRC should fund an ambitious “Zero TB country” strategy, which will allow:

- Recruiting the skilled staff able to work with a clear mandate approved by the ministry of health.

- Implementing the technical tools (IT systems, diagnostic tools, ...) in all places where it would be justified
- Partnering with local clinics and communities, and provide these with a reasonable payment for the work performed
- Transparently reporting the spending and the impact associated with their intervention

Conclusion and perspectives

To date, the international community has failed to imagine and implement sufficient solutions to stop the TB epidemic. Today, despite the fact that TB transmission and death can be prevented by the prompt initiation of highly effective treatments, the absence of a comprehensive approach for fighting TB in high-burden settings has led to alarming results (153). In 2016, over 10 million people were made ill by active TB disease. In 2015, 42% of patients who died of TB lived in Africa²¹.

TB mainly affects low-resource countries and particularly the people living in poverty. Considering this context and the market-driven logic of most innovation, countries like the Democratic Republic of Congo often have little other choice than to follow WHO guidelines and adopt the innovations financially supported by the Global Fund and aid agencies from developed countries rather than invest in innovations driven by local stakeholders based on context-specific demands, opportunities and constraints. During six years, with partners from different African countries, we explored the impact of end-user innovations as a complementary approach to generic TB control strategies.

In the first two chapters, we approached the major problem of TB under-detection. Taking into account that in many high TB burden countries, over 50% of patients with TB are believed to be undetected, improving access to care for these patients has high priority. We propose innovative re-analysis of standard notification report data that allows the level of PedTB under-detection to be predicted (67). We further demonstrated that people infected with TB and their relatives could be trained to find other patients with TB in their communities. We showed how these screeners allowed over 650.000 people living in hard-to-reach communities to be screened for TB. We further highlighted that these patients progressively improved their efficiency during the intervention.

In chapters three and four, we described microbiological and technical issues associated with the large-scale introduction of the

²¹ <http://www.tbfacts.org/tb-statistics/>

Xpert MTB/RIF assay. In addition to the operational aspects associated with the utilization of this tool in rural African settings, we discuss the limits of this test for TB and drug resistance detection. We showed how large-scale utilization of Xpert MTB/RIF led to the selection and spread of multi-drug resistant MTBc strains that are not detected by this test. We further demonstrated how re-analysis of raw test results collected through laboratory connectivity solutions allowed improved detection of NTM, TB and MDR-TB, compared to the built-in software of GeneXpert (117, 133).

In chapter five, we discuss how laboratory connectivity has become standard in low-resource settings, and focus on the efforts still to be made to transform these technical tools into potent medical breakthroughs. We illustrate how connectivity software developed in Africa has created the foundation for a new generation electronic medical records and surveillance systems.

During the last decade, there have been many demonstrations that market-driven innovations alone fail to improve the health of populations (146, 154, 155). Ending the TB epidemic will also require opportunities for those affected by TB to be creators as well as recipients of TB control interventions. We demonstrated how end-user innovations are valuable for optimizing the accessibility and quality of care, and for deploying international TB strategies in difficult contexts.

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Figure 3.11: Mechanism of selection by MGIT-based DST (A) and Xpert MTB/RIF (B) of strains harbouring undetected RIF-resistance mechanisms. The fact that MDR-TB and INH resistance are ruled-out based on a “RIF sensitive” test result contributes to the increased epidemic potential of these strains.

Figure 3.12: Screening results for 277 MTB strains reported as INH mono-resistant using current programmatic algorithms in South-Africa. A total of 37 strains were found to harbour the Ile491Phe *rpoB* mutation.

Figure 3.13: Origin of 37 MDR-TB strains previously reported as INH mono-resistant by current diagnostic algorithms and presenting the *rpoB* Ile491Phe mutation.

Figure 3.14: Hypothetical explanation for the emergence of *rpoB* Ile491Phe-associated MDR-TB outbreaks in South-Africa.

Figure 4.1: Installing GeneXpert machines in 10 different cities of the South-Kivu province required to ship these automates across the province. In many cases, there were no roads allowing travelling from Bukavu to these cities. (Photo: Crossing a river on the way from Bukavu to Baraka with a GeneXpert machine and Xpert MTB/RIF cartridges in the back of the car)

Figure 4.2: Proportion of failed Xpert MTB/RIF tests among early users of the technology from nine different countries (Modified from Creswell et al, 2015)

Figure 4.3: Illustration of the electrical installation required for using the GeneXpert in the Shabunda hospital. From the left to the right: battery of the solar panel installation, charger-converter, truck battery, stabilizer, UPS, laptop with internal battery and GeneXpert).

Figure 4.4: Utilisation and error rate of GeneXpert instruments (vertical axis) and their respective analytical modules (horizontal axis) in the South-Kivu province. The size of the circles represent the number of tests performed, and the colour represents the proportion of failed tests (error rate). This figure illustrates that high error rates do not prevent a massive utilization of failing analytical modules (e.g. instrument 803384, module 632793). Furthermore, it appears that failing analytical modules appear in nearly all laboratories. (Source: E. Renard and E. André, 2015)

Figure 4.5: Evolution of the proportion of failed tests between the modules that have later been identified as deficient due to a high mean error rate in the retrospective analysis (red) and those with a mean error rate < 10% (blue). Number of days of utilization are represented

in the horizontal axis. It appears that deficient modules have already present signs of higher error rates from the first month of utilization.

Figure 4.6: Evolution of the utilisation of modules. Deficient modules are represented in red and those with a mean error rate <10% are represented in blue. Number of days of utilization are represented in the horizontal axis. It appears that it end-users can continue using deficient modules for more than 6 months without self-reporting a technical issue.

Figure 4.7: Distribution of Cycle threshold (Ct) values obtained for the internal SPC control of the Xpert MTB/RIF valid tests performed in the South-Kivu province. (Source: C. Vande Kerckhove and E. André, 2015). The normal distribution observed suggests a satisfactory stability of reagents despite un-controlled storage conditions.

Figure 4.8: Mean percentage of failed tests per Xpert MTB/RIF reagent lots and number of samples analysed with cartridges of each lot.

Figure 4.9: Evolution of the number and proportion of valid (orange) and invalid (red) Xpert MTB/RIF test results over de time in the South-Kivu province of DRC. In total, 2.047/19.335 (10,6%) tests were not associated with a valid result. Most of these invalid tests were associated with the utilization of failing analytical modules of the Genexpert automates.

Figure 4.10: Utilization and yield of Xpert MTB/RIF in the South-Kivu province of DRC for the detection of TB among patients suspected of having TB despite a first negative smear result

Figure 4.11: Trends in positivity rate of Xpert MTB/RIF tests performed in the South-Kivu province of DRC among patients suspected of having TB despite a first negative smear result.

Figure 4.12: Percentage of RIF-resistant TB samples in the South-Kivu province of DRC

Figure 4.13: In the South-Kivu province, samples detected as RIF-resistant TB have to be transported from the peripheral health facilities to Kinshasa. After arriving in Bukavu, samples are transferred to the national reference laboratory located at over 2,000 km in Kinsahsa. The process of sample transportation generally takes several weeks. (Photo: Jean-Paul Chirambiza, biologist at the provincial TB program ini Bukavu, holding a package with RIF-resistant samples to be sent to Kinshasa)

Figure 4.14: Bacteriological confirmation of RIF-R cases detected by Xpert MTB/RIF in the South-Kivu province of DRC between 2012 and 2016. The implementation of an LPA laboratory at the Provincial level allowed a 350% increase in the number of cases confirmed with MDR-TB over the evaluation period.

Figure 4.15: Data capture proposed by the GenXchange software (2013 version) following a positive Xpert MTB/RIF test results. This screen automatically pops-up on the screen of the GeneXpert computer when a test is finalized. Following the capture of this information, the data are transferred to a central database together with the raw Xpert MTB/RIF test results.

Figure 4.16: Proposed sputum collection procedure for the national drug resistance survey to overcome the technical challenges. This system allowed solving most barriers preventing this study to be operationalized.

Figure 4.17: Performance of the Deeplex MycTB assay based on the microscopic examination grading. (n=300). Source: Genoscreen

Figure 4.18: Overview of information generated for each test by the Deeplex MycTB assay (Genoscreen, France)

Figure 4.19: Resistance profile of 79 samples from the national drug resistance survey in DRC presenting at least one resistance-associated mutation. The results were obtained with the Deeplex MycTB assay (Genoscreen, France).

Figure 4.20: Frequency of hetero-resistance among samples presenting a resistance mutation. The results were obtained with the Deeplex MycTB assay (Genoscreen, France).

