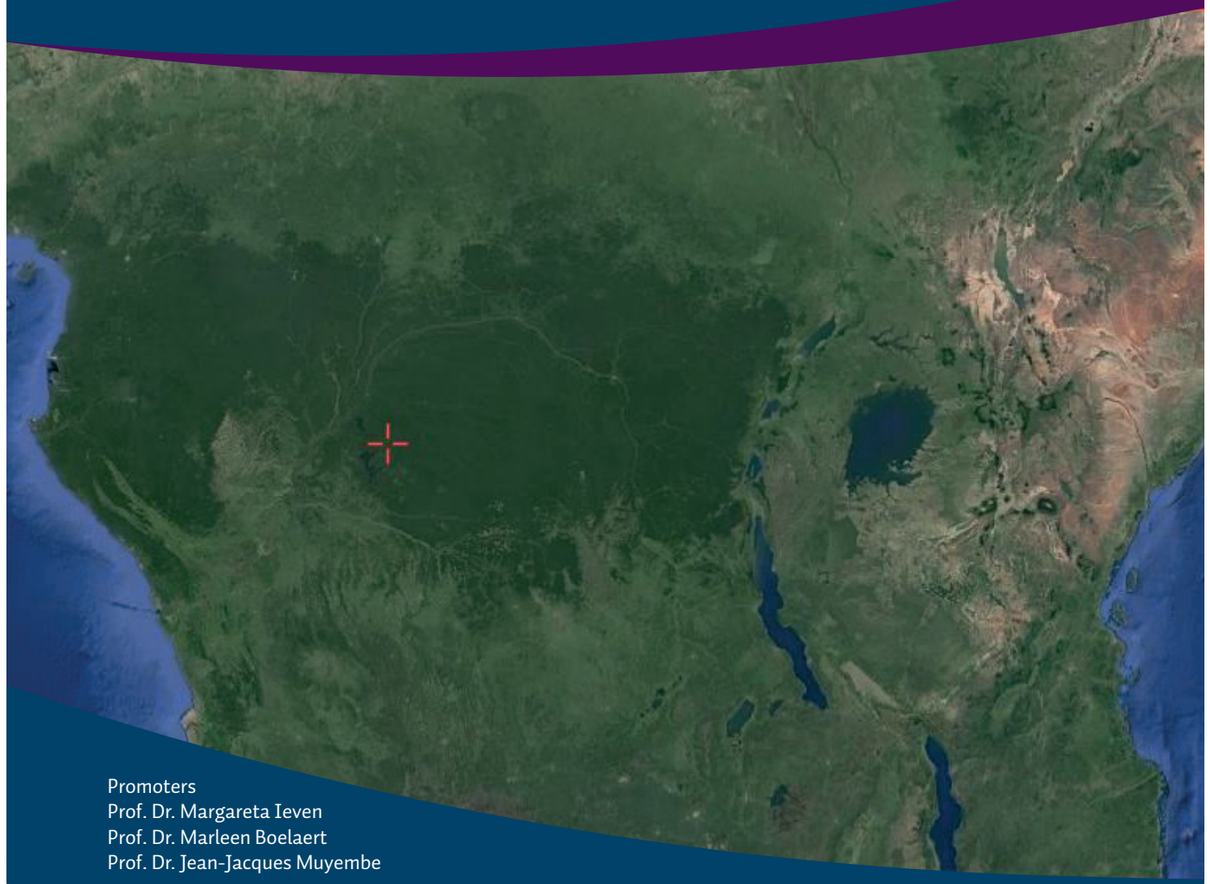
Diagnosis of Multi-Drug Resistant (MDR) tuberculosis in the Democratic Republic of the Congo

Dissertation submitted for the degree of Doctor
of Medical Sciences at the University of Antwerp

Michel Kaswa Kayomo



Promoters
Prof. Dr. Margareta Ieven
Prof. Dr. Marleen Boelaert
Prof. Dr. Jean-Jacques Muyembe

Faculty of Medicine
and Health Sciences
Antwerp 2018

INSTITUTE OF TROPICAL MEDICINE ANTWERP

University of Antwerp

Diagnosis of Multi-Drug Resistant (MDR) tuberculosis in the Democratic Republic of the Congo

Michel Kaswa Kayomo

Diagnosis of Multi-Drug Resistant (MDR) tuberculosis in the Democratic Republic of the Congo

Michel KASWA KAYOMO

ISBN 9789057285981
Legal deposit number D/2018/12.293/23

Cover design by Anita Muys (UA)

Description of cover photo
The cover picture shows a spatial view of the Democratic Republic of Congo. The red mark indicates the Bandundu Province where the first study was carried out.

Diagnosis of Multi-Drug Resistant (MDR) tuberculosis in the Democratic Republic of the Congo

Diagnose van Multiresistente tuberculosis in de Democratische Republiek Congo

Dissertation submitted for the degree of Doctor of Medical Sciences

Michel KASWA KAYOMO

Promoters

Prof.Dr. Margareta Ieven
University of Antwerp
Antwerp, Belgium

Prof.Dr. Marleen Boelaert
Institute of Tropical Medicine
Antwerp, Belgium

Prof.Dr. Jean-Jacques Muyembe
Institut National de Recherche Biomédicale
Kinshasa, République Démocratique du Congo

Antwerp – May 2018

Mentors

Prof. Dr. Bouke De Jong
Institute of Tropical Medicine
Antwerp, Belgium

Dr A.Van Deun
Institute of Tropical Medicine
Antwerp, Belgium

Dr E.Hasker
Institute of Tropical Medicine
Antwerp, Belgium

Dr T.Battaglioli
Institute of Tropical Medicine
Antwerp, Belgium

Individual Doctorate Committee

Chair:
Prof.Dr. Bob Colebunders
University of Antwerp
Antwerp, Belgium

Internal Jury Members:
Prof.Dr. Bob Colebunders
University of Antwerp
Antwerp, Belgium

Prof.Dr. Lut Lynen,
Institute of Tropical Medicine
Antwerp, Belgium

External Jury Members:
Prof.Dr. Patrick Van der Stuyft
University of Ghent
Ghent, Belgium

Prof.Dr. Frieda Behets
University of North Carolina
Chapel Hill, North Carolina, USA

M.Kaswa benefited from a Ph.D. grant of the Institute of Tropical Medicine (ITM) funded by the Belgian Development Cooperation (DGD) in the framework of institutional cooperation.

A Dieu

Recommande à l'Eternel tes œuvres et tes projets se réaliseront (Proverbes 16 : 3).

Le Seigneur reconnaît la valeur de mon service.

Mon Dieu est ma force (Esaïe 49 :4b)

Mon Dieu

Tu m'as permis de réaliser mon rêve celui de devenir Médecin, spécialiste et expert en maladies infectieuses, qui dédie tout son temps aux malades et aux personnes affectées recherchant des soins de qualité.

Merci !

Dédicace

A toi Nana MBONZE BOSANCI, ma très chère et tendre épouse. Jamais ces mots n'ont eu autant de valeur et de signification car tu les incarnes en toi. Tu as consenti tant de sacrifices et accepté stoïquement mes longues absences, pour que demain soit meilleur…. Merci pour tes encouragements incessants ;

A vous mes enfants bien-aimés Jody, Wivine et Stan, qui ont enduré tant de privations de la part de Papa. Retenez que tous ses efforts ont été faits pour vous exhorter et encourager à devenir demain utiles à la communauté ;

A mon Père le Professeur Docteur Jean KASWA KASIAMA, votre ténacité, l'éducation et l'instruction que vous nous avez gratifié, nous a permis d'aboutir à ce résultat. Ce travail est un hommage de plus à toute la famille. Je suis fier de toi Papa car tu as fait de moi un homme équilibré ;

A Son Excellence Docteur Oly ILUNGA KALENGA, pour le soutien à 100% que vous m'avez accordé pour cette grande étape du parcours de ma vie. Ensemble, faisons triompher la vie !

Au regretté Docteur Georges BAKASWA NTAMBWE, qui nous a inoculé le virus de la générosité dans l'effort. Ton slogan : « rendons les soins de la tuberculose accessibles à tous » demeurera ma motivation. Tu as fait des émules, je poursuis le combat !

A mon regretté Oncle le Colonel Guy KAYOMO ESSUEL qui, dans les moments de doute et questionnements, renforça notre conviction que la Médecine fut notre apostolat ;

A la mémoire de ma regrettée Maman Wivine KAYOMO LEMISA, son amour, sa générosité et son attention sont là les meilleurs atouts de succès qu'elle nous a légués.

« Mum, your spirit will live forever »

A mes frères et sœurs ;

Que plus d'amour nous unisse pour le progrès.

A toute ma grande famille.

Je dédie ce travail.

Table of Contents

Abbreviations

AC	Amplification control
AFB	Acid-Fast Bacilli
ART	Antiretroviral Therapy
BCG	Bacille Calmette-Guérin (vaccine)
CC	Conjugate Control
CDC	Centers for Disease Control and Prevention (US)
CFR	Case Fatality Ratio
CPT	Co-trimoxazole Preventive Therapy
DOT	Directly Observed Treatment
DOTS	Directly Observed Treatment, Short Course
DRS	Drug resistance surveillance
DR TB	Drug resistant tuberculosis
DST	Drug susceptibility testing
EQA	External quality assurance
FIND	Foundation for Innovative New Diagnostics
FQ	Fluoroquinolone
GFATM	Global Fund to Fight AIDS, Tuberculosis, and Malaria
GFX	Gatifloxacin
GLC	Green-Light Committee
HBC	Highest-Burden Countries
HIV	Human Immunodeficiency Virus

INH	Isoniazid
INRB	Institut National de la Recherche Biomédicale
ITM	Institute of Tropical Medicine
IUATLD	International Union Against Tuberculosis and Lung Disease
Km	Kanamycin
LJ	Lowenstein-Jensen
MDR-TB	Multidrug-resistant tuberculosis
NTM	Non tuberculous mycobacteria
PIH	Partners In Health
PPD	Purified protein derivative
PPM	Public-private mix
TB	Tuberculosis
MTBC	Mycobacterium Tuberculosis Complex
MUT	Mutation
NPV	Negative Predictive Value
NRL	National Reference Laboratory
NTM	Non-Tuberculosis Mycobacteria
NTP	National Tuberculosis Program
PI	Principal Investigator
PNB	Para-nitro-benzoic acid
PPV	Positive Predictive Value
RMP	Rifampicin
RR	Rifampicin-resistance

SLID	Second-line Injectable Drug
SOP	Standard Operating Procedures
SRL	Supranational Reference Laboratory
UHC	Universal Health Coverage
UNAIDS	Joint United Nations Programme on HIV/AIDS
WRD	WHO-recommended Rapid Diagnostics
WHO	World Health Organization
WT	Wild-type
ZN	Ziehl-Neelsen

Summary

Multidrug-Resistant Tuberculosis (MDR-TB), defined as the resistance of clinical isolates of *Mycobacterium tuberculosis* strains against Rifampicin (RMP) and Isoniazid (INH) is considered as a serious threat which jeopardizes the worldwide efforts to control Tuberculosis (TB). Conventional methods for diagnosing MDR-TB are slow and cumbersome requiring at least two months for test execution, and the treatment is complicated. In Low-Income Countries such as the Democratic Republic of the Congo (DRC), the challenge posed by MDR-TB is vast. Over the two last decades, the history of the DRC has been rife with civil unrest, which has led to the collapse of the health system and the recrudescence of TB and other infectious diseases. DRC is considered as one of the 27 countries with a high burden of MDR-TB, but the actual data on the magnitude, trends, and the distribution of MDR-TB in DRC are scanty. In 2008, the World Health Organization (WHO) estimated that the total number of MDR-TB cases in DRC was 5600 (95%CI: 530-11 000). However, less than 2% of this estimated number was detected and put on specific treatment during that same year. Extremely long turn-around times for laboratory results to reach the treating clinician increase the risk of the spread of resistant strains. The goal of this thesis was to provide the National TB Program (NTP) of DRC with evidence and guidance on how to improve the programmatic management of MDR-TB.

The first manuscript in the thesis reports an investigation of a cluster of MDR-TB cases in the Mosango health district, in the Kwilu Province (previously Bandundu-south Province), DRC in 2008. We collected two sputum specimens from each presumptive MDR-TB patient and his/her contacts for culture on solid medium Löwenstein Jensen (LJ) followed by detection of RMP and INH resistance by conventional Drug Susceptibility Testing (DST) on solid medium and further DNA sequencing. Phenotypic Drug Sensitivity Testing and DNA sequencing were performed on 18 sputum specimens collected from 4 presumptive MDR-TB patients and five household contacts. Sequencing data confirmed that the four presumptive patients were indeed Rifampicin resistant cases. Sequencing of the *rpoB* gene showed that 3 cases had a single mutation encoding a substitution to 526Tyr, 531Trp and 526Leu respectively. Patient C had a double mutation encoding a change to 531Leu and 633Leu. Two of the investigated cases died within four months of a second-line treatment course. The Mosango study underlined the need for novel tools to assess drug resistance and provided the rationale for the subsequent work.

We then assessed the diagnostic accuracy and feasibility of molecular line probe assays for rapid detection of RMP, INH, fluoroquinolone (FQ) and aminoglycoside resistance: the Genotype® MTBDR *plus* assay and the Genotype® MTBDR *sl* assay (version 1.0 & 2.0), Hain Lifescience, Nehren Germany. The double *gyrA* mutation 80Ala and 90Gly represented 57% of all FQ mutations identified from MDR-TB patient sputum samples, as confirmed by DNA sequencing. This double mutation was previously found to be associated with susceptibility to FQ, yet leads to absent hybridization of a wildtype band in the MTBDR *sl* and is thus falsely scored as

resistance. Our findings suggest interpreting MTBDR *sl* results with caution when the interpretation is solely based on the absence of a wildtype band without confirmation by visualization of a mutant band.

Building on the results of this validation study, we decided to analyze the burden of drug-resistant TB and to address the surveillance of TB resistance in Kinshasa, DRC in the third paper. For the period 2005-2010, among 2 349 retreatment patients sampled, 1 609 (68%) had a valid phenotypic Drug Susceptibility Result (DST), 282 (17.5%) of those were MDR-TB, and 6 (2.3%) of the MDR-TB were resistant to FQs. For the period 2011-2013, among 192 retreatment patients, 157 (82%) had a valid molecular DST, including 64 (40.7%) with MDR-TB, of whom 4 (7.0 %) also had FQ resistance. The number of first failure cases that was screened reached 409 (17%) and 71 (37%) for the periods 2005-2010 and 2011-2013, with 3.8% and 7.0% RMP resistance detected, of whom 2.0 % and 5% also had FQ resistance. Although there seemed to be a trend towards increased resistance, this was not confirmed by triangulation of our findings with registered recurrence cases.