Figure 4.21: Drug resistance profile of the 29 RIF-resistant samples tested for the drug resistance survey in DRC

Figure 4.22: Drug resistance profile of the 46 INH-resistant samples tested for the drug resistance survey in DRC

Figure 5.1: Overview of RIF resistance mutations captured using (A) Xpert MTB/RIF, (B) MTBDRplus and (C) Genoscholar TMNTM+MDRTB II. This representation should allow reference laboratories and automated analysis software to enhance the possibility to track RIF-resistant TB transmission using raw data generated by rapid molecular tests. (Source: Kamela Ng, 2017. Unpublished)

Figure 5.2: Illustration of the DataToCare (Savics, Belgium) Electronic Medical Record system. Different tests performed at different times in different laboratories can be automatically merged into a same electronic medical record system. The concentration of all medical records in national servers should allow experts to monitor patients who escape the “normal” process of diagnosis and follow-up or who require adapted therapy.

Table 0.1: Modelled yield of different algorithms when screening 100 000 persons in a population with a 1% prevalence of culture positive pulmonary TB (1 000 cases). (It is assumed that the final diagnosis uses results from sputum-smear microscopy or the Xpert MTB/RIF test, and there is no further diagnostic evaluation) (Source: Systematic screening for active TB, WHO 2013)

Table 0.2: Weighted mean and range number needed to screen (NNS) to find one case of TB in selected risk categories. All screening approaches are aggregated within each risk group. NS: No study. VCTC: Voluntary counselling and testing for HIV. (Source: Systematic screening for active TB, WHO 2013)

Table 1.1: Budgeted expenses associated with the patient-driven ACF intervention. NA: not applicable; USD: United States Dollars

Table 1.2: Cost of the 3-year ACF intervention and cost-per new SS+PTB case found. NA: not applicable; USD: United States Dollars

Table 3.1: Melting temperature (T_m) profile for a panel of RIF-resistant TB strains tested with the Xpert MTB/RIF Ultra assay (Cepheid, USA). The T_m is modified in the presence of an underlying mutations (Source: Alland D et al, 2015). The data presented suggest that some specific changes in the same may alter differently the T_m (e.g. in position 531). This level of precision may be later exploited to suggest or exclude transmission of TB among patients.

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Annex:

NGS sequencing results of MDR-TB strains from South-Africa and Swaziland
harbouring the *rpoB* 491 mutation

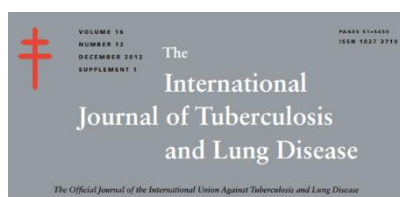
Country	Province	District	Local Municipality	Clinic	Country ID	UCL ID	Spoligotype International type (SIT)	Gene	AA Change	Drug resistance
RSA	North-West	Bojanala	Rutensburg	Job Shirmine Tabane Hospital	79	44-50	34	embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
RSA	North-West	Bojanala	Rutensburg	Thlabane PHC Clinic	274	43-7	34	tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								fabG1	K Lys-41-Q Gln	n/a
								fabG2	K Lys-41-M Met	n/a
								katG	S Ser-315-T Thr	INH
RSA	Gauteng	Tshwane	Tshwane	Schoongu T Clinic	277	44-7	34	pncA	H His-51-D Asp	PSA
								rpoB1	L Leu-452-Q Gln	n/a
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
RSA	Gauteng	Tshwane	Tshwane	Schoongu T Clinic	277	44-7	34	gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								rpoB1	F Phe-424-L Leu	n/a
								rpoB1	T Thr-427-I Ile	n/a
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
RSA	Gauteng	Tshwane	Tshwane	Karen Park Clinic	278	45-7	34	rpoB1	F Phe-424-L Leu	n/a
								rpoB1	T Thr-427-I Ile	n/a
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								Rv0678F	A Ala-124-E Glu	n/a
RSA	North-West	Bojanala	Rutensburg	Thlabane PHC Clinic	285	44-4	34	embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
Swaziland	DRS	DRS	DRS	DRS	4248-09	42-50	34	embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
Swaziland	DRS	DRS	DRS	DRS	4725-09	43-50	34	tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								fabG1	K Lys-41-Q Gln	n/a
								fabG2	K Lys-41-M Met	n/a
								katG	S Ser-315-T Thr	INH
RSA	North-West	Bojanala	Moses Kotane	Madikwe Clinic	216	11-R	34	pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
RSA	North-West	Bojanala	Rutensburg	Bapong Clinic	218	12-R	34	katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
RSA	North-West	Bojanala	Rutensburg	Boitekong Clinic	220	13-R	34	gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
RSA	Gauteng	Tshwane	Tshwane	Schoongu T Clinic	234	14-R	34	embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
RSA	North-West	Bojanala	Rutensburg	Boitekong Clinic	242	15-R	34	embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
RSA	North-West	Bojanala	Moses Kotane	Mabesraal Clinic	243	16-R	34	tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
RSA	North-West	Bojanala	Rutensburg	Thlabane PHC Clinic	245	17-R	34	rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								Rv0678	C Cys-464-R Arg	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
RSA	North-West	Bojanala	Rutensburg	Thlabane PHC Clinic	249	18-R	34	pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106
								gyrA	S Ser-95-T Thr	n/a
RSA	North-West	Bojanala	Rutensburg	Boitekong Clinic	251	19-R	34	katG	S Ser-315-T Thr	INH
								pncA	H His-51-D Asp	PSA
								rpoB1	I Ile-491-F Phe	SP
								rplL	K Lys-43-R Arg	SM
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-Ila	E106

Country	Province	District	Local Municipality	Clinic	Country ID	UCL ID		Spoligotype international type (BIT)	Gene	AA Change	Drug resistance
RSA	North-West	Bojanala	Rutensburg	Monakato PHC Clinic	42	1-R	34	fabD51	K Lys-41-Q Gln	n/a	
								fabD51	K Lys-41-M Met	n/a	
								fabD51	K Lys-41-N Asn	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
RSA	North-West	Bojanala	Rutensburg	Thabane PHC Clinic	290	22-R	34	katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
RSA	Gauteng	Tshwane	Tshwane	Soshanguve T Clinic	296	24-R	34	rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
RSA	North-West	Bojanala	Rutensburg	Monakato PHC Clinic	325	25-R	34	gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
RSA	North-West	Bojanala	Rutensburg	Botlokong Clinic	327	26-R	34	rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								Rv0678	A Ala-84-S Ser	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
RSA	North-West	Bojanala	Moses-Kotane	Mabesiraal Clinic	333	27-R	34	tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
RSA	North-West	Bojanala	Rutensburg	Botlokong Clinic	334	28-R	34	katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulhali Hospital	340	29-R	34	rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
RSA	North-West	Bojanala	Moses-Kotane	Mabesiraal Clinic	34	2-R	34	gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
Swaziland	DRS	DRS	DRS	DRS	7175-09	31-R	34	rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								Rv0678	M Met-146-T Thr	n/a	
Swaziland	DRS	DRS	DRS	DRS	4298-09	32-R	34	embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
Swaziland	DRS	DRS	DRS	DRS	4721-09	33-R	34	phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								Rv0678	M Met-146-T Thr	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
Swaziland	DRS	DRS	DRS	DRS	5109-09	34-R	34	phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								Rv0678	M Met-146-T Thr	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
Swaziland	DRS	DRS	DRS	DRS	7185-09	35-R	34	rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								Rv0678	M Met-146-T Thr	n/a	
Swaziland	DRS	DRS	DRS	DRS	10436-09	37-R	34	embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
								phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	
								embB	M Met-306-I Ile	n/a	
								gyrA	S Ser-95-T Thr	n/a	
								katG	S Ser-315-T Thr	INH	
Swaziland	DRS	DRS	DRS	DRS	4700-09	38-R	34	phoA	H His-51-D Asp	PZA	
								rpoB1	I Ile-491-F Phe	SM	
								rpsL	K Lys-43-R Arg	SM	
								tyrA	L Leu-11-L Leu	n/a	

Country	Province	District	Local Municipality	Clinic	Country ID	UCL ID	Spoligotype international type (BIT)	Gene	AA Change	Drug resistance
Swaziland	DRS	DRS	DRS	DRS	4723-09	39-R	34	tybA	L1eu-11-L1eu	n/a
								embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
tybA	L1eu-11-L1eu	n/a								
RSA	North-West	Bojanala	Rutensburg	Botetong Clinic	85	3-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
Swaziland	DRS	DRS	DRS	DRS	10438-09	40-R	34	gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
								Ru0678	M Met-146-T Thr	n/a
Swaziland	DRS	DRS	DRS	DRS	11023-09	41-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
Swaziland	DRS	DRS	DRS	DRS	4709-09	42-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
Swaziland	DRS	DRS	DRS	DRS	4724-09	43-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
Swaziland	DRS	DRS	DRS	DRS	6944-09	44-R	34	gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
								Ru0678	M Met-146-T Thr	n/a
Swaziland	DRS	DRS	DRS	DRS	10451-09	45-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Bojanala	Rutensburg	Botetong Clinic	88	4-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Bojanala	Rutensburg	Botetong Clinic	103	5-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Bojanala	Rutensburg	Job Shimane Tabane Hospital	112	6-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Ngodi	Ngodi	Ngodi Clinic	122	7-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Bojanala	Rutensburg	Karlens Park Clinic	198	8-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	Mpumalanga	Ngangala	Thembiile	Vaalbaags 2 Clinic	206	9-R	34	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	North-West	Bojanala	Rutensburg	Monakato FHC Clinic	93	45-50	92	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulharni Hospital	222	42-7	92	embB	M Met-306-I1a	DRG
								gyrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	W04
								pncA	H His-51-D Asp	PZA
								rpoB1	I1le-491-F Phe	SIF
								rpsL	K Lys-43-R Arg	DM
								tybA	L1eu-11-L1eu	n/a

Country	Province	District	Local Municipality	Clinic	Country ID	UCL ID	Spotlight type international type (SIT)	Gene	AA Changes	Drug resistance
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulhali Hospital	347	30-R	92	gtrA	S Ser-95-T Thr	n/a
								katG	S Ser-315-T Thr	INH
								pncA	n/a	PZA
								rpoB1	I Ile-491-F Phe	RF
								tyxA	L Leu-11-L Leu	n/a
RSA	Mpumalanga	Niangala	Thembiile	Vlaaiagte 1 Clinic	281	20-R	92	embB	M Met-306-I Ile	EMB
								gtrA	S Ser-95-T Thr	n/a
								gtrB	L Leu-91-R Arg	n/a
								gtrB	V Val-135-V Val	n/a
								gtrA	S Ser-95-T Thr	n/a
RSA	North-West	Bojanala	Rutensburg	Bafokeng PHC Clinic	288	23-R	156 (probable mix mutation)	katG	S Ser-315-T Thr	INH
								pncA	n/a	PZA
								rpoB1	I Ile-491-F Phe	RF
								tyxA	L Leu-11-L Leu	n/a
								embB	M Met-306-I Ile	EMB
RSA	North-West	Bojanala	Moses-Kotane	Madikwe Clinic	211	10-R	N/A (low test coverage)	gtrA	S Ser-95-T Thr	n/a
								pncA	H His-51-D Asp	PZA
								rpoB2	L Leu-314-L Leu	n/a
								rpoB1	I Ile-491-F Phe	RF
								rpoC1	I Lys-49-R Arg	RF
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulhali Hospital	291	23-R	N/A (low test coverage)	embB	M Met-306-I Ile	EMB
								gtrA	S Ser-95-T Thr	n/a
								gtrB	L Leu-91-R Arg	n/a
								gtrB	V Val-135-V Val	n/a
								gtrA	T Thr-145-K Lys	n/a
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulhali Hospital	291	23-R	N/A (low test coverage)	gtrA	S Ser-95-T Thr	n/a
								pncA	n/a	PZA
RSA	Gauteng	Tshwane	Tshwane	Dr Georges Mulhali Hospital	291	23-R	N/A (low test coverage)	rpoB1	I Ile-491-F Phe	RF
								rpoB1	I Ile-491-F Phe	RF

Conference paper #1 (2012): Alcohol-based conservation of sputum: quantitative and qualitative impact among referred samples from distant centres for GeneXpert MTB/RIF analysis



ABSTRACT BOOK

43rd World Conference
on Lung Health of the
International Union Against
Tuberculosis and Lung Disease (The Union)

KUALA LUMPUR - MALAYSIA
13-17 NOVEMBER 2012

PC-282-15 Alcohol-based conservation of sputum: quantitative and qualitative impact among referred samples from distant centres for GeneXpert® MTB/RIF analysis

E Andre,¹ J P Chirambiza,² R Nyota,² D Kalumuna.²
¹Microbiology, Université Catholique de Louvain, Brussels, Belgium; ²Coordination Provinciale Lépre et Tuberculose du Sud-Kivu, Programme National de Lutte contre la Tuberculose, Bukavu, Congo-Kinshasa.
e-mail: emmanuel.andre@ucdouvain.be

Background and challenges to implementation: GeneXpert® MTB/RIF technology has been developed to

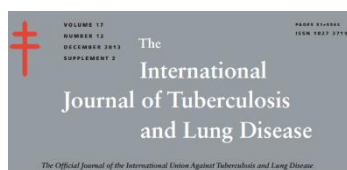
increase TB case detection in low-income and high-burden countries. These countries generally face major problems regarding the transportation of samples from peripheral centers to central labs due to bio-safety issues, long distances and high temperatures. These issues could diminish the overall impact of the GeneXpert MTB/RIF technology in low income countries.

Intervention or response: To facilitate the transport of samples from peripheral health centers to central GeneXpert-equipped labs (TB Reach funding), we validated a protocol of alcohol-based conservation of the samples and implemented it in the South-Kivu Province.

Results and lessons learnt: Sputum conserved by a 1/1 dilution of 95° ethanol can be analyzed by GeneXpert even after 3 months and after spending 48 h at 37°C. The use of this protocol in the South-Kivu province permitted a considerable increase in term of efficiency of the GeneXpert technology.

Conclusions and key recommendations: A major problem faced by low income countries is the transportation of samples due to long distances and high temperatures. Alcohol-based conservation of samples permits to overcome bio-hazard, time and bad conservation issues. In addition, the spread of this protocol permits a considerable increase of the impact of the implementation of a GeneXpert MTB/RIF machine in the population.

Conference paper #2 (2013): Former TB patients improve TB case detection in rural and mining communities of Eastern Democratic Republic of Congo



ABSTRACT BOOK

44th World Conference
on Lung Health of the
International Union Against
Tuberculosis and Lung Disease (The Union)

PARIS - FRANCE
30 OCTOBER-3 NOVEMBER 2013

Eastern Democratic Republic of Congo

E André,¹ O Bahati Rusumba,² A Codlin,³
E Mulume Musafiri,⁴ D Kalumuna,⁴ J Creswell,⁵
M Delmée,¹ Z Kashongwe Munogolo.⁶ ¹SSS/IREC/MBLG,
UCLouvain, Brussels, Belgium; ²Ambassadeurs de la lutte
contre la Tuberculose, Bukavu, Democratic Republic of Congo;
³Interactive Research and Development, Karachi, Pakistan;
⁴Division Provinciale de la Santé du Sud-Kivu, Coordination
Provinciale Lépre et Tuberculose du Sud-Kivu, Bukavu,
Democratic Republic of Congo; ⁵TB Reach Secretariat, World
Health Organization/Stop TB Partnership, Geneva, Switzerland;
⁶Comité scientifique, Programme National de Lutte contre la
Tuberculose, Kinshasa, Democratic Republic of Congo.
e-mail: emmanuel.andre@uclouvain.be

Background: In the context of rural and mining communities of the South-Kivu province in the Democratic Republic of Congo (DRC), the province has case notification rates that are 25% of the national incidence estimates possibly due to poor access to health services. Active case finding (ACF) consists of screening for TB outside the traditional health facilities. This approach has shown to be very effective to increase TB detection in other African settings with low detection rates. Through a TB REACH grant, we involved former TB patients to actively screen people for TB symptoms in their community. State the setting, methods, desired outcomes, procedures and techniques used to collect and analyse information. Include a description of participants, procedures, measures and appropriate statistical analyses.