We also carried out an epidemiological analysis to determine the burden and the trends of drug resistance, particularly RMP and FQ resistance, in the patients with recurrence (failure and relapse/reinfection) after primary treatment of tuberculosis in Kinshasa, the capital of DRC. In parallel, we evaluated the feasibility of a simplified and more efficient strategy for monitoring of drug resistance in DRC.

In the meantime, a new molecular tool for diagnosing TB and resistance to rifampicin had been introduced in DRC, the Xpert® MTB/RIF assay (Cepheid, USA). This test provides results within 2 hours. WHO recommended it in 2010 for the diagnosis of pulmonary TB in adults and later, in 2013, for use in children and to diagnose specific forms of extra-pulmonary TB. We used it in 2015 to document the emergence of TB/MDR-TB cases in a prison setting in Mbuji-Mayi in DRC to guide appropriate treatment and infection control measures. The outcomes of this assessment include the total burden of TB in the prison, the drug susceptibility patterns of *M. tuberculosis* isolated from inmates in this prison, and the associated risk factors. On clinical screening, we found 475 presumptive TB patients among 918 inmates and collected a fresh sputum sample from all of them. The overall prevalence rate of confirmed TB among the 918 prisoners incarcerated at the time of our investigation was 21% (199/918). Among those, there were 14 TB rifampicin resistant (TB-RR) cases. All TB and TB-RR have been put on treatment. Overcrowding and poor nutritional status were the major risks factors explaining the high TB prevalence in this prison.

All studies were approved by the Ethics Committee of the University of Antwerp, Belgium and the National Tuberculosis Program in DRC.

In conclusion, this thesis provides key information on the emergence of drug-resistant TB in a high-burden country, DRC, its public health impact, experience gained in patient management and strategies for addressing drug resistance within NTP in DRC. Laboratory services, although crucial for national disease control programmes, are often the weakest link in the health system, receiving low priority and inadequate resources. For TB control, quality-controlled bacteriological examination is essential for the diagnosis and management of TB patients. Laboratory strengthening is a priority, including improved access to and use of existing diagnostics as well as the development and implementation of appropriate new technologies.

Samenvatting

Multidrug resistente tuberculose (MDR-TB), gedefinieerd als de resistentie van klinische isolaten van Mycobacterium tuberculosis-stammen tegen Rifampicine (RMP) en Isoniazide (INH), is een ernstige bedreiging voor de wereldwijde inspanningen om tuberculose (TB) te bestrijden. Conventionele diagnostische methodes voor MDR-TB zijn omslachtig, vereisen ten minste twee maanden voor de uitvoering, en de behandeling van MDR-TB is complex. In lage inkomenslanden, zoals de Democratische Republiek Congo (DRC), is de uitdaging die MDR-TB stelt enorm. Gedurende de afgelopen twee decennia werd DRC getekend door maatschappelijke onrust en gewelddadige confrontaties, wat heeft geleid tot de ineenstorting van het gezondheidssysteem en de heropflakkering van TB en andere infectieziekten. DRC wordt beschouwd als één van de 27 landen met een groot MDR-TB probleem, maar data over de feitelijke omvang, trends en distributie van MDR-TB in DRC zijn schaars. In 2008 schatte de Wereldgezondheidsorganisatie (WHO) dat het totale aantal MDR-TB gevallen in DRC, 5600 bedroeg (95% CI: 530-11 000). In datzelfde jaar werd echter minder dan 2% van dat geschatte totaal ook werkelijk gedetecteerd door het programma en daarna behandeld met aangepaste geneesmiddelen. De extreem lange doorlooptijden vooraleer de laboratoriumresultaten de behandelende arts bereiken, verhogen het risico van de verspreiding van resistente stammen. Het doel van dit proefschrift was om het Nationale TB programma van de DRC te voorzien van wetenschappelijke evidentie en richtlijnen voor het verbeteren van de bestrijding van MDR-TB.

Het eerste manuscript in dit proefschrift rapporteert een onderzoek naar een cluster van MDR-TB gevallen in de gezondheidszone Mosango, in de Kwilu provincie (voorheen zuid-Bandundu), DRC in 2008. We verzamelden twee sputum specimens van elke vermoedelijke MDR-TB patiënt met symptomen en tekens die op TB kunnen wijzen, en zijn / haar contacten, voor cultuur op vast Löwenstein Jensen (LJ) medium gevolgd door detectie van RMP- en INH-resistentie via conventionele Drug Susceptibility Testing (DST) op vast medium gevolgd door DNA-sequencing. Fenotypische DST en DNA-sequencing werden uitgevoerd op 18 sputum specimens verzameld van 4 vermoedelijke MDR-TB gevallen en 5 contacten in hun gezin. Sequencing-gegevens bevestigden dat deze 4 inderdaad Rifampicine-resistente gevallen waren. Sequentiebepaling van het rpoB-gen toonde aan dat 3 gevallen een enkele mutatie hadden die codeerde voor een substitutie naar respectievelijk 526 Tyr, 531 Trp en 526Leu. Patiënt C had een dubbele mutatie die codeerde voor een verandering naar 531 Leu en 633 Leu. Twee van de onderzochte gevallen stierven binnen 4 maanden na een tweedelijns behandeling. Het Mosango-onderzoek onderstreepte de behoefte aan nieuwe instrumenten om de resistentie tegen geneesmiddelen te beoordelen en legde de basis voor het daaropvolgende werk.

Vervolgens hebben we de accuraatheid en gebruiksvriendelijkheid van moleculaire lijntesten voor snelle detectie van RMP-, INH-, fluoroquinolone- (FQ) en aminoglycoside-resistentie beoordeeld: de Genotype® MTBDR plus en de Genotype® MTBDR sl assay (versie 1.0 & 2.0),

Hain Lifescience, Nehren Duitsland. De dubbele gyrA-mutatie 80Ala en 90Gly vertegenwoordigde 57% van alle FQ -mutaties die werden aangetoond in MDR-TB-sputummonsters, zoals bevestigd door DNA-sequencing. Deze dubbele mutatie bleek eerder geassocieerd te zijn met gevoeligheid voor FQ, maar leidt tot afwezigheid van hybridisatie van een wildtype band in de MTBDR sl en wordt dus vals gescoord als resistentie. Onze bevindingen suggereren om MTBDR sl-resultaten met de nodige voorzichtigheid te interpreteren wanneer de interpretatie uitsluitend gebaseerd is op de afwezigheid van een wildtype band zonder bevestiging door visualisatie van een mutante band.

Gebaseerd op dit validatieonderzoek, hebben we vervolgens de frequentie van resistente TB geanalyseerd op basis van de beschikbare data van de surveillance van tbc-resistentie in Kinshasa, DRC . Voor de periode 2005-2010 hadden bij 2 349 retreatment-patiënten die werden bemonsterd, 1 609 (68%) een geldig DST resultaat, 282 (17.5%) daarvan waren MDR-TB en 6 (2.3%) van de MDR-TB waren resistent tegen FQ. Voor de periode 2011-2013 hadden van de 157 patiënten die opnieuw behandeld werden 157 (82%) een geldige moleculaire DST, waaronder 64 (40.7%) met MDR-TB, van wie er 4 (7.0%) ook FQ-resistentie hadden. Het aantal patiënten met een primair therapie falen dat werd gescreend bereikte 409 (17%) en 71 (37%) voor de perioden 2005-2010 en 2011-2013, en we detecteerden RMP-resistentie in 3.8% en 7.0% ervan, van wie 2.0% en 5% ook resistent waren aan FQ. Hoewel er een tendens naar verhoogde resistentie leek te zijn, werd dit niet bevestigd door triangulatie van onze bevindingen met geregistreerde recidiefgevallen.

Intussen was een nieuwe moleculaire methode voor de diagnose van TB en resistentie tegen rifampicine geïntroduceerd in DRC, de Xpert® MTB / RIF-test (Cepheid, VS). Deze techniek levert resultaat binnen de twee uur en werd in eerste instantie aanbevolen (in 2010) voor de diagnose van longtuberculose bij volwassenen. Sinds 2013 is ze ook aanbevolen voor gebruik bij kinderen en om specifieke vormen van extra-pulmonaire TB te diagnosticeren. We hebben de Xpert gebruikt om een probleem van MDR-TB in een gevangenisomgeving te documenteren om daarna de aangewezen behandelings- en infectiecontrolemaatregelen te kunnen nemen. Deze studie beschrijft de frequentie van TB en MDR-TB in de gevangenis, de resistentiepatronen van de *M. tuberculosis* stammen die geïsoleerd werden bij de gedetineerden, en de bijbehorende risicofactoren. Bij klinische screening vonden we 475 vermoedelijke TB-patiënten bij 918 gedetineerden en verzamelden een vers sputumstaal van elk van hen. Het totale aantal bevestigde TB gevallen onder de 918 gevangenen tijdens ons onderzoek bedroeg 21.7% (199/918). Onder hen waren er 14 rifampicine-resistente gevallen. Alle TB en TB-RR gevallen zijn behandeld geworden. Overbevolking en een slechte voedingsstatus waren de belangrijkste onderliggende risicofactoren voor de hoge TB-prevalentie in deze gevangenis.

Alle studies werden goedgekeurd door de ethische commissie van de Universiteit van Antwerpen, België en het nationale tuberculoseprogramma in de DRC.

Concluderend geeft dit proefschrift belangrijke informatie over de opkomst van resistente TB in een land met hoge TB prevalentie, de DRC, de gevolgen voor de volksgezondheid, de ervaring tot dusver opgedaan met het diagnostisch en therapeutisch beleid en strategieën voor de aanpak van resistente TB binnen het NTP in de DRC. Laboratoriumdiensten, hoewel cruciaal voor nationale ziektebestrijdingsprogramma's, zijn vaak de zwakste schakel in het gezondheidssysteem en krijgen lage prioriteit en ontoereikende middelen. Voor TB-controle is bacteriologisch onderzoek met kwaliteitscontrole essentieel voor de diagnose en behandeling van patiënten met TB. Versterking van het laboratorium is een prioriteit, zowel wat betreft de toegang tot en gebruik van bestaande diagnostiek als de ontwikkeling en implementatie van nieuwe technologieën.

Chapter 1. General introduction

TB kills more than five thousand children, women and men each day and leaves no country untouched. It is one of the leading killers among people of working age which creates and reinforces a cycle of ill-health and poverty, with potentially catastrophic social and economic consequences for families, communities, and countries. While recognizing the higher prevalence of TB among men, women, and children are also vulnerable to the consequences of TB due to gender- and age-related social and health inequalities, such as poor health literacy, limited access to health services, stigma and discrimination,...

Multidrug-resistant TB (MDR-TB) accounts for one-third of all antimicrobial resistance (AMR)-related deaths, making the global AMR agenda central to tackling TB. TB is also the principal cause of death among people living with HIV/AIDS.

The global TB targets will not be met without new and more effective tools and innovative approaches for prevention, diagnosis, treatment, and care. Persistent funding gaps impede progress towards ending TB.

Although a concern to all people, TB disproportionately afflicts the poorest and the most vulnerable populations. Tobacco smoking, harmful use of alcohol and other substance abuse, air pollution, exposure to silica dust, living with HIV/AIDS, diabetes and malnutrition increase the risk of TB. Stigma and discrimination remain critical barriers to TB care".

Moscow Declaration to End TB. Moscow, November 2017. First global ministerial conference on Tuberculosis.

Declaration available from
http://www.who.int/tb/features_archive/Online_Consultation_MinisterialConferenceDeclaration/en/
Accessed on April 23 2018

Chapter 1. General introduction

1.1 TUBERCULOSIS, AN OVERVIEW

Tuberculosis (TB) is an infectious disease affecting the lungs (pulmonary TB) or other tissues (extrapulmonary TB) and is caused by slowly growing mycobacteria, of the species *Mycobacterium tuberculosis*. Though evidence of TB can be found throughout history for several millennia, the causative pathogen was only detected in 1882 by Robert Koch. Typically only a small proportion of those infected with *M. tuberculosis* will develop overt TB over their lifetime; many will remain latent carriers. The probability to progress to TB is higher in those co-infected with HIV and in those otherwise immunesuppressed through conditions as diabetes, malnutrition or other. The disease has a very protracted course and is often fatal without treatment.

TB is a major cause of illness and death worldwide, especially in Asia and Africa. The World Health Organization (WHO) estimates that in 2016, there were 10.4 million new cases of TB, and 1.7 million deaths (including 0.4 million among people with HIV) (1). Although the incidence of TB has decreased in many countries over the past decade, in many sub-Saharan African countries it continues to rise, as a consequence of the HIV epidemic. In the past 10-15 years, TB case numbers have increased 300-400% in some high HIV-prevalence countries of Africa, mainly because HIV increases the risk of disease reactivation. With poor treatment outcomes due to multidrug resistance (MDR) and the collapse of the health system, Eastern Europe has also seen an increased magnitude of TB in recent years. On a positive note, WHO estimates that between 2000 and 2016, 53 million lives were saved through TB diagnosis and treatment.

However, the global burden of TB is mostly concentrated in the developing world, and 80% of all cases occur in the 22 so-called Highest-Burden Countries (HBCs). In 2016, the list of HBCs was updated and brought to 30 HBCs for TB, 30 for MDR-TB and 30 for TB-HIV co-infection for the period 2016-2020. Over 95% of all TB deaths occur in low- and middle-income countries. TB causes more deaths than any other infectious disease worldwide and is a serious threat to global health security. Ending the TB epidemic by 2030 is among the health targets of the Sustainable Development Goals (SDG).

This mycobacterial infection is spread when people with active disease cough, sneeze or spit, and others get infected by inhaling the contaminated droplets. TB patients with a positive sputum smear are the most infectious and hence the main drivers of TB transmission. TB control programs concentrate therefore on pulmonary TB as control is based on the assumption that

treating all patients who discharge TB bacilli will interrupt the transmission chain in a community (2,3).

Sputum smear microscopy, first used in the diagnosis of TB in 1882, as the standard method in low-resource settings for diagnosing TB. It identifies the most infectious patients, who are also at greatest risk of dying from TB. However, the role of smear microscopy for TB control has become controversial since it lacks sensitivity especially in the context of HIV co-infection with its paucibacillary specimens (4,5). In recent years, the development of new diagnostic tools was set as a priority for the research agenda and WHO and the Stop-TB Partnership have strongly advocated for the improvement of TB diagnostics (6,7). Several promising TB diagnostic tests are now in the pipeline and are expected to have an impact on the detection and global control of tuberculosis. There is a consensus that a novel TB diagnostic test, more sensitive and simple to perform, is needed to improve case finding.

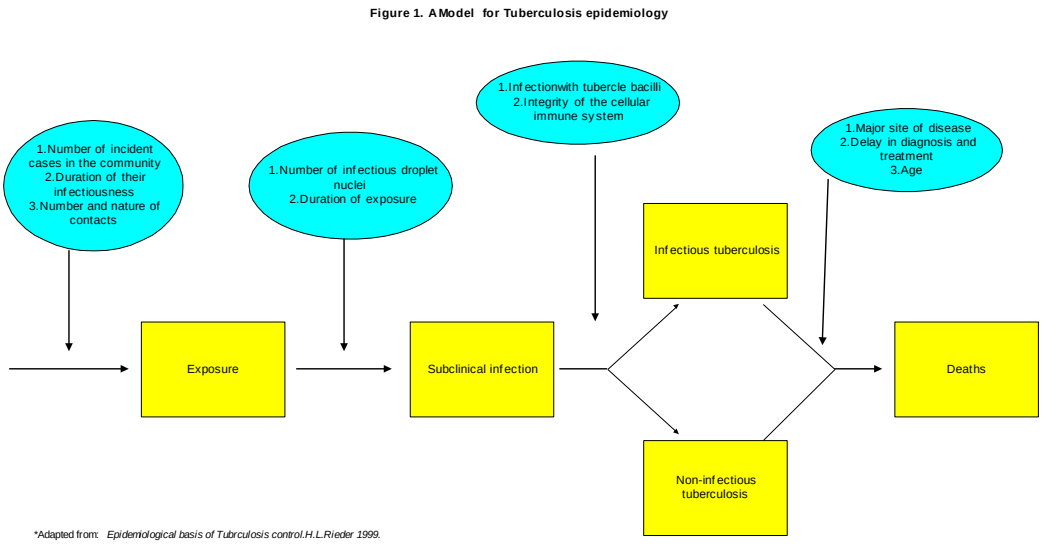
The development of drug resistance in *Mycobacterium tuberculosis* was first documented in the late 1940s, soon after streptomycin monotherapy was introduced for tuberculosis treatment. Trials conducted by the British Medical Research Council established that patients infected with drug-resistant strains were less likely to be cured but that combination chemotherapy could prevent the emergence of drug resistance(8). Nevertheless, it was only in the early 1990s that drug-resistant tuberculosis began to receive global attention as a public health threat. This coincided with the detection of outbreaks of multi-drug resistant (MDR) tuberculosis (defined as resistance to at least rifampicin and isoniazid) that were associated with high mortality among patients co-infected with HIV, such as the one in New York in 1991 (9). MDR-TB is now considered as a serious threat which jeopardizes the worldwide efforts to control TB. Conventional methods for diagnosing MDR-TB are slow and cumbersome, requiring at least two months for test execution and the treatment is complicated. Recently, mycobacterial strains have emerged that are XDR or Extensively Drug-Resistant, meaning they are on top of being INH and RIF-resistant, also resistant to Fluoroquinolones (FQ) and at least one of the second-line injectable drugs (10). Patients with MDR-TB require particular attention to avoid the development of XDR-TB, and those who have XDR-TB require rigorous and individualized treatment, with the frequent addition of third-line agents. Available evidence on XDR-TB and its treatment is limited, but experience in some well-controlled settings shows significantly lower treatment success rates compared to MDR-TB.

We review in this chapter the epidemiological evidence, the current diagnostic and therapeutic options for TB control as recommended by WHO, the global programmatic approaches to TB control as well as the challenges at country level in the Democratic Republic of the Congo (DRC). We used the peer-reviewed literature, WHO policy documents and our own experience as Director of the National TB Control Program in the DRC to compile this review.

1.2 EPIDEMIOLOGICAL BASIS OF TUBERCULOSIS CONTROL

Efficient TB control activities depend on a correct understanding of the etiologic agent, the clinical presentation of TB, the epidemiology of TB, and how to apply the tools currently available for the control of TB. Important to remember is that infection with *M. tuberculosis* does not always lead to active TB disease. Even if the infection evolves to overt clinical disease, it does – in the vast majority of cases- not do so immediately, but after a considerable lag time. **Figure 1** shows a basic transmission model of TB that was adapted from a classification of the American Thoracic Society and the Center for Disease Control and Prevention.

Figure 1. An epidemiological model of tuberculosis (Adapted from H.L. Rieder 1999)



According to this model, four distinct steps in TB pathogenesis can be identified: exposure, infection, disease, and death. Once an individual is exposed, there are factors which determine the risk of infection, disease, and death from TB.

1.2.1 Exposure to TB bacilli

The risk of exposure to TB bacilli is determined by the number of new infectious TB cases in the community, the duration of their infectiousness and the number and nature of the interaction between a case and a susceptible contact per unit of time of infectiousness.

The duration of infectiousness of a TB patient is the main determinant of the risk of the general population to become exposed to a case. A short duration of infectiousness lowers the risk of others to get exposed, but that risk increases if the infectiousness of a case is prolonged. At the time the diagnosis of a sputum smear-positive index case is made, the proportion of contacts around him/her found to be already infected is around 30 to 40 percent (11).

Several factors could influence the number and nature of possible case-contact interactions in the community. These factors will vary mainly according to individual behavior. These factors are population density, family size, differences in climatic condition, the age of the source of infection and the gender (12).

1.2.2 Infection with TB bacilli

Transmission of *Mycobacterium tuberculosis* is airborne by infectious droplet nuclei. Talking, coughing, sneezing or singing by a pulmonary TB patient all can produce infectious droplets. Experiments by Loudon and Roberts have demonstrated that from the droplet nuclei produced by one cough, about half were still suspended in air thirty minutes later (13). The infection with TB bacilli depends on the number of infectious droplet nuclei present per volume of air and the duration of exposure of a susceptible individual to that particle density.

The tuberculin test – based on the intradermal injection of a tiny volume of purified protein and measuring the delayed-type hypersensitivity reaction- allows ascertaining that a previously healthy individual got infected by *M. tuberculosis*. However, as stated above, not every infected person develops acute disease. The risk of progression (or reactivation) from latent infection to active disease is greatest within the first 2 years after infection and subsequently declines over time but does not disappear (14). While the risk of becoming infected with tubercle bacilli is primarily exogenous and determined by the characteristics of the source case, the environment and the duration of exposure, in contrast, the risk of developing the disease is endogenous, determined by the cellular immune system (12). Among the predisposing factors, recent infection and HIV infection are the strongest determinants for progression to active disease. Infection with HIV reduces cell-mediated immunity, and this facilitates the development of clinical TB (15,16). In an HIV-positive person, the annual risk of developing active TB disease

ranges from 5% to 15% depending on the degree of immune suppression, as opposed to a 10 % lifetime risk in an immune competent person.

1.2.3 The disease

TB usually presents as pulmonary TB and less frequently as extra-pulmonary. The classic symptoms of pulmonary TB are a chronic productive cough with blood-tinged sputum, fever, night sweats and weight loss. In case of active pulmonary TB, the number of acid-fast bacilli found in the sputum specimens correlates well with the potential infectiousness of the case. With a diligent technique, smear microscopy can identify smear-positive patients who are the most potent sources of transmission of TB bacilli in the community. A TB patient co-infected with HIV produces sputum that contains less bacilli (called paucibacillary sputum), and in this group of patients, the sensitivity of smear microscopy is therefore lower. Sputum smear-negative pulmonary TB cases are also relatively less infectious than a smear-positive case**.** The number of infections generated by a smear-negative pulmonary but culture-positive TB case was about 10-20% of that generated by a smear-positive (17-21). Recently, these findings were confirmed using DNA fingerprinting (22).

Extra-pulmonary TB (without associated lung involvement) accounts for 15–20% of TB in populations with a low prevalence of HIV infection (15,16). Extra-pulmonary TB can affect any organ and does not play a role in transmission.

1.2.4 Death

The case fatality rate of TB is largely determined by HIV infection, the site, and type of disease and by the type and timeliness of interventions. Pulmonary TB is of major public health importance, because it accounts for the majority of TB deaths and, secondly because these patients will transmit the disease. Smear-positive pulmonary TB has a much higher fatality rate than sputum smear-negative TB (12,20). Untreated sputum smear-positive TB will lead to death in about 30 to 40% of cases within one year, and cumulatively kills about 50 to 70% of patients within 5 to 7 years. These two factors explain why TB control programs all over the world focus heavily on the detection and treatment of smear-positive TB.

1.3 TB AND HIV COINFECTION

TB is a major death cause among people living with HIV/AIDS. Although sub-Saharan Africa carries the highest burden of the TB-HIV epidemic, the rapidly increasing HIV rates in eastern Europe and in China will also increase the number of people with TB resulting from HIV infection (1).

Infection with HIV carries an increased risk of progression of recent *M. tuberculosis* infection and of reactivation of latent *M. tuberculosis* infection (5–15% annually), depending on the degree of immune deficiency. It also increases the rate of recurrence of TB, both relapse (reactivation of latent TB) and reinfection (newly acquired infection). HIV is responsible for a large increase in the proportion of patients with smear-negative pulmonary and extrapulmonary TB (23, 24). These patients have inferior treatment outcomes, including excessive early mortality, compared with HIV-positive, smear-positive pulmonary TB patients. Tackling this problem requires rapid diagnosis of smear-negative pulmonary and extra-pulmonary TB in settings with high HIV prevalence.

HIV infection causes reduced immune competence and the consequent loss of ability to prevent the spread of the tubercle bacilli from localized granulomas (due to a decline in the number of CD4+ T cells) (16). Rapid progression from initial infection to TB disease may also occur in markedly immunosuppressed patients. Patients with active TB who are HIV-positive have a higher risk of dying from TB than those without (16,24).

Within the health system, the clinical and the programmatic management of co-infection with M. *tuberculosis* and HIV involves many challenges that call for effective collaboration between national TB and AIDS control programmes. A mechanism for collaboration between the TB and AIDS control programmes should be put in place (24, 25, 26).

1.4 DRUG-RESISTANT TUBERCULOSIS

1.4.1 Definitions

Drug-resistant TB is a major public health concern in many countries as it compromises TB care and prevention. The definitions of drug-susceptible and drug-resistant TB are given in **Table 1.** 'Pan-susceptible TB' is defined as TB caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) strains that are susceptible to all first-line anti-TB drugs.

MDR-TB is defined as resistance to the two key first-line anti-TB drugs, isoniazid (INH) and rifampicin (RMP/RIF), the two most powerful anti-TB drugs; and requires treatment with a second-line drug regimen (27-29).

The term XDR-TB appeared in the literature for the first time in March 2006, in a report jointly published by WHO and CDC, to describe a very aggressive form of disease characterized by high mortality rates (10). It is presently defined as TB caused by strains of *M. tuberculosis*, which are the following: (a) resistant to at least INH and RMP (i.e. MDR-TB), (b) plus any fluoroquinolone, and (c) to at least one of three injectable drugs used in anti-TB treatment, capreomycin, kanamycin or amikacin (30).

The term "totally drug-resistant TB" is not recognized by WHO, as it is impossible to test and ascertain in the laboratory.

Table 1. Definitions of drug-susceptible and drug-resistant TB (Adapted from G. B. Migliori et al.2010).

Type	**Definitions**
Pan (totally)-susceptible TB	TB caused by *M. tuberculosis* strains that are susceptible to all first-line anti-TB drugs;
INH monoresistant TB	TB caused by *M. tuberculosis* strains resistant only to INH;
Rifampicin Resistant –TB (RR-TB).	TB caused by *M. tuberculosis* strains resistant only to RMP;
Multidrug-resistant tuberculosis (MDR-TB)	TB caused by *M. tuberculosis* strains resistant to at least two first-line anti-TB drugs INH and rifampicin (RMP);
Extensively drug-resistant tuberculosis (XDR-TB)	TB caused by *M. tuberculosis* resistant to RMP and INH plus any fluoroquinolone, and at least one of the 3 following injectable drugs: capreomycin, kanamycin or amikacin;
Pan (Totally)-resistant TB	TB caused by *M. tuberculosis* resistant to all first and second-line anti-TB drugs.

1.4.2 Causes

Although its causes are multiple (microbiological, clinical and programmatic); drug-resistant TB is essentially a man-made phenomenon. From a microbiological perspective, resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacilli. An inadequate or poorly administered treatment regimen allows drug-resistant mutants to become the dominant strain in a patient infected with TB. **Table 2** lists the common causes of inadequate treatment, that can be summarized as inadequate regimens prescribed, inadequate supply/quality of drugs and inadequate drug intake

Table 2. Factors leading to drug-resistant TB .

Health-care provider-related	**Drug-related**	**Patient-related**
Inadequate regimens because of absent or inappropriate guidelines, non-compliance with guidelines Poor training No monitoring of treatment Poorly organized or funded TB control programmes	Poor quality Unavailability of certain drugs (stock-outs or delivery disruptions) Poor storage conditions Wrong dose or combination	Poor adherence (or poor DOT) Lack of information Lack of money (no treatment available free of charge) Lack of transportation Adverse effects Social barriers Malabsorption Substance dependency disorders

1.4.3 Mechanisms of drug resistance in Mycobacterium tuberculosis

Until the end of the 19th century, the diagnosis of pulmonary tuberculosis marked a death sentence for patients. The discovery of *Mycobacterium tuberculosis* as the causative agent of TB by Robert Koch in 1882 did pave the way for the first treatments (30). Streptomycin (SM) in 1943 and para-aminosalicylic acid (PAS) in 1946 were among the first clinically important drugs after sulfonamides (31). They were rapidly followed by isoniazid (INH) and pyrazinamide (PZA) in 1952, ethambutol (EMB) in 1961 and rifampicin (RIF) in 1966, during what is called " the golden age"

of antituberculous drugs (31). The introduction of these medicines allowed for a decline in the incidence of TB worldwide. However, TB suddenly resurged in the 1980s in association with the growing epidemic of Acquired Immunodeficiency Syndrome (AIDS) and the emergence of drug-resistant forms (32).The emergence and spread of resistant TB is now recognized as one of the most dangerous threats to the global fight against tuberculosis (33). Patients with TB-resistant strains, multidrug resistant (MDR) TB strains i.e resistant to at least RIF and INH, or extensively drug-resistant (XDR) with the MDR phenotype having in addition resistance to at least one fluoroquinolone (FQ) and one of the 3 injectable molecules of 2nd line (kanamycin, capreomycin, amikacin), require long, toxic and expensive drug treatment with unsatisfactory clinical outcomes (34,35). In **Table 3,** we summarize the molecular mechanisms leading to resistance to the main first- and second-line TB drugs: rifampicin, isoniazid, pyrazinamide, ethambutol, the fluoroquinolones and the aminoglycosides. Further research is needed on the relationship between drug resistance in *M. tuberculosis* strains and their virulence and transmissibility.