Results: The intervention was implemented in 19 sub-districts that had no history of participation with ACF interventions. These areas provide care to an estimated population of 1.2 million, comprising rural and mining communities. Starting progressively in April 2012, 240 volunteer former TB patients were recruited to perform verbal screening for TB symptoms in their community and referral of suspects for smear microscopy and GeneXpert testing. Volunteers

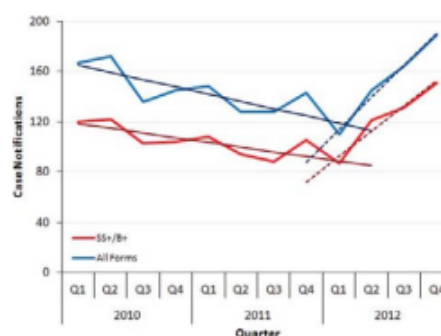


Figure Trends in case notification pre/post ACF intervention for bacteriologically confirmed (SS+/B+) cases and all forms of TB.

received financial support for the logistical costs linked to their activities (mainly reimbursement of local transport costs). Project staff supervised them regularly. National TB Program (NTP) notification data from these facilities were modeled to measure changes in notification trends pre- and post-ACF intervention. During quarters 3 and 4 of 2012, 18 261 verbal screening events were conducted by former TB patients, resulting in the detection of 1361 (7.5%) suspects with symptoms suggestive of TB. All newly identified TB suspects were referred for smear microscopy testing. The intervention significantly increased the rate of case notification. Regression analysis shows a 3.7% declining trend over two years was replaced by an estimated 20.2% growth quarter on quarter post-implementation ($P < 0.0001$). These activities resulted in a 46.1% increase in new smear-positive TB notifications in NTP data in the last six months of 2012 compared with the same period in 2011.

Conclusion: This study shows that involvement of volunteer former TB patients groups for active case finding is an effective way to improve case detection. This intervention should be considered for scale-up to improve TB case detection, especially in areas of difficult access and poor case detection rates.

Conference paper #3 (2014): High prevalence of TB-HIV and rifampicin-resistant TB among military populations of Eastern Democratic Republic of Congo



ABSTRACT BOOK

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and poor medical education. This project was supported by TB REACH.

Design/Methods: Patients clinically suspected of pulmonary TB with no prior history of TB treatment were included in this study. One sample per patient was tested after three negative smear results. We analysed 468 valid Xpert results and the HIV status of patients when available.

Results: 30 patients (6%) tested positive for TB, with a proportion decreasing from 16% (year 1) to 2.25% (year 3). Among TB-positive patients, 8 (27% (95%CI 14 – 46%)) were Rifampicin resistant. HIV test results were available for 25% (116/468) of the patients, with an HIV prevalence of 19% (95%CI 12 – 27%). Among the 102 TB negative patients with HIV results, HIV prevalence was much lower than among TB positive patients, in whom the prevalence was 71% (95%CI 40 – 90%).

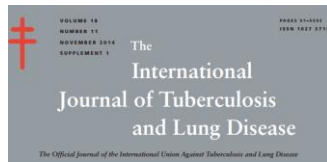
Conclusion: Very high rates of TB, HIV and TB/HIV infections are prevalent among military communities. Xpert testing permitted the diagnosis of 6% of patients with TB symptoms but negative smear results. 27% rifampicin resistance was observed among these patients with no prior history of TB. This specific professional group and their families should urgently be targeted with specific active strategies in order to prevent dissemination of diseases both inside and outside their community.

High prevalence of TB-HIV and rifampicin-resistant TB among military populations of Eastern Democratic Republic of Congo

E Andre,¹ J-P Chirambiza,² E Musafiri,² D Kalumuna,² D Bahati Rusumba,³ D Kikobya,³ J Creswell,⁴ O Le Polain De Waroux.⁵ ¹Pôle de Microbiologie, Université Catholique de Louvain, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium; ²Coordination Provinciale Lèpre et Tuberculose du Sud-Kivu, Programme National de Lutte contre la Tuberculose, Bukavu, ³Médecine Interne, Hôpital Provincial FARDC, Bukavu, Democratic Republic of Congo; ⁴TB REACH secretariat, Stop TB Partnership, Geneva, Switzerland; ⁵Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK.

Background: Although the prevalence of HIV and incidence of TB are known to be high among military communities in South Kivu (DRC), quantitative data are lacking. We here report the results from a TB screening programme conducted between 2012 – 2014 at the Provincial Military Hospital in Bukavu, South Kivu, using a rapid molecular test (Xpert MTB/RIF). Prior qualitative studies have shown this group is exposed to poverty, promiscuity, sexual behaviours at risk of HIV

Conference paper #4 (2014): MDR-TB management in a low-income country: GeneXpert versus Hain Lifescience assays for TB



ABSTRACT BOOK

45th World Conference
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BARCELONA - SPAIN
28 OCTOBER-1 NOVEMBER 2014

PD-572-30 MDR-TB management in a low-income country: GeneXpert™ versus Hain Lifescience™ assays for TB

Z Kashongwe Munogolo,¹ B Bisimwa,¹ S Callens,² E Andre.³ ¹Kivu, ISTM Bukavu, Bukavu, Democratic Republic of Congo; ²Gent University, Gent, ³Université Catholique de Louvain, Bruxelles, Belgium. e-mail: zkashongwe@yahoo.fr

Background: South Kivu is a post conflict area in the East part of DR Congo. Effective TB control is challenged by the weak socioeconomic status of the country and the concomitant HIV epidemic.

Opportunity: In 2013, a screening network MDR TB was initiated under the governance of WHO TB Reach initiative and the Flemish VLIR_UOS project. Ten GeneXpert® Kits were distributed in different health districts all over the Province and a molecular biology laboratory using Hain Lifescience® assays for TB was installed in the main city Bukavu

Methods: 82 sputum specimens were collected from high risk groups (military, police, PLHIV, minors, retreatment cases) from september 2013 to march 2014; Then 20 rifampicin resistant on GeneXpert underwent screening for rifampicin and INH resistance by Hain MTB plus assay.

Results: 14 (70%) specimens showed resistance to Rifampicin and INH, 5 (25 %) were Rifampicin

resistance only and 1(5%) was identified as atypical mycobacterium.

Conclusion: GeneXpert is an efficient mean for Rifampicin resistance detection and suspicion of MDR_TB, but it needs complementary tests to confirm MDR_TB

Conference paper #5 (2015): Active case finding activities performed by former TB patients: experience from South-Kivu Province, Democratic Republic of Congo



ABSTRACT BOOK

46th World Conference
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CAPE TOWN - SOUTH AFRICA
2-6 DECEMBER 2015

OA-497-06 Active case finding activities performed by former TB patients: experience from South-Kivu Province, Democratic Republic of Congo

E Andre,¹ D Bahati Rusumba,² E Musafiri,² D Muzigo,² D Kasereka,² D Kalumuna,² O Le Polain De Waroux³
¹Université de Louvain, Brussels, Belgium; ²Coordination Provinciale Lèpre et Tuberculose du Sud-Kivu, Bukavu, Democratic Republic of the Congo; ³London School of Hygiene & Tropical Medicine, London, UK. e-mail: olivier.lepolain@lshtm.ac.uk

Background: Late and under-detection of TB is one of the most important challenges towards TB control in the Democratic Republic of the Congo. We here report the results of the first 14 months of implementation of an Active Case Finding (ACF) programme in the South Kivu Province (population ~ 6 million), where former TB

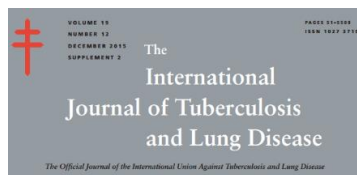
patients are being trained to undertake ACF in their communities. **Material/**

Methods: ACF activities were performed by screeners in their close environment, before extending it geographically ('stone in the pond' approach). Information on socio-demographic characteristics were recorded for each individual screened, as well as information on cough lasting for ≥ 15 days. Cases with a cough were offered a laboratory test (Ziehl Nielsen). We performed descriptive statistics and explored differences in the prevalence of cough and TB by socio-demographic characteristics, through the use of negative binomial regression.

Results: A total of 14 043 individuals from 2900 households were screened by the 141 screeners in communities living in 15 of the 34 health districts of the Province. Overall, 2,103 (15%) individuals reported a cough for ≥ 15 days. This proportion increased linearly with age from 4.5% in <5 y olds to 28.6% among adults aged 40 years and above, was higher in males than females ($P < 0.0001$), and cases tended to be clustered within households. About 92% of individuals with a cough accepted to be tested, of whom 38 (1.8%) were confirmed with smear positive pulmonary TB (SPP). TB incidence increased linearly with age and was higher in males, with a 5% increase in the proportion of diagnosed cases by 5 year age increase (adjusted incidence rate ratio for age (aIRR) 1.05 (95%CI 1.04 – 1.06)) and sex (aIRR males 1.31 (95%CI 1.01 – 1.69). The cumulative incidence of SPP TB in the province, as reported through the national surveillance system, was 53 per 100 000 (95%CI 50 – 55). In comparison, the incidence of SPP TB from the ACF activities over the same period was 231 per 100 000 (95%CI 154 – 334).