Table 3: Overview of genetic mutations related to drug resistance in TB in first-line and second-line TB drugs, and their effect

	Gene	Effect
First-line TB drugs		
INH	*inhA*	Low-level resistance to isoniazid can be overcome by normal doses of the drug (76-85)
	katG	Moderate to high level of INH resistance. While normal doses of isoniazid cannot overcome the level of resistance conferred by mutations in *katG*, evidence exists that high-dose isoniazid is beneficial in MDR-TB patients, who experienced faster conversion than patients receiving normal-dose isoniazid or placebo (Katiyar et al., 2008). When a mutation in *katG* occurs together with a mutation in *inhA*, the level of resistance is even higher, and it becomes unlikely that isoniazid continues to be of benefit (36-45).
Rifampicin	*rpoB*	High level resistance to rifampicin (46-53)
Pyrazinamide	*pncA*	Pyrazinamide (PZA) resistance testing is challenging. Phenotypic testing is prone to technical errors, including the amount of bacteria added and the pH of the medium, which should be low for pyrazinamide to work, but not so low that the bacterial growth is inhibited even without resistance. Molecular testing is difficult as well, because of the large variety of mutations found across *pncA*, of which the clinical significance is not always known, even though the vast majority of non-silent *pncA* mutations seems to induce phenotypic resistance. Despite the difficulties in testing for pyrazinamide resistance, when these tests are performed carefully, the phenotypic and molecular results show excellent correlation (54-73).
Ethambutol	*embB*	Discordant results between phenotypic testing and the detection of mutations are relatively frequent. It is not clear which testing approach (phenotypic versus molecular) is most predictive of clinical benefit (74-88).

Second-line TB drugs		
Fluoroquinolones	*gyrA*	Mutations in *gyrA* explain about 85-90% of resistance to FQs overall, and about all high-level FQ resistance. Emerging findings suggest that mutations at position 94 of *gyrA*, except for those resulting in an alanine amino acid, confer the highest FQ resistance (Rigouts et al., 2016, see repository).
	gyrB	Mutations in *gyrB* are less frequent (occur in up to 5% of phenotypically resistant isolates), and appear to be associated with low-level rather than high-level FQ resistance (89-103).
Second Line Injectable Drug	*eis*	Mutations in *eis* typically correlate with low-level kanamycin resistance and cause slightly raised MICs for capreomycin and amikacin.
	rrs	Mutations in *rrs* region 1400 are associated with high-level resistance to 2LI. Mutations at position 1402, however, confer resistance to kanamycin and capreomycin, but not to amikacin. Remember that streptomycin resistance-conferring mutations in the *rrs* gene are located in the regions 530 and 912 of the gene (104-116).
Ethionamide/prothionamide	*inhA*	Mutations in *inhA* and its promotor do not only confer low-level resistance to INH, but also effect resistance to ethionamide and prothionamide. However, absence of an *inhA* mutation does not necessarily mean thioamide susceptibility, other mechanisms exist and may be more frequent (such as *ethA* mutations).

Source: ITM course module on TB-HIV, courtesy of L.Lynen and references 75-88.

1.5 TB DIAGNOSIS

"The effective detection of TB cases by health services requires that affected individuals are aware of their symptoms, have access to health facilities and are examined by health care workers (doctors, nurses, medical assistants, clinical officers) who can recognize the symptoms of TB. Health workers must have access to a reliable laboratory and ensure that the necessary specimens are sent to that laboratory." (The lab has to communicate the results timely and the health worker has to act upon them…).

This is a complex set of activities and behaviours, and failure at any stage can cause delays in diagnosis or misdiagnosis.

Source: WHO Handbook for National Tuberculosis Control Programmes [1].

1.5.1 Clinical suspicion of TB

Coughing for more than two weeks is the most common symptom of pulmonary TB and it is therefore the major criterion for defining presumptive TB in control programs (117).

Apart from cough and sputum production, the patient may present other r**espiratory symptoms** such as shortness of breath, chest pain with coughing or breathing, back pains, haemoptysis; and non-specific **general symptoms:** loss of appetite, unintentional weight loss, fever, night sweats, fatigue.

A patient with extrapulmonary TB will present symptoms related to the specific extrapulmonary sites of the infection, such as lymph nodes, pleura, larynx, meninges, genitourinary and intestinal tracts, bone, spinal cord, eye and skin. E.g., TB of the spine will cause back pain, and renal TB might cause hematuria.

Unfortunately, in many countries with a high TB burden, diagnostic delays, i.e. the time between onset of symptoms and the day the diagnosis is made, are often huge for lack of diagnostic services.

[1] Case Detection - Implementing The WHO Stop Tb Strategy.
https://www.ncbi.nlm.nih.gov/books/NBK310769/ (accessed May 06, 2018).

1.5.2 Diagnostic tools of TB

1.5.2.1 Smear microscopy

Although microscopic examination of clinical specimens has changed little over the past 100 years, it remains the cornerstone for detection of active TB cases in low- and middle-income countries. The cell wall of *Mycobacterium spp* has a high content in mycolic acid, and shows a pink color when stained with acid-fast stains such as Ziehl-Neelsen (ZN) or Kinyoun stains. The term "acid-fast bacilli" (AFB) has therefore become a synonym for mycobacteria. A sputum smear coloured by ZN stain identifies the most infectious TB patients (118), though its sensitivity is not perfect. In research settings comparing sputum smear to culture in immunocompetent individuals, its sensitivity was often less than 60 percent (119). It requires some 5,000 TB bacilli in 1ml of sputum to yield a positive smear examination with a reasonable chance (17) and some 10,000 TB bacilli to identify a smear as positive with 95 percent probability (18). To increase the sensitivity of the technique, the optimum number of sputum specimens to establish a diagnosis has been evaluated. The first specimen was found positive in 83–87% of all patients in whom acid-fast bacilli (AFB) were ultimately detected; the second specimen was positive in an additional 10–12% and the third specimen in a further 3–5% (120). On this basis, WHO now recommends the microscopic examination of two sputum specimens (previously three).[2] WHO further recommends that at least one specimen should be obtained from an early morning sample because the yield of AFB appears to be greatest then. Repeated sputum smear microscopy can diagnose pulmonary TB in up to two-thirds of active cases. In nearly all clinical circumstances in settings of high TB prevalence, identification of AFB by microscopic examination is highly specific for the *M. tuberculosis* complex (117).

Sputum specimens should be obtained for microscopic examination from all presumptive pulmonary TB patients. Sputum collection inevitably leads to droplet production that are highly infectious if the patient has untreated pulmonary TB. Sputum collection should therefore be organized in areas with good ventilation or, if not available, outside the building. Sputum smear specimens should ideally immediately be examined by microscopy and at any rate no later than 5 to 7 days after they have been collected (117).

Sputum smear microscopy is not adequate for the diagnosis of extrapulmonary TB as the appropriate specimens are more difficult to obtain as relatively few *M. tuberculosis* organisms are present in extrapulmonary sites. For example, microscopic examination of pleural fluid in

[2] A reduction in the number of specimens examined for screening TB suspects from three to two was recommended by WHO and endorsed by the Strategic Technical and Advisory Group for Tuberculosis in June 2007.

tuberculous pleuritis and tuberculous meningitis detects AFB in only about 5–10% of cases (121,122). Given the low yield of microscopy, both culture and histopathological examination of tissue specimens, such as those that may be obtained by needle biopsy of lymph nodes, are important diagnostic tests for extrapulmonary TB (117).

Compared with conventional direct microscopy, fluorescence microscopy offers advantages as time savings and lower workload . In fact at least 1minute is recommended for reading a negative smear with auramine staining rather than 5 minutes for ZN staining (119). A microscopist can properly examine at least 100 smears per days by fluorescence microscopy compared with only 20-30 ZN-stained smears per day. Equipment costs might limit the wider use of fluorescence microscopy. The classical fluorescence microscope costs between $10,000 and $20,000 and uses an intense ultraviolet light source, such as a high-pressure mercury lamp, which typically costs $200–$300 and lasts only 100–200 h (119). A further disadvantage of mercury lamp fluorescence microscopy is that a continuous supply of standard electrical power with minimal voltage fluctuations is needed. These requirements are often difficult to reach in developing countries. Light-emitting diodes (LED) have brought many advantages such as a lifespan of 20,000 hours and replacement costs that may be less than $5. Moreover, they also excite dyes such as auramine and rhodamine to fluoresce (119). Recently to overcome the classical conventional and fluorescence microscopy limits on TB detection, National TB Program DRC has introduced the Light Emitting Diode (LED) fluorescence system on the conventional microscopes. Under routine health centers conditions confronted with a high prevalence of HIV and an important workload in Kinshasa, this LED system has shown an increased sensitivity of 20% over the conventional ZN microscopy after a study evaluation period of 2 years (non-published data, NTP, Kinshasa). Contrary to the classical fluorescence microscopy, the LED system was well accepted by the users.

1.5.2.2 Radiography

Though smear microscopy is the cornerstone of TB diagnosis in low-and-middle income settings, chest X-ray (CXR) remains an important tool for triaging and screening for pulmonary TB, and it is also useful to help diagnosis when pulmonary TB cannot be confirmed bacteriologically. Although recent diagnostic strategies have given specific prominence to bacteriology, CXR can be used for selecting individuals for referral for bacteriological examination, and the role of radiology remains important when bacteriological tests cannot provide a clear answer. For smear-negative and extrapulmonary TB, CXR combined with clinical information is often essential to reach a diagnosis. As no chest radiographic pattern is absolutely specific for pulmonary TB, the diagnosis of smear-negative TB is always presumptive and must be based on other clinical and epidemiological information, including failure to respond to a course of broad-

spectrum antibiotics and exclusion of other pathology (117). Radiographic examination is thus most useful when applied as part of a systematic clinical approach to evaluate patients whose symptoms and/or findings suggest TB but whose sputum smears are negative.

Recent advances in radiological techniques, such as computer-assisted digital radiography, portable devices, wireless techniques and electronic data archiving and retrieval, have allowed more opportunities in the TB management. However presumptive TB cases in low-income countries have limited access to these technologies due to their prohibitively high costs.

1.5.2.3 Culture techniques

Growth detection techniques based on the culture of TB bacilli are considered as the reference standard for TB diagnosis (123,124). AFB culture on selective media remains the most sensitive method for detecting Mycobacterium tuberculosis complex in clinical specimens and allows subsequent strain identification and drug susceptibility testing (DST). The most commonly used type of culture media are egg-based (Löwenstein-Jensen), agar-based (Middlebrook 7H10 or 7H11 medium) and liquid (Middlebrook 7H9 and 7H12). Mycobacterial growth seems to be slightly better on the egg-based medium but more rapid on the agar medium if one compares only the solid media. Growth in broth media is faster and has a higher yield than growth on solid media.

Recently some commercial systems for mycobacterial growth detection on liquid media were introduced. Semi-automated or fully automated culture systems such as BACTEC 460 (Becton Dickinson Microbiology Systems, Sparks, MD), mycobacterial growth indicator tube (MGIT) systems and ESP (Extra Sensing Power) Myco-ESP culture System II (Trek Diagnostic Systems, Inc., Westlake, OH) use Middlebrook 7H12 broth supplemented with antibiotics and material for mycobacteria detection (radiometric or colorimetric systems). Liquid media systems, whether semi- or fully automated, bring a substantial benefit in terms of recovery rate and turnaround time in TB detection The recovery rates in BACTEC 460 were ranked from 89.4 to 99.6%, from 73.8 to 96.4% in MGIT compared to 54.9 to 100% in the classical Löwenstein-Jensen (LJ) (125-129). The radiometric system BACTEC 460 had the highest sensitivity in the detection of *Mycobacterium tuberculosis.* Table 4 summarizes the recovery rate of *Mycobacterium tuberculosis* from sputum specimens with liquid and solid media.

Table 4 Recovery rate of Mycobacterium tuberculosis from sputum specimens with known infection in BACTEC 460, MGIT 960 and Löwenstein-Jensen (LJ) medium

	N	**BACTEC 460**	**MGIT 960**	**LJ**
Pfyffer et al.(123)	1500	89.4	81.4	75.2
Somoskovi et al.(124)	387	92.7	96.4	81.3

Macondo et al.(125)	531	-	91.9	54.9
Diraa et al.(126)	405	-	73.8	100
Cruciani et al.(127)	14745	99.6	85.8	76.0

- : No data available

Non-commercial or "home-made" liquid culture growth detection methods, which depend on very inexpensive reagents, have been developed. They might be suitable for wider use in regional reference laboratories. However, they lack standardization and cross-contamination is frequent. Therefore there is insufficient data at present to support their use. We have some personal experience to report in this regard, that is however not published. At the Institut National de Recherche Biomédicale (INRB) in Kinshasa, the Democratic Republic of the Congo (DRC), Kaswa et al. evaluated the performance of the inexpensive homemade 7H9 broth for the recovery rate and time to detection of MTBC and compared these results with those of the MGIT 960, BACTEC460TB and Lowenstein-Jensen (LJ) media (unpublished data). The 7H9 tubes were weekly centrifuged, an aliquot was stained, and considered positive when cord formation was detected by smear examination. BACTEC460 TB was considered as the reference test. The 7H9 broth showed a sensitivity of 96% and a specificity of 58%. The figures were 94% and 93%, respectively with MGIT 960. Due to the frequent manipulations to retrieve AFB using smear microscopy, the percentage of false positives was quite high in 7H9. Managing liquid media manually involves more technical manipulations which may lead to an increased risk of cross-contamination.

A very important parameter is the turn-around time of a test, i.e. the time required to get results. The evidence we retrieved on the average number of days required for detection of all *Mycobacterium tuberculosis* isolates with the liquid and solid media in our literature review is summarized in **Table** 5. The radiometric BACTEC 460 was the fastest system in detection of *Mycobacterium tuberculosis*. 7H9 "homemade" presented similar TTD to the liquid culture systems.

Table 5 The mean time to detection (days) of all Mycobacterium tuberculosis isolates (smear-negative and smear-positive for two commercial liquid media compared to Löwenstein-Jensen (LJ).

	BACTEC 460	MGIT 960	LJ
Pfyffer et al.(52)	13.5	14.0	23.1

Somoskovi et al.(53)	16.8	13.2	36.2
Macondo et al.(54)	-	10.5	20.1
Diraa et al.(55)	-	15.3	25.4
Cruciani et al.(56)	15.2	13.2	25.8

For an accurate and rapid response on the challenge of co-infection HIV and TB, smear-negative pulmonary TB has become an important issue. Usually patients with a high degree of immune suppression provide paucibacillary sputa that smear microscopy cannot detect. Since culture is more sensitive than microscopy, it is useful for the detection of smear-negative patients. **Figure 2** shows that the mean TTD of smear-negative sputum specimens was much longer than smear-positive specimens. In the meta-analysis by Cruciani et al.(127), TTD in smear-negative pulmonary TB patients was 18 days in BACTEC460, 16.5 days in MGIT960 and 33.7 days in LJ medium. In the Pfyffer et al. (123) study the TTD in smear-negative patients was 18.1 days in BACTEC460, 15.6 days in MGIT960 and 28.4 days in LJ medium. In both studies the mean TTD in smear-negative specimens were not shorter than 2 weeks.

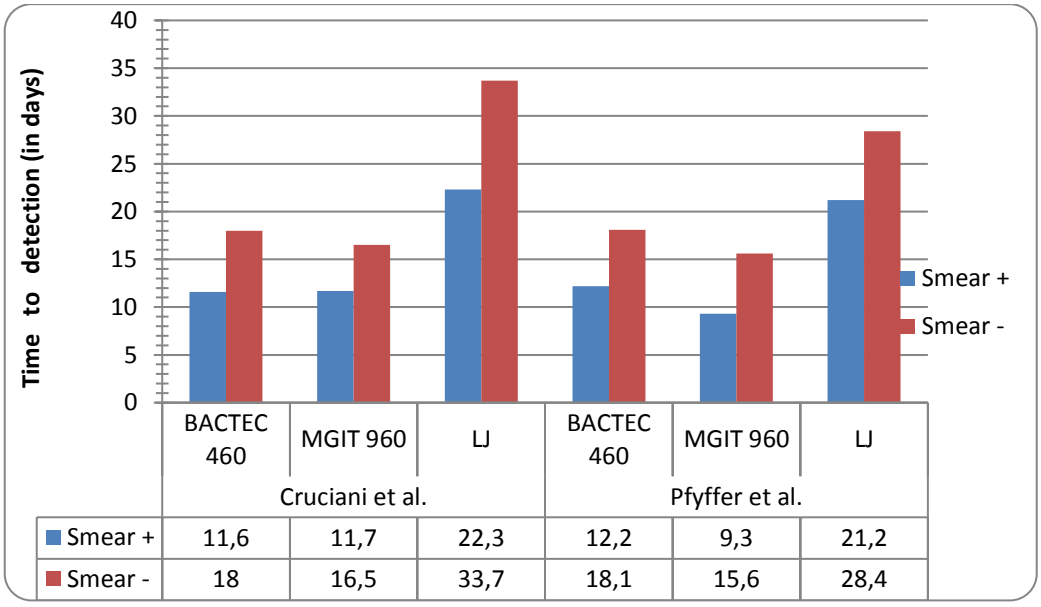


Figure 2. The mean time to detection (in days) comparing smear-positive versus smear-negative pulmonary TB in BACTEC 460, MGIT960 and LJ

In conclusion, the major advantage of liquid media is that they allow rapid detection of mycobacteria growth within 1-3 weeks, where growth does take 3-8 weeks with solid media (128). Even if the performance of growth detection methods is highly depending of the correct use of techniques, nevertheless, culture of AFB on both solid and liquid media is the most sensitive method for the isolation of *Mycobacterium tuberculosis*. All those phenotypic growth methods could be used for drug susceptibility testing (DST).

In recognition of their superior speed and sensitivity, BACTEC 460, and other radiometric liquid culture systems have been in wide use in high biosafety mycobacteriology laboratories in developed countries for more than a decade. The difficulties involved in working with radioactive waste, the necessity of expensive equipment for the detection of radioactive gas, and the cost of materials tend to limit the use of these systems in low-resource settings.

Overall, the major limitations of growth detection methods are the long turn-around time. Infrastructure and staff limitations are also crucial bottlenecks which limit not only the use but also the accessibility of these techniques, especially in developing countries where the majority of patients are poor. Recently, alternative growth detection methods for liquid culture employing oxygen quenching have been described and commercialized that show an accuracy comparable to BACTEC 460TB (125,127-128). Although all these automated and semi-automated techniques offer an attractive enhancement (not replacement) for solid culture media, the cost of these commercial systems is currently considered too high for most developing countries health systems. However a more affordable price for liquid medium and other consumables has been negotiated by the Foundation for Innovative New Diagnostics (FIND) especially for countries depending on outside funding for TB control activities.

1.5.2.4 Nucleic Acid Amplification Techniques

To address the issue of rapid and accurate diagnostic tests for the control of TB, Nucleic Acid Amplification Techniques (NAATs) have been developed for direct detection and identification of *Mycobacterium tuberculosis* in clinical specimens. These techniques reduce the diagnostic turnaround time from weeks to hours (58).

- Performance of NAAT

The performance of some available commercial methods which are capable of detecting *Mycobacterium tuberculosis* directly from respiratory specimens are summarized in Table 6.

Sensitivity of the several NAATs varied widely whereas specificities have been shown to be extremely high. The overall test sensitivity in sputum specimens (compared with culture and clinical diagnosis) ranged from 75.0 to 100% and was shown to be higher for smear-positive specimens (84.2 to 100%) while dropping to 33.3 % in smear-negative specimens. The overall test specificity ranged from 96.5 to 100%. An important advantage of NAATs is the shorter turnaround time. Most of the results were available within 2-7 hours (129-139). The variable sensitivity of NAATs, especially in smear-negative specimens is a serious drawback. Also, in the context of HBC, with the huge sample load to examine in often precarious laboratory conditions, these techniques may be prone to massive cross-contamination. Because of the needs of adequate infrastructure, the high price of consumables and the need for highly trained personnel, this technology is currently only available in reference or national laboratories and it is unlikely that the current formats will be accessible one day in the remote areas where the majority of patients are located.

Table 6: Performance of some commercial direct amplification tests for detection of Mycobacterium tuberculosis in respiratory specimens

STUDIES	Method	No. of specimens	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%) for:	
							Smear-positive	Smear-negative
Rajalahti et al. (129)	AMPLICOR	324	83	99	97	95	90	68
Reischl et al. (130)	AMPLICOR	643	84.2	99.1	NA	NA	95.4	50
Bogard et al. (131)	AMPLICOR	5077	85.2	99.7	96.4	98.8	96.1	71.7
Gamboa et al. (132)	AMPLICOR	755	92.4	100	100	96.5	100	59.6
Piersimoni et al. (133)	LCx	273	75.7	98.8	96.4	90.5	91.6	58.8
	AMTD 2	273	92.8	99.4	98.5	97	100	85.3
Wang and Tay (134)	LCx	230	100	99.3	98.7	100	100	100
	PCR	230	96.1	100	100	98.1	96.9	91.7
	AMTD	230	98.6	99.4	98.6	99.4	100	87.7
Brown et al. (135)	LCx	42	79.2	100	100	77.2	84.2	55.6

STUDIES	Method	No. of specimens	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%) for:	
							Smear-positive	Smear-negative
	PCR	42	75.0	100	100	77.5	84.2	33.3
Scarparo et al. (136)	AMTD 2	296	85.7	100	100	90.4	91.7	65.5
	PCR	296	94.2	100	100	96	98.9	95
Della-Latta & Whittier (137)	AMTD 2	1385	97.1	99.5	NA	NA	100	92.9
	PCR	1380	96.7	100	NA	NA	97.4	95.9
Piersimoni et al. (138)	AMTD 2	331	88	99.2	NA	NA	93.4	62.5
	DTB	331	94.5	99.6			98.7	75
Pfyffer et al. (139)	DTB	799	97.9	96.5	63.9	99.9	100	92.3

PPV: Positive predictive value; NPV: Negative predictive value; NA : Not available; AMTD: Amplified Mycobacterium tuberculosis; LCx: Ligase Chain reaction; PCR: Polymerase chain reaction.

DTB: Becton Dickinson Probe Tec Direct Assay.

- **Other molecular tools**

In recent years, rapid and sensitive tests based on molecular methods, the loop-mediated isothermal amplification (TB-LAMP) test (Eiken Chemical, Tokyo, Japan) has become available to complement existing conventional tests for detecting *Mycobacterium tuberculosis* complex. LAMP technology requires less equipment than conventional PCR. WHO has also approved the urine-based lateral flow lipoarabinomannan (LF-LAM) assay (140) to assist with the diagnosis of TB among seriously ill people living with HIV/AIDS (PLHIV) (See **Figure 3**). This test can be used as a point-of-care test and provides results in just minutes.

Figure 3. ***The TB LAM Ag test***

1.5.3 Drug resistance testing (DST)

The emergence of resistant strains justifies the determination of TB susceptibility in all TB cases. DST determines whether a TB strain is susceptible to particular antibiotics: a result indicating that the strain is susceptible to particular agents means that treatment with those antibiotics will most likely be successful; a result indicating that a strain is resistant means that there is a high possibility that treatment with those agents will fail and, therefore, other agents should be used. Thus, using standardized and reliable DST for *M. tuberculosis* provides guidance on treating a patient. For this purpose, two types of methods are available: phenotypic methods that rely on the detection of growth of *M. tuberculosis* strains on culture media supplemented with

antibiotics, and genotypic or molecular methods that rely on the detection of mutations target genes, recognized as responsible for resistance to anti-TB drugs.

1.5.3.1 Phenotypic methods

Phenotypic methods involve culturing *M. tuberculosis* in the presence of anti-TB agents to detect growth (which indicates resistance) or inhibition of growth (which indicates susceptibility). Phenotypic DST methods are performed as direct or indirect tests in solid or liquid media. Direct testing involves inoculating drug-containing and drug-free media directly with a concentrated specimen. Indirect testing involves inoculating drug-containing media with a pure culture grown from the original specimen. Three methods are commonly used: the proportion, absolute concentration and resistance ratio methods. For first-line anti-TB agents, the results obtained do not differ significantly among the three methods.

The most commonly used phenotypic method for measuring TB susceptibility is the proportion method, which is performed on solid medium (Löwenstein-Jensen medium, Middlebrook medium 7H11 or Middlebrook 7H12) or liquid medium (BactecTM medium, Middlebrook medium 7H9 or "mycobacteria growth indicator tube" medium (MGIT) to determine the proportion of bacilli resistant to each anti-TB drug in a given bacillary population. The Löwenstein-Jensen (LJ) solid-state antibiogram, or Canetti's ratio method is the reference method (30).

1.5.3.1.1 DST on solid media

The LJ solid-medium antibiogram principle is to seed several dilutions of the bacillary suspension on culture media with or without (controls) antituberculous drugs, which allows to measure the proportion of bacteria resistant to a given concentration of antibiotic. A strain is said to be susceptible if the proportion of resistant mutants is less than 1% for isoniazid, rifampicin, streptomycin, ethambutol, and less than 10% for pyrazinamide. The results are obtained **8 to 10** weeks after the sampling when the antibiogram is carried out from colonies of the initial culture (indirect antibiogram). This delay can be shortened between 4 and 6 weeks if the antibiogram is done directly from the positive sample on microscopic examination. The antibiogram in liquid medium allows for a faster result, in 10 days. Here the result is read by growth inhibition in vials containing antibiotics. It is compared to the growth observed in control vials without antibiotics inoculated under the same conditions as the test (100% controls) and the control vials inoculated with the 1/100 dilution (1% controls) (141-142).

1.5.3.1.2 DST on liquid media

The Mycobacterial Grown Indicator Tube (MGIT) system is based on the inoculation of tubes containing critical concentrations of the different antituberculous agents and control tubes, followed by a daily fluorescence reading at 365 nm. A strain is said to be resistant if the fluorescence is positive within 2 days relative to the control tube (fluorescence occurring during the decrease of the O2 concentration of the medium in case of bacterial growth). The results are obtained in 3 to 14 days (on average 5 days) compared with the 28–42 days needed for conventional solid media. Because liquid culture systems have increased sensitivity and reduce delays in diagnosis, they may contribute significantly to improving patient management (30).These results are correlated with those of the proportion method correlated with those of the method of proportions (143,144).

The study of the antibiotic susceptibility of *M. tuberculosis* can also be carried out by measuring the minimal inhibitory concentrations (MIC) by the E-test method (bioMérieux, France) on Middlebrook 7H11 agar supplemented with the Oleic Albumin Dextrose Catalase culture supplement. This method, mainly used for the measurement of antibiotic MICs of non-tuberculous mycobacteria, provides results for the main anti-tuberculosis drugs in 5 to 7 days. These results are correlated with those of the proportion method (145,146).

The implementation of phenotypic DST techniques within a country requires that a laboratory with minimal biosafety conditions should be set up.WHO recommends that formal links be established between the TB Supranational Reference Laboratory (SRL) Network and National Reference Laboratories (NRL) to ensure that DST is available for both first-line and second-line anti-TB agents. Since 1999, the SRL Network has been coordinated by the Institute of Tropical Medicine in Antwerp, Belgium, and it currently comprises more than 33 laboratories distributed across all continents. Countries wishing to offer DST should seek advice from the TB SRL Network to ensure they have continuous, adequate expert input into the requirements for laboratory design, the transportation of specimens, processes, biosafety standards, standard operating procedures, schedules for maintaining equipment, and processes for external quality assessment. Current lack of capacity to put MDR-TB patients on treatment should not delay countries to build capacity for DST (147).

1.5.3.1.3 DST for first-line anti-TB agents

Phenotypic culture-based DST techniques are most accurate in detecting susceptibility to RMP and INH; results are less reliable and reproducible for SM, EMB and PZA. At a minimum, national TB-control programmes should establish sufficient laboratory capacity to detect rifampicin-resistant TB (RR-TB) or MDR-TB (MDR-TB is TB that is resistant to at least isoniazid and rifampicin). For many groups of patients, RR-TB is a proxy for MDR-TB in many settings. Persons

at risk for MDR-TB should be targeted as a priority for rapid DST. Phenotypic culture-based DST methods, using the critical concentrations recommended by WHO are the current reference standards for rifampicin resistance (148). However, a number of recent studies have raised concerns about using phenotypic DST to detect rifampicin resistance, in particular the automated liquid system (149). If rifampicin resistance has been detected, DST for resistance to isoniazid and second-line anti-TB agents should be performed, following WHO's recommendations (150).

1.5.3.1.4 DST for second-line anti-TB agents

DST for second-line anti-TB agents is more complex and cumbersome to implement. Routine DST for second-line agents is not recommended unless laboratory infrastructure and capacity have been established, rigorous quality assurance is in place and sustained proficiency has been demonstrated (151).

The recommended gold standard for DST for second-line anti-TB agents is the automated liquid system (152). Methods for the absolute concentration or resistance ratio methods on solid media for second-line anti-TB agents have not been validated.

Phenotypic DST for second-line injectable agents (kanamycin, amikacin, capreomycin) and fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin, gatifloxacin) is generally reliable and reproducible across various settings (152).

The susceptibility of *M. tuberculosis* to all fluoroquinolones used by a national TB programmes should be tested to guide the choice of the most appropriate agent for treatment.

Routine DST for other second-line agents (such as ethionamide, prothionamide, cycloserine terizidone, p-aminosalicylic acid, clofazimine, amoxicillin/clavulanic acid, clarithromycin and linezolid) is not recommended because the reliability and reproducibility tests for these anti-TB agents cannot be guaranteed.

The WHO SRL network is currently developing and validating DST methods for the new and re-purposed second-line agents (bedaquiline, delamanid, clofazimine, linezolid).

1.5.3.1.5 Non-commercial methods

Non-commercial methods of culture and DST are less expensive than commercial systems but are prone to errors due to a lack of standardization and to local variations in the methods. The performance of these methods is highly operator-dependent; therefore, it is imperative that good laboratory practices are followed, good microbiological techniques are used, and there is adequate quality assurance, supported by adequate training. Similar to the conditions needed

with commercial systems, noncommercial systems require the implementation and enforcement of stringent laboratory protocols, SOPs and internal quality controls.