Discussion: Our preliminary results suggest that almost 2% of individuals reporting a cough lasting for more than 15 days were diagnosed with SPP TB. The higher incidence among communities targeted by ACF activities is likely due to a combination of increased detection, as well as the targeting of more high risk communities, and suggest ACF activities are important to help curtail the burden of TB in South Kivu.

Conference paper #6 (2015): Failing Xpert MTB/RIF modules: When? What warranty is needed from the customer's perspective? Experience in the DR Congo



ABSTRACT BOOK

46th World Conference
on Lung Health of the
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CAPE TOWN - SOUTH AFRICA
2-6 DECEMBER 2015

PC-817-04 Failing Xpert[®] MTB/RIF modules: When ? What warranty is needed from the customer's perspective? Experience in the DR Congo

E Renard,¹ E Andre^{2,3} ¹Université Catholique de Louvain,
Louvain-la-Neuve, ²Université Catholique de Louvain,
Brussels, ³Cliniques Universitaires Saint-Luc, Brussels,
Belgium. e-mail: emmanuel.andre@uclouvain.be

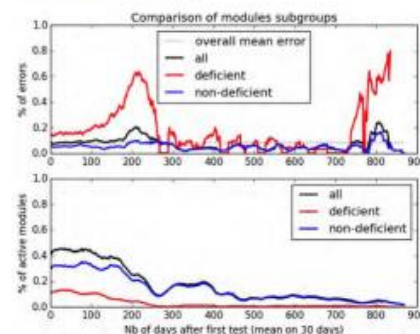
Background and challenges to implementation: Between 2012 and 2014, the Democratic Republic of Congo has gradually implemented 22 GeneXpert machines. The overall proportion of non-valid results (i.e. ((error)), ((invalid)) and ((no result))) was 8%. Among the 68 analytical modules composing the 22 GeneXpert, 32% presented a significantly higher rate of non-valid results during the first 3 years of implementation, leading to unnecessary work and consumption of reagents as well as loss of diagnostic opportunities. The objective of this study was to determine whether it was possible to early identify modules presenting sub-optimal performance.

Intervention or response: Data was collected using the GenXchange (UC Louvain, Belgium; NTP, DRC) and XpertSMS (IRD, Pakistan) technology. We separated the modules in two groups: 22 modules identified with a high proportion of non-valid results, and 46 modules presenting normal figures. For each group, we computed the proportion of non-valid results based on time after implementation of the GeneXpert. We used a moving average on 30 days.

Results and lessons learnt: The figures below represent the percentage of non-valid results between the two groups of modules and the progressive decrease in activity of the modules with the time. In the group with a higher rate of non-valid results, all showed signs of dysfunction (proportion of not-valid results higher than 10%) from the first month after implementation and increased significantly with a peak of over 60% around month 7 (210 days). After 7 months of activity, virtually none of these modules was still in activity, compared to the "non-failing" group of modules, which continued reporting tests.

Conclusions and key recommendations: It had been previously reported that 32% of GeneXpert MTB/RIF

modules presented signs of dysfunction during the first three years after implementation. We showed that failing modules present early signs of dysfunction which can be identified as early as the first month after implementation by using adequate automatic reporting systems. We conclude that the one-year free-of-charge warranty from the manufacturer will cover the vast majority of costs related to sub-optimal equipment delivered. Nevertheless, as failing modules can be difficult to identify, it is recommended to implement the adequate surveillance solutions in order to maximize the benefit that can be made from this warranty.



Conference paper #7 (2015): Stability of Xpert MTB/RIF reagents in sub-optimal storage conditions: evaluation based on three years' experience in the Democratic Republic of Congo



ABSTRACT BOOK

46th World Conference
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CAPE TOWN - SOUTH AFRICA
2-6 DECEMBER 2015

PC-818-04 Stability of Xpert® MTB/RIF reagents in sub-optimal storage conditions: evaluation based on three years' experience in the Democratic Republic of Congo

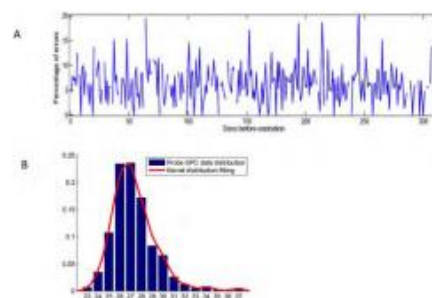
C Vande Kerckhove,¹ E Andre^{2,3} ¹Université Catholique de Louvain, Louvain-la-Neuve, ²Université Catholique de Louvain, Brussels, ³Cliniques Universitaires Saint-Luc, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be

Background and challenges to implementation: Since 2012, the Democratic Republic of Congo (DRC) has performed 23,227 GeneXpert MTB/RIF tests. "Cartridges" are the main consumables for this technology and must be stored between 2 and 28°C. In DRC, these instructions cannot be fully controlled due to local climatic and infrastructure constraints. Based on international quality standards, medical laboratories must perform additional verifications when storage instructions cannot be strictly observed. The objective of this study was to determine the impact of the sub-optimal storage on the quality of reagents.

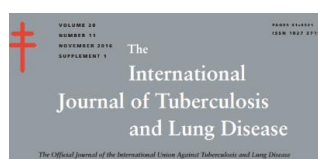
Intervention or response: Out of 22 lots of cartridges, 3 were considered as defective based on hypothesis testing and were excluded from analysis. Data was collected using the GenXchange (UCLouvain, Belgium; NTP, DRC) and XpertSMS (IRD, Pakistan) technology. We focused on two quality indicators. First, the evolution in the proportion of non-valid results (i.e. "Error", "Invalid", "No result"): we computed for each test the

remaining days left before the expiration date of the cartridge and whether the result was valid or non-valid. The second indicator was the distribution of the internal quality control (Cycle threshold or Ct of the SPC) among all tests.

Results and lessons learnt: Figure A represents the evolution of the proportion of non-valid results over time. The expiration date is the graph origin for all lots of cartridges. There was no significant increase of non-valid results observed when the expiration date was closer. The Histogram (Figure B) represents the Ct distribution for the SPC internal quality control. The empirical probability density function was obtained by fitting a distribution to the data histogram. The Ct values of SPC ranged between 23 and 37, with a mean value at 27.3. We observed a normal distribution of these values. **Conclusions and key recommendations:** In this study, we evaluated the quality of Xpert MTB/RIF reagents during their life-cycle post-shipment by the manufacturer. We conclude that sub-optimal storing conditions did not seem to impact the quality of reagents over time, as there was no significant increase of non-valid results in the time and the distribution of SPC Ct was considered as normal, although we cannot definitively exclude from this analysis that high SPC values were not linked to cartridges exposed to particularly sub-optimal storage conditions or linked to cartridges approaching expiry date.



Conference paper #8 (2016): When ex-TB patients start looking for other patients: results after 28 months in the South-Kivu Province of DRC



ABSTRACT BOOK
47th World Conference
on Lung Health of the
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Tuberculosis and Lung Disease (The Union)

LIVERPOOL - UNITED KINGDOM
28-29 OCTOBER 2016

EP-150-28 When ex-TB patients start looking for other patients: results after 28 months in the South-Kivu province of DRC

E Andre,^{1,2} O Bahati Rusumba,³ E Mulume Musafiri,⁴ O Le Polain de Waroux⁵ ¹Université Catholique de Louvain, Pôle de Microbiologie, IREC, Brussels, ²Cliniques Universitaires Saint-Luc, Service de Microbiologie, Brussels, Belgium; ³Ambassadeurs de Lutte contre la Tuberculose ASBL, Bukavu, ⁴Coordination Provinciale de Lutte contre la Tuberculose du Sud-Kivu, Bukavu, Democratic Republic of the Congo; ⁵London School of Hygiene & Tropical Medicine(LSHTM), Department of Infectious Disease Epidemiology, London, UK. e-mail: oliverus.mcd@gmail.com

Background and challenges to implementation: An adequate response to chronic under-detection of TB cannot rely on fragile health systems overwhelmed by current activities. In order to respond to the need for additional human resources required for implementing active case-finding activities at a large scale, the National TB Program of the South-Kivu province in the Democratic Republic of the Congo (population of 6 million) has initiated an innovative collaboration with an organization of ex-TB patients. The rationale for involving ex-TB patients is to facilitate access to remote areas that the health system traditionally does not reach by involving people familiar with both the disease, its symptoms, and the communities to reach.