The evidence base for selected non-commercial methods of culture and DST has been reviewed by WHO, and the performance of these methods has been found to be acceptable in the specific context of NRLs in selected settings only when stringent laboratory protocols are followed (153). The methods evaluated include the Microscopic Observation Drug-Susceptibility (MODS) assay, the Colorimetric Redox Indicator (CRI) methods, and the Nitrate Reductase Assay (NRA). The WHO recommendations for their use are listed below:

- MODS is a microcolony method that uses liquid culture. Drug-free and drug-containing media are inoculated, and this is followed by microscopic examination of early growth. MODS is recommended as a direct or indirect test for rapid screening of patients suspected of having MDR-TB;
- CRI methods are indirect methods. A coloured indicator is added to liquid culture medium on a microtitre plate after *M. tuberculosis* strains have been exposed to anti-TB agents in vitro. Resistance is detected by a change in the colour of the indicator, which is proportional to the number of viable mycobacteria in the medium. CRI methods are recommended for use as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDR-TB; however, the method is slower in detecting MDR-TB than conventional DST methods using commercial liquid culture and molecular LPAs, but it is less expensive;
- NRAs can be used as direct or indirect methods on solid culture. NRAs are based on the ability of M. tuberculosis to reduce nitrate, which is detected by a colour reaction.NRAs are recommended for use as direct or indirect tests to screen patients suspected of having MDR-TB; however, indirect NRA is not faster in detecting MDR-TB than conventional DST using solid culture.

Both commercial and non-commercial culture and DST systems and methods are suitable for use only by central or regional reference laboratories. Non-commercial methods are recommended for use only as an interim option while capacity is being developed for rapid genotypic DST. Furthermore, non-commercial methods have not been validated for use with second-line agents.

1.5.3.1.6 Disadvantages and limitations of phenotypic DST

Phenotypic DST techniques have some disadvantages and limitation,as listed below:

Disadvantages

- Phenotypic DST methods take longer to produce results;
- These methods are suitable for use only at the central reference laboratory level, given the need for appropriate laboratory infrastructure (particularly biosafety precautions) and the technical complexity of the techniques and methods;
- Liquid DST fails to detect some clinically relevant "borderline rifampicin resistant strains" with *rpoB* mutations which is part of the group called "disputed" mutations described previously by Van Deun (154).

Limitations

The accuracy of phenotypic DST varies according to the anti-TB agent being tested.

However, phenotypic DST is still needed as current molecular methods cannot replace phenotypic DST for second-line agents because there is incomplete cross-resistance among second-line injectable agents. Current molecular methods cannot identify resistance to specific second-line injectable agents; thus, they cannot be used to guide the choice of second-line agents included in individualized MDR-TB regimens (155).

1.5.3.2 Genotypic methods

Genotypic methods target specific molecular mutations associated with resistance against individual anti-TB agents. Understanding the mechanisms of drug resistance in M. tuberculosis did contribute to the development of rapid molecular diagnostic tools and furnish possible insights into new drug development for the treatment of TB. The use of genotypic methods that detect the mutations of the genes involved in tuberculosis resistance of M. tuberculosis is interesting because the results are available in a few days whereas with the phenotypic methods of determining the sensitivity to antibiotics, they are obtained within 5 days (BactecTM) to 21 days (proportion methods). Most genotypic methods rely on a polymerase chain reaction (PCR) reaction of a gene region or the entire gene involved in resistance. Then, the demonstration of the mutations can be realized by different methods. Another important advantage of molecular tools is that there is now a mix of diagnostic methods and resistance tests.In fact, some diagnostic methods also detect resistance as GeneXpert MTB / RIF® and Line Probe Assay (LPA).

1.5.3.2.1 Real-time amplification

Real-time amplification is used routinely for the detection of major *rpoB* gene mutations associated with rifampicin resistance. It combines PCR amplification and hybridization of fluorescent probes in an automated system that also extracts the nucleic acids in a closed system, limiting as much as possible the risk of cross-contamination of the samples. This approach provides detection of *M. tuberculosis* and resistance to RMP within 60 minutes. This approach is marketed by Cepheid (GeneXpert MTB / RIF®) and has become the standard in this field (156).

Recently Xpert® MTB/RIF was introduced. It is revolutionizing TB control by contributing to the rapid diagnosis of TB disease and drug resistance. The test simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and resistance to rifampicin (RIF) in less than 2 hours. RIF resistance is a proxy for MDR-TB because it usually co-exists with resistance to INH. Rapid diagnosis of RIF resistance facilitates an early start on effective treatment, contrasting with the long turn-around time of conventional drug susceptibility testing (DST). In comparison, standard cultures can take 2 to 6 weeks or MTBC to grow and conventional drug resistance tests can add 3 more weeks. The information provided by this novel test assay aids in selecting treatment regimens and reaching infection control decisions quickly.

The Xpert® MTB/RIF assay, as shown on **Figure 4**, is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multi-disease GeneXpert platform. The Xpert®MTB/RIF assay is performed directly on sputum, processed sputum sediment and selected extra pulmonary specimens from adults and children. GeneXpert instruments are modular, and options include systems with the capacity to have 1, 2, 4, 16, 48 or 80 independently functioning modules.

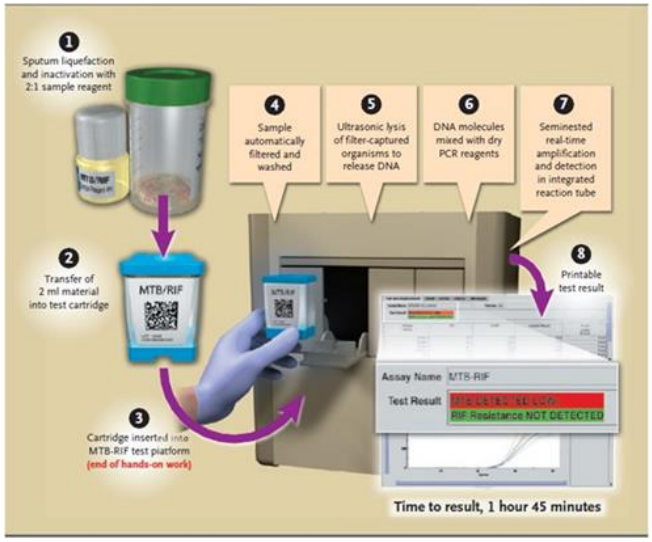
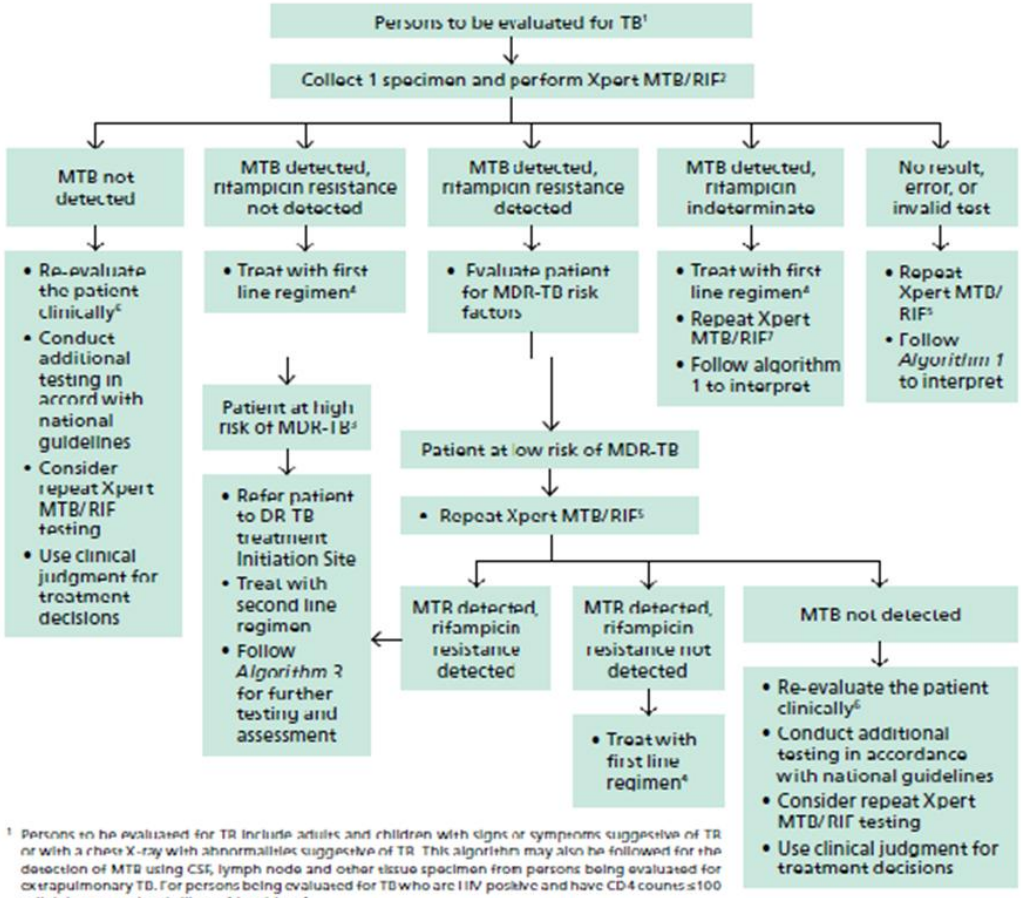


Figure 4 . Sequence of procedures on Xpert MTB/RIF. MTB/RIF is an automated, cartridge-based nucleic acid amplification test (NAAT) that uses the multi-disease GeneXpert platform

For smear positive TB, the sensitivity of the Xpert® MTB/RIF assay for detecting TB is similar to that of liquid culture. A Cochrane review published in 2014 estimated the pooled sensitivity of the assay to be 89% and the specificity to be 99%. For smear-positive, culture-positive TB, Xpert® MTB/RIF pooled sensitivity was 98% (95% CI 97% to 99%; 21 studies, 1936 participants).For smear-negative culture-positive TB, the pooled sensitivity of Xpert® MTB/RIF was 68%.The superior performance of Xpert® MTB/RIF in detecting TB over that of microscopy makes it a particularly useful tool for case-finding among people living with HIV. As a tool for detecting rifampicin resistance, Xpert® MTB/RIF had a sensitivity of 95% and specificity of 98% when compared with phenotypic DST(157).

The biosafety precautions required for Xpert® MTB/RIF are similar to those for smear microscopy, and the training is minimal, which allows the technology to be used at relatively low levels of the health system within the national laboratory network.

Figure 5 shows the recommended algorithm for MTB testing in individuals being evaluated for pulmonary TB and incorporates the goals of the End TB Strategy for the use of WHO-recommended rapid TB diagnostics (WRDs) and universal DST. This algorithm is feasible when a GeneXpert instrument is available on site or when Xpert® MTB/RIF testing can be accessed through a reliable referral system with short turnaround time. This algorithm may also be used for the detection of MTB using cerebrospinal fluid (CSF), lymph nodes and other tissue types from persons being evaluated for extrapulmonary TB.



[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node and other tissue specimen from persons being evaluated for extrapulmonary TB. For persons being evaluated for TB who are HIV positive and have CD4 counts ≤100 cells/µl or are seriously ill, see Algorithm 4.

[2] Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the Xpert MTB/RIF test. The second specimen may be used for the additional testing described in this algorithm. For persons being evaluated for pulmonary TB, sputum is the preferred specimen.

[3] Patients at high risk for multidrug-resistant TB (MDR-TB) include previously treated patients including those who had been lost to follow-up, relapsed, and failed a treatment regimen, non-converters (smear positive at end of intensive phase), MDR-TB contacts, and any other MDR-TB risk groups identified in the country.

[4] Patients should be initiated on a first-line regimen according to national guidelines. A sample may be sent for molecular or phenotypic DST for isoniazid if the patient has been previously treated with isoniazid or if there is a high prevalence of isoniazid resistance not associated with rifampicin resistance (i.e., isoniazid mono- or poly-resistance) in this setting or for DST for rifampicin if rifampicin resistance is still suspected.

[5] Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second Xpert MTB/RIF test for clinical decisions.

[6] Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Xpert MTB/RIF testing, or culture.

[7] Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Use the rifampicin result of the second Xpert MTB/RIF test in this algorithm for a decision(s) regarding choice of regimen (first line or second line regimen).

Figure 5. Preferred algorithm for rapid testing to detect MTB and rifampicin resistance

GLI Model TB Diagnostic Algorithms. Geneva, Stop TB Partnership, 2017. Available from: http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf

However, the GeneXpert platform has some limitations:

- It requires a stable, uninterrupted electrical supply; in settings where extended power outages may occur, uninterruptable power devices (UPS) and additional batteries may be needed to provide up to 2 hours of power;
- The ambient temperature cannot exceed 30 °C when in operation, and cartridges must be stored at less than 28 °C;
- The modules require annual calibration; if modules fail the calibration test, using a specific calibration cartridge, they must be exchanged, which entails the importation of additional modules and exportation of the faulty modules;
- The use of Xpert® MTB/RIF does not eliminate the need for microscopy, culture, and DST, which are required to monitor the progress of treatment and to detect resistance to other TB drugs ;
- Under routine field condition in the vast majority of low-income countries, the Xpert® MTB/RIF algorithm cannot be used.

1.5.3.2.2 PCR-RFLP

The PCR-RFLP (restriction fragment length polymorphism) consists in digesting the PCR product of the gene concerned, with a restriction enzyme having a cleavage site at a potential mutation site. It is an inexpensive method, easy to implement, but for which the presence of restriction sites at the level of mutations is essential and thus does not allow to detect all the mutations (158).

1.5.3.2.3 Hybridization (or Line Probe Assays)

Performing Line Probe Assays (LPA) involves extracting DNA from *M. tuberculosis* isolates (culture/indirectly) or directly from clinical specimens and using polymerase chain reaction (PCR) to amplify the resistance-determining region of the *rpoB* gene using biotinylated primers. Subsequently, labelled PCR products are hybridized with specific oligonucleotide probes immobilized on a strip. Colorimetric development of the captured and labelled hybrids enables the presence of *M. tuberculosis* complex to be detected as well as the presence of wildtype *M. tuberculosis*. It also detects mutations associated with drug resistance. If a mutation is present in one of the target regions, the amplicon will not hybridize with the relevant probe. Therefore,

mutations are detected by a lack of binding to wild-type probes as well as by binding to specific probes for the most commonly occurring mutations. These are the principles of the MTBDR *plus* and MTBDR*sl* (RM22052) line probe assay kits of Hain GenoType® (marketed by Hain Lifescience, Nehren, Germany) which are being widely used and implemented in Kinshasa. The post-hybridization reaction leads to the development of coloured bands on the strip at the site of probe binding, and it can be read by the laboratory technician (159).