Intervention or response: Supported by TB REACH and CHALLENGE TB, ex-TB patients have been trained to perform door-to-door screening to identify patients with a cough lasting for more than 15 days, and to refer those individuals to the health facilities for a bacteriological investigation of sputum samples. These screeners provide monthly reports, which are validated by the health structure that ensures the medical follow-up of the patients as per national guidelines.

Results and lessons learnt: During the first 28 months of this collaboration, the organisation and the NTP performed 476 days of training and supervision in the 100 TB centers of the province. During this period, an average of 330 ex-TB patients reported screening activities each month. Altogether, they screened 683 088 individuals, among whom 10% (70 222) presented a cough for more than 15 days. 61% of these attended to the closest health facility for smear microscopy, and 8.3% of those tested were diagnosed with a smear positive pulmonary TB. The positivity rate remained relatively stable throughout those 28 months, although differences by regions were observed.

Conclusions and key recommendations: During a 28 months period, over 10% of the South-Kivu population has been screened for TB thanks to the implication of ex-TB patients. Incidence of bacteriologically confirmed pulmonary TB was 526/100 000, a figure about 6 fold higher than usually reported by the NTP when perform-

ing passive case finding of TB. This experience and the high figures reported tend to show the utility of this innovative collaboration.

Conference paper #9 (2016): Different sensitivities of rapid phenotypic and genotypic methods for low-level rifampicin resistance conferring *rpoB* mutations



ABSTRACT BOOK

47th World Conference
on Lung Health of the
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LIVERPOOL · UNITED KINGDOM
26-29 OCTOBER 2016

OA-306-27 Different sensitivities of rapid phenotypic and genotypic methods for low-level rifampicin resistance conferring *rpoB* mutations

G Torrea,¹ M Gumusboga,¹ E André,² D Affolabi,² L Rigouts,¹ A Van Deun,¹ BC de Jong¹ ¹Institute of Tropical Medicine, Mycobacteriology Unit, Antwerp, ²Université Catholique de Louvain, Louvain, Belgium; ³Laboratoire de Référence de Mycobactéries, Cotonou, Benin. e-mail: gtorrea@itg.be

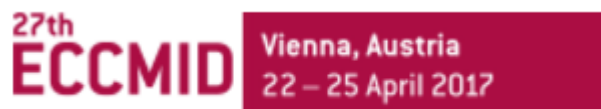
Background: Rifampicin-resistant tuberculosis was diagnosed by phenotypic drug susceptibility testing (DST) until WHO approval of molecular methods, revealing discordant results, especially for 'disputed mutations' in the *rpoB* gene, responsible for low-level rifampicin resistance yet poor clinical outcome. We aimed at evaluating the capacity of the rapid phenotypic and genotypic methods to detect 'disputed mutants'.

Methods: A panel of 39 *Mycobacterium tuberculosis* isolates harboring 'disputed mutants', 5 with the *rpoB*531 mutation (positive control) and 15 wildtype strains were tested in MGIT960 (at 0.125, 0.25, 0.5 and 1.0 µg/ml with routine and extended incubation time), Nitrate Reductase Assay (NRA), Resazurin Microtiter Assay (REMA), Thin Layer Agar (TLA), Microscopic Observation Drug Susceptibility Assay (MODS, Sensititre (TREK Diagnostics), Genotype MTB-DRplus V2 and Xpert MTB/RIF. We calculated the accuracy of the tests, including whether lowering the critical concentration and/or extended incubation time in MGIT960 could increase its sensitivity. Two different lots of the Genotype MTB-DRplus V2 were evaluated in their ability to detect *rpoB* WT8.

Results: MODS and NRA detected >50% of disputed mutants with good specificity (92.3% and 100%). Mutant 511Pro was hardly detectable by any phenotypic method, while the remaining mutants were ≥50% detected by MODS and NRA, including mutation 572Phe not covered in genotypic methods. Concordance between MTB-DRplus (74.4% detection rate) and Xpert MTB/RIF (79.5% detection rate) was 96.5% due to 2 false susceptible Genotype MTB-DRplus results (511Pro). Mutation 533Pro (absence of WT8) was detected by the newest lot of Genotype MTB-DRplus. MGIT extension to 21 days at the critical concentration increased its sensitivity without affecting specificity.

Conclusion: Genotypic methods perform well for the region covered, with Xpert MTB/RIF being slightly more sensitive than Genotype MTB-DRplus V2 for mutation 511Pro. None of the phenotypic methods can detect all 'disputed mutants'. MGIT960 at 21 days of incubation detects mutation 572Phe not covered in molecular DSTs.

Conference paper #10 (2017): Identification of *Mycobacterium avium* and *Mycobacterium intracellulare/chimaera* in clinical practice using probe data of the Xpert MTB/RIF assay



Identification of *Mycobacterium avium* and *Mycobacterium intracellulare/chimaera* in clinical practice using probe data of the Xpert MTB/RIF assay

Background: Identification of non-tuberculosis mycobacteria (NTM) remains a major challenge in clinical practice, and requires specific tools such as sequencing, MALDI-TOF MS or commercial molecular assays for accurate diagnosis. *Mycobacterium avium* and *M. intracellulare* are regularly involved in human infections, particularly among severely immunocompromised patients. *M. chimaera* has recently been reported as a cause of health-care associated endocarditis, and is very similar genetically to *M. intracellulare*. In our clinical practice, we performed Xpert MTB/RIF® PCR on all MGIT cultures after confirmation of the presence of a mycobacterium by fluorescence microscopy. The principle of this test is based on the amplification of the Rifampicin Resistance Determining Region (RRDR) and the binding of 5 different segments by molecular-beacon probes names A, B, C, D and E. In our experience, approximately 50% of positive cultures are negative for *M. tuberculosis* (MTB), and identification of NTM is performed by *rpoB* sequencing or MALDI-TOF MS.

Material/methods: We analysed the raw Probe results of 31 Xpert MTB/RIF tests performed on positive MGIT cultures for which an NTM was later confirmed and at least one Probe was positive (A, B, C, D or E).

Results: All results could be classified within 3 different profiles determined by which of the 5 probes were positive. Among the 8 strains presenting an amplification of Probe A only, all were identified as *M. intracellulare*, *M. chimaera* or *M. marseillense*, three closely related species. Among the 14 strains which presented an amplification of Probe C only, different species were identified including *M. gordonae* (8), *M. xenopi* (3), *M. simiae* (1), *M. fortuitum* (1) and *M. avium* (1). Among the 9 strains presenting an amplification of both Probe C and Probe E, all were identified as *M. avium*.

Conclusions: In our clinical experience, the analysis of Xpert MTB/RIF probe results allowed a correct identification of 3 important pathogenic NTM. These results were supported by the fact that there is a partial overlap of the RRDR region of the *rpoB* gene of MTB with other mycobacterial species. Further studies would be needed to better understand the clinical value of this observation.

Conference paper #10 (2017): Missed opportunities for detecting MDR-TB in South-Africa. Increasing epidemiological importance of a rifampicin-resistance mutation undetected by commercial molecular assays



Background: Rapid molecular assays to detect rifampicin resistance in *Mycobacterium tuberculosis* (MTB) have revolutionized the detection of resistance to this most important drug. However, up to one third of the multi-drug resistant tuberculosis (MDR-TB) cases in Swaziland harbor the Ile491Phe *rpoB* mutation (previously reported as Ile572Phe), which is unrecognized by the commercial molecular assays designed to detect *rpoB* mutations located within the 81 bp so-called rifampicin resistance determining region (RRDR). Furthermore, the resistance associated with this specific mutation has been reported as difficult to identify by phenotypic drug susceptibility tests, in particular when using the phenotypic BACTEC MGIT 960 test (Beckton Dickinson). These tests are widely used in South-Africa, where the Xpert MTB/RIF assay (Cepheid) has recently been introduced as first-line test replacing smear microscopy. Studies performed prior to the introduction of the Xpert MTB/RIF did not reveal an epidemiological importance in regard to the Ile491Phe *rpoB* mutation in South-Africa. Our aim was to evaluate if the wide-spread utilization of commercial molecular assays that miss the Ile491Phe *rpoB* mutants played a role in the selection of such mutants.

Material/methods: We selected 277 positive cultures from the Dr George Mukhari tertiary laboratory, Gauteng province in South-Africa. All these MTB strains had been reported as INH mono-resistant strains according to both the Hain MTBDR_{plus} test (Hain Life science) and the BACTEC MGIT 960 phenotypic assay. We performed a MAS-PCR developed at Université catholique de Louvain that specifically targets the Ile491Phe *rpoB* mutation. All resistant and undetermined PCR results were confirmed by *rpoB* Sanger sequencing.

Results: Out of the 259 strains for which a PCR result was available (93.5% of total), 200 (77,2%) presented a wild-type result. Resistance was reported for 40 strains (15,4%) among which 35/38 (92,1%) were confirmed by Sanger. In total, 37/259 (14.3%) INH mono-resistant strains were re-classified as MDR-TB due to the presence of the Ile491Phe *rpoB* mutation.

Conclusions: We showed that a significant proportion MDR-TB cases in the Gauteng province of South-Africa are not detected by current diagnostic strategies, which rely mainly on commercial molecular assays that do not detect the Ile491Phe *rpoB* mutation. This mutation is associated with poor rifampicin based treatment outcome at similar frequency as the common *rpoB* mutations. This high frequency of the Ile491Phe mutation highlights the limits of current commercial solutions, and stresses the importance for other South-African provinces and countries to monitor the presence and the evolution of this mutation and adjust their diagnostic algorithms as needed. The frequency of this mutation among MDR-TB cases may be even higher, as it is probable that a proportion of strains harbouring the *rpoB* Ile491Phe mutation were detected as resistant by the phenotypic BACTEC MGIT 960 test, and therefore not included in this study.

Conference paper #11 (Submitted, 2017): Evaluation of WHO-endorsed rapid diagnostic tests for potential application as surveillance tools for rifampicin resistant tuberculosis transmission

Evaluation of WHO-endorsed rapid diagnostic tests for potential application as surveillance tools for rifampicin resistant tuberculosis transmission

Kamela S. Ng^{1*}, Conor J. Meehan¹, Léonie Goeminne², Gabriela Torrea¹, Maren Diels¹, Leen Rigouts¹, Bouke C. de Jong¹, Emmanuel André^{2,3}

1) Mycobacteriology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

2) Pôle de Microbiologie Médicale, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium

3) Microbiology Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium

Background: Globally rising rates of rifampicin resistant tuberculosis (RR-TB) has been attributed to transmission. Its diagnosis which increasingly relies on WHO-endorsed rapid diagnostic tests (RDTs), produces large volumes of raw data which are not fully utilised. This study aimed to evaluate the discriminatory power, reproducibility, and concordance of RDTs to explore their potential utilisation for monitoring RR-TB transmission.

Methods: 108 non-clustered RR-TB strains representing 35 different *rpoB* mutations, including 32 located within the 81 bp hotspot, were selected from the Belgian Coordinated Collections of Microorganisms hosted at the ITM. These strains were subjected to Xpert MTB/RIF (Cepheid), MTBDRplusv2.0 (Hain), and GenoscholarTMNTM+MDRTB II (Nipro). Raw data obtained from these tests were compared and analysed through obtained values of Hunter Gaston Discriminatory Index (HGI), Shannon's Index of Diversity, and Wallace Coefficient (WC). The reproducibility of the RDTs was analysed using seven mutation profiles found in at least four strains, and four mutation profiles in two to three strains.

Results: Raw data of each RDT were evaluated to assess their ability to discriminate different mutant sequence profiles. Findings exemplified by distinct probe profiles for each mutant sequence are presented in Figure 1, in which the consensus numbering system for *M. tuberculosis* H37Rv has been adapted. The HGI for the tests ranged from 0.82-0.88 and the Shannon indices ranged from 3-3.5. The WC computed for the following reference-complementary RDT pairs – Hain-Xpert, Hain-Nipro, Nipro-Xpert, Nipro-Hain – were 0.417, 0.583, 0.625, and 0.700 respectively; and the average percent reproducibility for Xpert, Hain and Nipro were 87.94, 96.31, and 97.78 respectively.

Conclusions: Taken together, sufficient discriminatory power, reproducibility, and concordance of RDTs support the validity of its potential use as first line tool for suspecting RR-TB transmission. For definitive confirmation, complementary testing with gold standard genotyping methods is recommended.

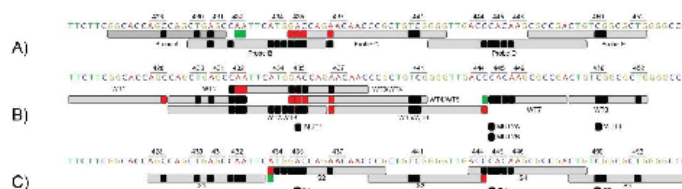


Figure 1. Overview of RR-TB RDT captured mutations. The observed results for each *rpoB* mutation (to scale) are overlaid on claimed probe coverage (light gray) for A) Xpert MTB/RIF, B) MTBDRplusv2.0, and C) GenoscholarTMNTM+MDRTB II. Mutations captured outside claimed probe boundaries are highlighted in green with dashed borders; mutations that were expected to be captured by a specific probe but were not are in red.

*Corresponding author: Kamela S. Ng

Conference paper #12 (Submitted, 2017, accepted then suppressed after demand from DRC NTP): Impact of the introduction of LPA assays on the confirmation of MDR-TB among RIF-resistant TB cases in the South-Kivu province of DRC

Impact of the introduction of LPA assays on the confirmation of MDR-TB among RIF-resistant TB cases in the South-Kivu province of DRC

B Bisimwa¹, J-P Chirambiza², R Nyota², Z Kashongwe¹, S Callens³, E André⁴

¹ISTM, Bukavu, Congo (Democratic Rep.), ²CPLT Sud-Kivu, Bukavu, Congo (Democratic Rep.), ³UGent, Gent, Belgium, ⁴Université catholique de Louvain, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be

Background and challenges to implementation: The Democratic Republic of Congo lies among the countries with the highest burden of tuberculosis (TB) and multi-drug resistant TB (MDR-TB). Despite this alarming situation, Drug Susceptibility Testing is still performed only in the National Reference Laboratory of Kinshasa. The South-Kivu province, which lies on the other extremity of the country, has identified 183 new patients with rifampicin resistant tuberculosis (RR-TB) following the introduction of Xpert MTB/RIF in 2012. Among these, results of drug susceptibility testing (DST) only confirmed eight cases (4,4%) of multi-drug resistant TB (MDR-TB). For the rest of the patients, results were unavailable or did not confirm MDR-TB.

Intervention or response: From the fourth quarter of 2012, we implemented a LPA test (MTBDR_{plus}, Hain Lifescience) in Bukavu, the capital city of the South-Kivu province. This was the first time that such technology was introduced outside the capital of the country.

Results and lessons learnt: The introduction of a provincial LPA laboratory in Bukavu allowed confirming MDR-TB among 28 additional patients (+350%). Out of the 54 results which presented RIF resistance on the LPA test, 26 (48%) did not show any isoniazid (INH) resistance mutation on the *katG* or *inhA* genes.

Conclusions and key recommendations: Despite all patients could not receive a confirmatory test following the detection of RIF resistance, we demonstrated the feasibility and utility of introducing LPA confirmatory testing at the provincial level in DRC. The high level of RIF mono-resistance found using this test should be confirmed by phenotypic drug susceptibility testing or Next-Generation sequencing methods in order to exclude INH caused by mutations in other genes, and further assess drug resistance for other TB drugs.

Option 2: Suited for public health practice submissions.

Conference paper #13 (Submitted, 2017): Technical validation of DataToCare® software as a mean to implement electronic medical records and improve the management of patients with tuberculosis in Rwanda

Technical validation of DataToCare® software as a mean to implement electronic medical records and improve the management of patients with tuberculosis in Rwanda

JD Umazimpaka¹, A Ghouti², JC Ngabonziza¹, J Vanvolsem², X Morelle², E André³

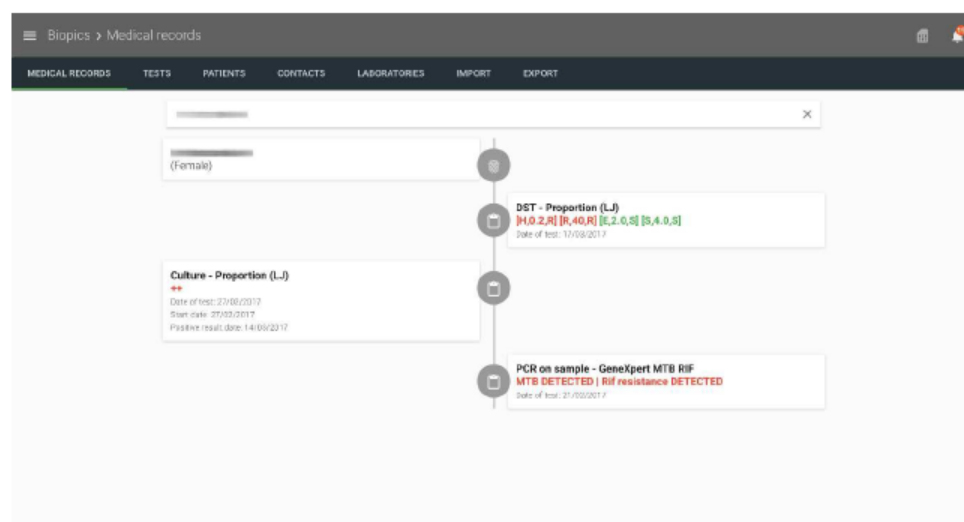
¹Rwanda Biomedical Center, National Reference Laboratory Division, Kigali, Rwanda, ²Savics, Brussels, Belgium,