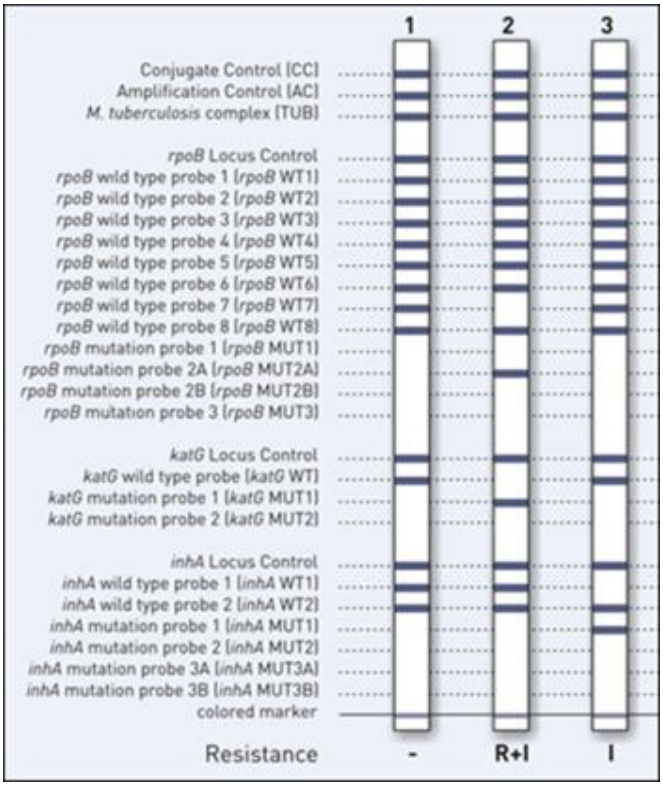


Figure 6. GenoType MTBDR *plus* assay is a genotypic method of resistance detection of isoniazid (*katG, inhA*) and rifampicin (*rpoB*). Resistance is detected when, for a gene, the MUT band is more intense than the corresponding WT (wild type) band: for *rpoB* mutation in MUT3 with attenuation of the WT8 band; for *katG*, mutation in MUT 1 and 2 with complete disappearance of the band *katG* WT; no mutation in *inhA*.

Another LPA hybridization marketed under the name InnoLiPA Rif® (Innogenetics), allows rapid and easy detection of the four most common mutations in the rpoB gene that are responsible for resistance to rifampicin (160-161). The InnoLiPA Rif ® is based on the reverse dot-blot principle. The biotin-labeled amplification products are hybridized with ten specific oligonucleotides, immobilized on a nitrocellulose membrane (a probe specific for the M. tuberculosis complex, five probes S, S1 to S5 and four probes R, R2, R4a, R4b and R5). The hybrids thus formed are detected by colorimetric method.

There is good correlation of these methods with phenotypic methods. The major interest of these techniques would be to detect the resistance of strains directly from the sample (sputum in particular) when the microscopic examination is positive.

WHO updated the policy recommendations on LPA for the detection of rifampicin resistance conferring mutations as well as utility of LPA in detection of resistance to fluoroquinolones (FQ) and second-line injectable anti-TB drugs (SLIDs) (159).

However molecular LPAs have some disadvantages as well:

- LPAs do not eliminate the need for conventional culture and DST specially for isoniazid as the sensitivity of LPAs to detect resistance to isoniazid is lower (approximately 85%) than that of culture methods;
- Available LPAs are recommended for use only on smear-positive sputum specimens and isolates of *M. tuberculosis*;
- LPAs are suitable for use at reference laboratory level; they have the potential to be used at the sub-national level if the appropriate infrastructure can be ensured (three separate rooms are required).

1.5.3.2.4 Sequencing

Genetic sequencing allows the determination of the nucleotide sequence, leading to the direct demonstration of the genetic modifications responsible for a resistance compared to the sequence of sensitive bacilli (162). The results are fast. This method, long considered to be very long and very expensive, now becomes applicable in routine thanks to the use of sequencing automata. Automatic sequencing, unlike the previous techniques, allows analysis of large DNA regions (about 1000 base pairs). Its major disadvantage is the high cost of the equipment. Genome sequencing is a highthroughput technology that is mainly used in research settings and for surveillance purposes in high-income countries. Recently, sequencing was evaluated as a method for surveillance of drug resistance in TB in resource-poor countries (163).

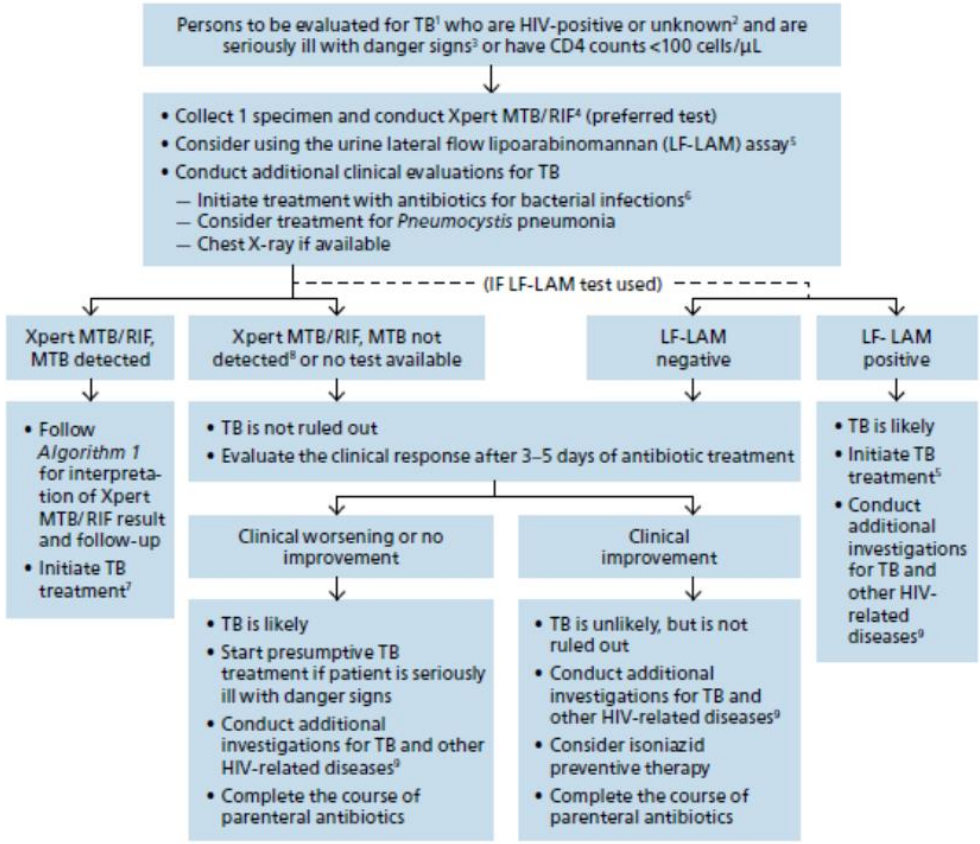
1.5.4 Diagnosis of TB in HIV/TB coinfection

HIV alters the clinical pattern of TB and complicates its diagnosis. Important differences exist in the diagnosis of TB in HIV-prevalent settings and settings of low HIV prevalence (24, 164-165).

In the early stages of HIV infection, before host immunity is significantly compromised, patients with TB have the typical symptoms of TB, and smear microscopy is usually positive. With more advanced HIV infection and compromised immune status, TB symptoms are atypical, and the smear is often negative. Paucibacillary (scanty) smears are also more frequent in HIV-infected TB patients. HIV-positive patients with smear-negative TB are more likely to die during or before diagnosis than HIV-negative smear-positive patients (24,166).

The use of chest radiography to diagnose pulmonary TB may be compromised by poor film quality, low specificity and difficulties with interpretation (165). HIV infection further diminishes the reliability of chest radiographs for the diagnosis of pulmonary TB because the disease commonly presents with an atypical pattern. Furthermore, the chest radiograph may be normal in a proportion of HIV-infected patients with sputum culture-positive TB (observed in up to 14% of such cases). However, chest radiography remains an important adjunct to the diagnosis of smear-negative pulmonary TB in people living with HIV (PLHIV).

Due to the complexity of TB diagnosis in the context of HIV, WHO recommends the use of **diagnostic algorithms** (166). An algorithm for evaluating TB among PLHIV who are seriously ill with danger signs or have CD4 counts ≤ 100 cells/µl is provided below **(Figure 7).**



[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node and other tissue specimen from persons being evaluated for extrapulmonary TB.

[2] PLHIV (People living with HIV/AIDS) include persons who are HIV positive or whose HIV status is unknown, but who present with strong clinical evidence of HIV infection in settings where there is a high prevalence of HIV or among members of a risk group for HIV. For all people with unknown HIV status, HIV testing should be performed according to national guidelines. For all adults living with HIV/AIDS regardless of CD4 cell count or clinical stage, ART should be recommended and initiating co-trimoxazole preventive therapy should be considered.

[3] Danger signs include any one of the following: respiratory rate >30 per minute, temperature>39°C, heart rate >120 beats per minute, or unable to walk unaided.

[4] The Xpert MTB/RIF test is the preferred initial diagnostic test. For persons being evaluated for pulmonary TB, sputum is the preferred specimen.

[5] The LF-LAM assay may be used to assist in diagnosing active TB in both in-and out-patients who are seriously ill with danger signs, regardless of CD4 count. Testing with the LF-LAM assay may be especially useful for patients unable to produce a sputum specimen. Whenever possible, a positive LF-LAM should be followed up with other tests such as Xpert MTB/RIF. While awaiting results of other tests, clinicians could consider initiating TB treatment immediately based on the positive LF-LAM and their clinical judgment.

[6] Antibiotics with broad-spectrum antibacterial activity (except do not use fluoroquinolones) should be used.

[7] Initiate a treatment with first-line or second-line TB drugs based on the Xpert MTB/RIF result. See Algorithm 1.

[8] If the Xpert MTB/RIF test does not detect MTB, the test can be repeated using a fresh specimen. See Algorithm 1 for a discussion of possible follow-up testing for an Xpert MTB/RIF result of MTB not detected.

[9] Further investigations for TB may include chest X-ray, additional clinical assessments, a repeat Xpert MTB/RIF using a fresh specimen, or culture. If the patient is being evaluated for extrapulmonary TB, extrapulmonary specimens should be obtained and sent for culture and abdominal ultrasound may be performed.

Figure 7. Algorithm for evaluating persons for TB, among PLHIV who are seriously ill with danger signs or have CD4 counts ≤ 100 cells/µl(166).

GLI Model TB Diagnostic Algorithms. Geneva, Stop TB Partnership, 2017. Available from: http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf

1.6 TREATMENT OF TUBERCULOSIS PATIENTS

Rapid and adequate treatment of all detected TB cases is life-saving and is one of the most effective interventions to control TB. Direct observed therapy (DOT) is the rule in WHO's TB-control paradigm. Recommended regimens have to be strictly followed, and differ in function of patient category. For treatment purposes, patients are categorized as previously untreated and previously treated.

In this section we summarize the current WHO guidelines for TB treatment that are quite detailed and elaborated. The source of the information in this section is thus mainly derived from WHO policy documents that guide the country programs. Our main source is the Handbook for National Tuberculosis Control Programmes (165). DRC's treatment policy is totally aligned with these WHO recommendations.

1.6.1 Drug-susceptible TB

1.6.1.1 New patients, previously untreated

The standardized regimens for anti-TB treatment in drug susceptible TB (first-line regimens) recommended by WHO include five essential medicines designated as "first line": isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E) and streptomycin (S) (167-169) . **Table 7** shows the recommended doses for adults and children according to WHO.

Table 7: WHO Recommended doses of first-line antituberculosis drugs for adults and children

Drugs	**Recommended dose**			
	Daily		**Three times weekly**	
	Dose and range (mg/kg body weight)	**Maximum (mg)**	**Dose and range (mg/kg body weight)**	**Daily maximum (mg)**
Isoniazid	5 (4–6)	300	10 (8–12)	–
Rifampicin	10 (8–12)	600	10 (8–12)	600
Pyrazinamide	25 (20–30)	–	35 (30–40)	–
Ethambutol	children 20 (15–25) adults 15 (15–20)	–	30 (25–35)	–
Streptomycin	15 (12–18)	–	15 (12–18)	–

A minority of TB patients (0.7–14%) treated with the above regimens experience adverse events, some major and requiring interruption of treatment, but mostly minor (165).

WHO recommends the use of fixed-dose combinations (FDCs) of drugs for the treatment of all TB patients. FDCs minimise prescription errors and patients have to ingest fewer tablets. The main advantage is that patients cannot voluntarily skip one of the drugs. Poor bioavailability of rifampicin has been found in some FDCs. The use of drug combinations of assured quality is therefore essential.

For treatment of new cases of pulmonary or extrapulmonary TB, WHO recommends a standardized regimen consisting of two phases. The *initial (intensive) phase* regimen consists of four drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) that need to be administered for two months. This is followed by a *continuation phase* with two drugs (rifampicin and isoniazid) for four months or, exceptionally, with two drugs (isoniazid and ethambutol) for six months when adherence to treatment with rifampicin cannot be ensured More details on the specific drug regimens per patient category can be found in reference 181.

Treatment guidelines for drug susceptible tuberculosis have been updated by WHO in 2017 (167). In all patients with drug-susceptible pulmonary TB, the use of thrice-weekly dosing **is not recommended in both the intensive and continuation phases of therapy and daily dosing remains the recommended dosing frequency.** However, in the field conditions in many countries, the feasibility of this recommendation is low.

1.6.1.2 Previously treated cases

A TB program deals with many TB cases who have to be retreated, for several reasons (relapse after succesful treatment, failure of initial treatment or interruption of treatment by a patient lost-to-follow-up. Basically all patients who have been treated for more than one month and have a positive sputum fall into this category of "previously treated cases".

Drug resistance is obviously more likely to be present in those previously treated patients. Ideally, they should be assessed for drug susceptibility before initiating chemotherapy (168-169). However, in settings where access to quality-assured culture and DST is limited, WHO recommended until not so long ago a standardized regimen for previously treated cases (165). Unfortunately, this standardized re-treatment regimen led to poor results in MDR-TB cases, with cure rates below 50%. This could mean that the patient would develop additional resistance to those drugs that were still effective at the start of treatment.

For this reason, treatment guidelines have been recently updated by WHO in 2017 (167). In patients who require TB retreatment, **the category II regimen should no longer be prescribed and drug susceptibility testing should be conducted to inform the choice of treatment regimen**. Patients eligible for retreatment should be referred for DST or a WRD (a WHO recommended rapid diagnostic test) to determine at least rifampicin resistance, and preferably also isoniazid resistance status.

On the basis of the drug susceptibility profile, a standard first-line treatment regimen (2HRZE/4HR) can be repeated if no resistance is documented; and if rifampicin resistance is present, an MDR-TB regimen should be prescribed according to WHO's recent drug-resistant TB treatment guidelines.