³Université catholique de Louvain, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be

Background and challenges to implementation: Medical management of Tuberculosis (TB), in particular MDR-TB, requires performing multiple laboratory tests including smear microscopy, rapid molecular tests, cultures and drug susceptibility testing (DST). In programmatic conditions, these tests are performed in different laboratories at different times. It is necessary for doctors to have access to these different test results in real-time and at from any TB health facility to which the patient refers. DataToCare® (Savics, Belgium) is a new diagnostics connectivity solution which has recently been introduced in Rwanda.

Intervention or response: The technical validation of DataToCare® included four main criteria: its ability to collect TB test results from different laboratories, to collect patient-related information, to merge different test results of a same patient based on the recognition of its identity, and to present these results in a manner that would be usable for doctors working in different health facilities.

Results and lessons learnt: DataToCare® was successfully validated for implementation in Rwanda. The system allowed uploading test results from Xpert, culture and DST laboratories. Tests performed for one same patient were automatically merged by the system and these results were presented for each patient in an individual electronic medical record



[EMR DataToCare(c)]

Conclusions and key recommendations: Improvement of patient management, in particular for those with MDR-TB, requires integrating a multitude of different diagnostic tests performed during the process of diagnosis and treatment. To make this complex information available and usable, tests results not only need to be successfully captured and transferred, but also presented in a format that will support clinical decision. DataToCare® fulfilled these requirements.

Conference paper #14 (Submitted, 2017): Exploring the potential of diagnostics connectivity solutions for improving tuberculosis laboratories quality management. Application of DataToCare© in Rwanda

Exploring the potential of diagnostics connectivity solutions for improving tuberculosis laboratories quality management. Application of DataToCare© in Rwanda

JD Umazimpaka¹, A Ghouti², JC Ngabonziza¹, J Vanvolsem², X Morelle², **E André**³

¹Rwanda Biomedical Center, National Reference Laboratory Division, Kigali, Rwanda, ²Savics, Brussels, Belgium, ³Université catholique de Louvain, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be

Background and challenges to implementation: Improving the quality of laboratories is a priority for African countries. In the field of tuberculosis (TB), WHO and reference laboratories support this process by organizing external quality assessments (EQA). These consist in sending a panel of samples to different laboratories with the aim to verify that these provide the same result despite using different methods and working in different environments. With the introduction of Xpert and the subsequent development of diagnostics connectivity solutions, reporting of these EQA results and their potential real-time sharing between laboratories offer the possibility to support laboratory quality assessment. The Rwanda national tuberculosis program recently introduced DataToCare© (Savics, Belgium) as a mean to improve the collection of patient test results performed within the different TB laboratories of the country.

Intervention or response: We evaluated the ability of DataToCare© to merge culture, drug susceptibility testing (DST) and Xpert MTB/RIF (Cepheid) results performed for EQA samples received from the ITM supra-national reference laboratory.

Results and lessons learnt: Test results of one same sample processed through different techniques (culture, DST and Xpert) were collected and merged automatically in DataToCare©.

Conclusions and key recommendations: DataToCare© allows collecting and merging patient test results into unique electronic medical records by using a unique patient identifier recognition system. The present evaluation demonstrated that this functionality can be used to collect and compare the performance of different laboratories or diagnostic technologies. In the future, national and international laboratories could use this possibility to improve the process of EQA, supporting quality of TB laboratories in programmatic conditions.

Conference paper #16 (Submitted, 2017): Peer-led active case finding of TB in high-risk groups in the South-Kivu province of DRC. Results working with miners, soldiers, prisoners and household contacts

Peer-led active case finding of TB in high-risk groups in the South-Kivu province of DRC. Results working with miners, soldiers, prisoners and household contacts

O Bahati Rusumba¹, A Ishara¹, E Marhegane Munguakankwa¹, C Habimana Ndwanyi¹, V Bola², L Kitete², JP Kabuayi³, **E André**⁴

¹*Ambassadeurs de Lutte contre la Tuberculose, Bukavu, Congo (Democratic Rep.)*, ²*Challenge TB (CTB) project, Bukavu, Congo (Democratic Rep.)*, ³*The International Union Against TB and Lung Disease, Kinshasa, Congo (Democratic Rep.)*, ⁴*Université catholique de Louvain, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be*

Background: Active case finding of patients with active tuberculosis (TB) disease in high-risk groups is an essential component for tackling the TB epidemic. In the South-Kivu province of DRC, we identified four high-risk groups: people living in the household of TB patients, people living in military camps, people working in the artisanal mining sector and people living in prisons.

Methods: We performed symptomatic screening of cough in the four pre-identified high risk groups. Suspects identified were tested for pulmonary TB using smear microscopy of rapid molecular tests.

Results: We performed symptomatic screening among 17.693 household TB contacts, 13.953 people living in military camps, 10.876 people working in the artisanal mining sector and 480 individuals living in prisons. The number of individuals needed to screen in order to find one bacteriologically confirmed TB case was 131, 71, 83 and 68 respectively.

Conclusions: In the South-Kivu province of DRC, we found that the incidence of smear-positive pulmonary TB was the highest among people living in prisons, in military camps and artisanal mining camps. These results suggest that an urgent attention should be given to these populations highly exposed to TB.

Conference paper #17 (Submitted, 2017): Complementing the bio-medical approach with patient-led active case finding. Evaluation of a large scale intervention in the Democratic Republic of Congo

Complementing the bio-medical approach with a patient-led active case finding. Evaluation of a large scale intervention in the Democratic Republic of Congo.

O Bahati Rusumba¹, A Ishara¹, C Habimana Ndwany¹, E Marhegane Munguakonkwa¹, V Bola¹, L Kitete², JP Kabuayi³, **E André⁴**

¹*Ambassadeurs de Lutte contre la Tuberculose, Bukavu, Congo (Democratic Rep.)*, ²*Challenge TB (CTB) project, Bukavu, Congo (Democratic Rep.)*, ³*The International Union Against TB and Lung Disease, Kinshasa, Congo (Democratic Rep.)*, ⁴*Université catholique de Louvain, Pôle de Microbiologie, Institut de Recherche Expérimentale et Clinique, Brussels, Belgium. e-mail: emmanuel.andre@uclouvain.be*

Background: Each year in DRC, it is estimated that 156.000 patients with TB infection do not reach the health system or remain undiagnosed despite seeking care.

In 2013, TB patients and TB-affected communities of the South-Kivu province of DRC created a non-profit organisation called "Ambassadeurs de Lutte contre la Tuberculose". The objective of this organisation is to tackle the burden of TB disease in their households and communities by accelerating the detection and treatment of patients with TB disease.

Between 2014 and 2016, TB-affected volunteers performed active case-finding activities.

The screening algorithm applied in this study consisted in providing a microscopy test to patients coughing for more than 15 days.

Methods:

The aim of our study was to evaluate the quality of the active case-finding intervention by assessing (1) their ability to identify symptomatic patients in their community, (2) their ability to convince symptomatic patients to get tested for TB and (3) their ability to detect new TB cases.

Results: In total, over 650.000 individuals were screened and the intervention contributed to the detection of 4.300 new smear positive pulmonary TB cases, representing 42% of the provincial notification during this period. The intervention allowed to increase the number of smear positive pulmonary TB cases diagnosed per quarter and per health facility ($p=0,006$). Furthermore, the screeners tended to improve their ability to detect new TB cases (mean of 0,71 per quarter and per screener, with a trend of +117% over 3 years ; $p=0,0051$).

Conclusions: This large-scale intervention allowed to demonstrate the feasibility and utility of active case-finding activities performed by TB-affected populations. Improving the clinical skills and the laboratory performance of the health system should allow to improve further the impact of this strategy.