1.6.2 Drug-resistant tuberculosis

Patients in whom drug-resistant TB is diagnosed and who require treatment with second-line drugs will require specialized regimens. The different therapeutic options in drug-resistant TB include standardized conventional, standardised short regimen and individualized regimens. Since 2016, empirical treatment and individualized treatment are no longer recommended. No single treatment policy can fit all situations, and the choice between these strategies will depend on many factors, including the operational context and laboratory capacity. **Table 8** shows the basic principles that should guide a country's policy for MDR-TB regimen design:

Table 8: Principles to design a treatment policy for drug-resistant TB (Source, WHO (165)

- Regimens should be based on the history of medicines taken by the patient;
- Drugs commonly used in the country and prevalence of resistance to first-line and second-line drugs should be taken into consideration when designing a regimen;
- Regimens should consist of at least four drugs with either certain or highly probable effectiveness. In the case of unclear evidence about its effectiveness, a drug can be part of the regimen but it should not be depended upon for success. More than four drugs may be started if the susceptibility pattern is unknown, if effectiveness is questionable for an agent(s) or if extensive, bilateral pulmonary disease is present;
- Drugs are administered at least six days per week. When possible, pyrazinamide, ethambutol and fluoroquinolones should be given once per day because the high peaks attained in once-a-day dosing may be more efficacious. Once-a-day dosing is permissible for other second-line drugs, depending on patient tolerance. However ethionamide/protionamide, cycloserine and P-aminosalicylic acid have traditionally been given in divided doses during the day;
- An injectable agent (an aminoglycoside or capreomycin) is used for a minimum of six months or for four months after culture conversion, whichever is longer;
- Each dose is given as DOT throughout the treatment, and a treatment card is marked for each observed dose;
- DST, when available and from a reliable laboratory, should be used to guide therapy. However, the quality and comparability of results in DST of some first-line and most of the second-line anti-TB drugs have not been fully assessed, and DST does not predict the effectiveness of a drug with complete certainty. Despite these limitations, regimens should include at least four drugs highly likely to be effective based on DST and/or drug history of the patient;
- Pyrazinamide can be used for the entire treatment if it is judged to be effective. Many MDR-TB patients have chronically inflamed lungs, which (theoretically) produce the acidic environment in which pyrazinamide is active.

In May 2016, WHO published a new classification of TB drugs and issued new recommendations for management of drug-resistant TB :

- For MDR-TB (or RR-TB) a short standard treatment of 9 to 12 months;
- For pre-TB (XDR), therapeutic regimens of 20 months including new drugs are now recommended.

In patients with rifampicin-resistant tuberculosis (RR-TB) or multidrug-resistant TB (MDR-TB), WHO recommends a treatment regimen of 7 drugs during the intensive phase (**Table 9)**:

- Five basic drugs ensuring the effectiveness of the regimen: 4 essential drugs (one of group A, one of group B, at least two of group C) and pyrazinamid (Z). In case this regimen of at least 5 drugs cannot be administered, an agent from group D2 and other agents from group D3 must be added;
- Complementary products: strengthen the regimen by adding high-dose isoniazid and/or ethambutol.

Table 9: WHO new classification of TB antibiotics for drug-resistant management (Source WHO).

A. Fluoroquinolones	Levofloxacin Moxifloxacin Gatifloxacin		**Lfx** Mfx Gtx
B. Second line injectables	Amikacin Capreomycin Kanamycin (Streptomycin)		**Am** Cm Km (S)
C. Other essential drugs in the treatment of drug resistant TB	Ethionamide / Prothionamid Cycloserine / Terizidon Linezolid Clofazimin		Eto / Pto Cs / Trd **Lzd** **Cfz**
D. Additional drugs (Not considered as essential in the treatment of MDR-TB but used for XDR-TB)	D1	Pyrazinamid Ethambutol Isoniazid in high dose	**Z** E **H^{h}**

	D2	Bedaquilin Delamanid	**Bdq** **Dlm**
	D3	Carbopenem + Amoxicillin-clavulanate (**I**mipenem-cilastatin, Meropenem, Ertapenem) P-aminosalicylique acid); (Thioacetazon)	**Mrp/Clav** PAS TB1

By their effectiveness, the drugs of the first 3 groups (A, B and C) are essential drugs for the constitution of second-line regimens. Drugs from other groups are additional drugs that are added to the regimen to increase effectiveness and / or because of their sterilizing effects.

1.6.3 TB in HIV-infected patients

For HIV-infected patients with active TB disease, the first priority is to initiate standardized anti-TB treatment. The optimal time for starting antiretroviral therapy (ART) in these cases is well known and the decision is based on risk–benefit considerations (186-188). The principles of anti-TB treatment are the same irrespective of HIV status (199). Although ethambutol and isoniazid are included in recommendations for the continuation phase, short-course regimens that contain rifampicin throughout have better outcome, and reduce the risk of TB recurrence. The use of thioacetazone is contra-indicated in HIV-infected individuals because of the risk of fatal hypersensitivity reactions and is discouraged by WHO because of the risk of severe toxicity. Ethambutol should replace thioacetazone, especially in areas where HIV is prevalent.

Patients in whom TB is diagnosed while receiving ART should start anti-TB treatment immediately to assess whether the development of active TB reflects a failure of ART treatment failure has to be ruled out, preferably by a viral load.

1.7 TB SURVEILLANCE AND MONITORING

1.7.1 Case definitions

Case registration and reporting in TB management is essential. This has to happen according to strict definitions. We summarize the most important elements, again derived from WHO policy documents (165, 174-175), as we are using the same definitions in the subsequent studies of the thesis, and are compiled here for easy reference.

Under the current WHO policy (issued 2013-revised in 2014), **a diagnosis of TB** should be followed by specification of the type of TB, i.e. **the case definition**, which is necessary for prescribing treatment according to standardized regimens, for patient registration and reporting, for cohort analysis of treatment outcomes and for determining trends.

A **presumptive TB patient** is defined as a patient who presents with symptoms or signs suggestive of TB (previously known as a TB suspect)

A bacteriologically confirmed TB case is a patient with a biological specimen positive by smear microscopy, culture or one of the WHO-endorsed Rapid Diagnostics (such as Xpert® MTB/RIF). All such cases should be notified, regardless of whether TB treatment has started.

A clinically diagnosed TB case is a patient who has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation. Clinically diagnosed cases subsequently found to be bacteriologically positive (before or after starting treatment) should be reclassified as bacteriologically confirmed.

Bacteriologically confirmed or clinically diagnosed cases of TB are also classified according to:

- anatomical site of disease;
- history of previous treatment;
- drug resistance;
- HIV status.

1.7.1.1 Classification based on anatomical site of disease

Pulmonary tuberculosis (PTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree. Miliary TB is classified as PTB because there are lesions in the lungs. Tuberculous intra-thoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constitutes a case of extrapulmonary TB. A patient with both pulmonary and extrapulmonary TB should be classified as a case of PTB.

Extrapulmonary tuberculosis (EPTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges.

1.7.1.2 Classification based on history of previous TB treatment (patient registration group)

New patients have never been treated for TB or have taken anti-TB drugs for less than 1 month.

Previously treated patients have received 1 month or more of anti-TB drugs in the past. They are further classified by the outcome of their most recent course of treatment as follows:

- **Relapse patients** have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection);
- **Treatment after failure patients** are those who have previously been treated for TB and whose treatment failed at the end of their most recent course of treatment;
- **Treatment after loss to follow-up patients** have previously been treated for TB and were declared lost to follow-up at the end of their most recent course of treatment. (These were previously known as treatment after default patients.)
- **Other previously treated patients** are those who have previously been treated for TB but whose outcome after their most recent course of treatment is unknown or undocumented.

Patients with unknown previous TB treatment history do not fit into any of the categories listed above. **New** and **relapse cases** of TB are **incident** TB cases.

1.7.1.3 Classification based on HIV status

HIV-positive TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a positive result from HIV testing conducted at the time of TB diagnosis or other documented evidence of enrolment in HIV care, such as enrolment in the pre-ART register or in the ART register once ART has been started.

HIV-negative TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has a negative result from HIV testing conducted at the time of TB diagnosis. Any HIV-negative TB patient subsequently found to be HIV-positive should be reclassified accordingly.

HIV status unknown TB patient refers to any bacteriologically confirmed or clinically diagnosed case of TB who has no result of HIV testing and no other documented evidence of enrolment in HIV care. If the patient's HIV status is subsequently determined, he or she should be reclassified accordingly.

1.7.1.4 Classification based on drug resistance

Cases are classified in categories based on DST of clinical isolates confirmed to be *M. tuberculosis:*

- Monoresistance: resistance to one first-line anti-TB drug only;
- Polydrug resistance: resistance to more than one first-line anti-TB drug (other than both isoniazid and rifampicin);
- Multidrug resistance: resistance to at least both isoniazid and rifampicin;
- Extensive drug resistance: resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance;
- Rifampicin resistance: resistance to rifampicin detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to rifampicin, whether monoresistance, multidrug resistance, polydrug resistance or extensive drug resistance.

These categories are not all mutually exclusive. When enumerating rifampicin-resistant TB (RR-TB), for instance, multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) are also included. While it has been the practice until now to limit the definitions of monoresistance and polydrug resistance to first-line drugs only, future drug regimens may make it important to classify patients by their strain resistance patterns to fluoroquinolones, second-line injectable agents and any other anti-TB drug for which reliable DST becomes available.

1.7.2 Outcomes of anti-TB treatment

The new treatment outcome definitions make a clear distinction between two types of patients:

- Patients treated for drug-susceptible TB;
- Patients treated for drug-resistant TB using second-line treatment

1.7.2.1 Treatment outcomes for TB patients (excluding patients treated for RR-TB or MDR-TB)

Table 10 shows the possible outcomes in patients treated for drug-susceptible TB (WHO,2014). All bacteriologically confirmed and clinically diagnosed TB cases should be assigned an outcome from this list **except** those with RR-TB or MDR-TB, who are placed on a second-line drug regimen (see section 1.7.2.2).

Table 10: Definitions of treatment outcomes for patients treated for drug-susceptible TB (WHO, 2014)

Outcome	Definition
Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion;
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable;
Treatment failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment;
Died	A TB patient who dies for any reason before starting or during the course of treatment;
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more;
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases “transferred out” to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit;
Treatment success	The sum of cured and treatment completed.

Patients found to have an RR-TB or MDR-TB TB strain at any point in time should be started on an adequate second-line drug regimen. These cases are excluded from the main TB cohort when

calculating treatment outcomes and included only in the second-line TB treatment cohort analysis (section 1.7.2.2). If treatment with a second-line drug regimen is not possible, the patient is kept in the main TB cohort and assigned an outcome from among those in **table 10** in section 1.7.2.1 above.

1.7.2.2 Outcomes for RR-TB/MDR-TB/XDR-TB patients treated using second-line treatment

Table 11 lists how outcomes should be reported after treatment of drug-resistant TB;

Table 11: Definitions of treatment outcomes for patients treated for drug-resistant TB (WHO, 2014)

Outcome	Definition
Cured	Treatment completed as recommended by the national policy without evidence of failure AND three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase[a];
Treatment completed	Treatment completed as recommended by the national policy without evidence of failure BUT no record that three or more consecutive cultures taken at least 30 days apart are negative after the intensive phase[a];
Treatment failed	Treatment terminated or need for permanent regimen change of at least two anti-TB drugs because of: lack of conversion[b] by the end of the intensive phase[a], or - bacteriological reversion[b] in the continuation phase after conversion[b] to negative, or - evidence of additional acquired resistance to fluoroquinolones or second-line injectable drugs, or – adverse drug reactions (ADRs);
Died	A TB patient who dies for any reason before starting or during the course of treatment;
Lost to follow-up	A patient whose treatment was interrupted for 2 consecutive months or more;
Not evaluated	A patient for whom no treatment outcome is assigned. (This includes cases "transferred out" to another treatment unit and whose treatment outcome is unknown);
Treatment success	The sum of cured and treatment completed.

[a] For Treatment failed, lack of conversion by the end of the intensive phase implies that the patient does not convert within the maximum duration of intensive phase applied by the programme. If no maximum duration is defined, an 8-month cut-off is proposed. For regimens without a clear distinction between intensive and continuation phases, a cut-off 8 months after the start of treatment is suggested to determine when the criteria for Cured, Treatment completed and Treatment failed start to apply.

[b]The terms "conversion" and "reversion" of culture as used here are defined as follows: Conversion (to negative): culture is considered to have converted to negative when two

consecutive cultures, taken at least 30 days apart, are found to be negative. In such a case, the specimen collection date of the first negative culture is used as the date of conversion. Reversion (to positive): culture is considered to have reverted to positive when, after an initial conversion, two consecutive cultures, taken at least 30 days apart, are found to be positive. For the purpose of defining treatment failed, reversion is considered only when it occurs in the continuation phase.

1.7.3 Global Surveillance of Antituberculosis-Drug Resistance

In 1994, the Global Tuberculosis Program of the WHO, with the support of the International Union against Tuberculosis and Lung Disease (the Union), established the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. The objectives of this Global Project are to gather data on drug resistance using a standard methodology and to determine the global magnitude of resistance to four first-line antituberculosis drugs (first-line drugs): isoniazid, rifampicin, ethambutol and streptomycin and finally to monitor trends (176). Since its existence, this project has been hosted by the WHO and supported by the TB SRL Network and several technical agencies. Proficiency testing is conducted annually, and results are published regularly (177-179).

WHO issued guidelines on standardized methods for conducting surveys of antituberculosis-drug resistance, which have been regularly updated (180-184). The standard methodology includes representative sampling of patients with adequate sample sizes, standardized data collection distinguishing between new and previously treated patients and quality-assured laboratory DST supported by the SRL Network. Surveillance data are collected either through continuous surveillance systems based on routine testing of all patients with TB or periodic surveys, which are discrete studies measuring drug resistance among a selected sample of patients who are representative of an entire population of patients with TB.These standardized methods allow comparability of data within countries over time as well as between countries. Rifampicin and Isoniasid were the major first line drugs which were under active surveillance. Starting in 2006, soon after the recognition of extensively drug-resistant (XDR) tuberculosis (defined as MDR tuberculosis plus resistance to a fluoroquinolone and at least one second-line injectable agent: amikacin, kanamycin, or capreomycin) as an emerging threat worldwide (184-186), the panel of drugs tested as part of the project was expanded to include fluoroquinolones and second-line injectable agents for all patients who had received a diagnosis of MDR TB.

Despite these surveillance data, the magnitude of drug resistance is not yet known in many areas of the world with high burden of TB. Nevertheless, evidence from half the world's nations confirms that drug resistance is a serious problem worldwide. The most recent data on the percentage of patients with newly diagnosed TB who have MDR-TB are shown in **Figure 8**. The percentage remains stable, at 3% or lower in most parts of the world. However, countries in Eastern Europe and central Asia have serious MDR-TB epidemics. Usually it recognized that the

severe disruptions of drug supply after the collapse of the Soviet Union may have led to mismanagement of patient care, generating high levels of MDR-TB. The highest measured levels of MDR-TB among patients with newly diagnosed TB are in Belarus (34.1% in 2014), Estonia (19.5% in 2014), Kazakhstan (25.2% in 2013), Kyrgyzstan (26.4% in 2011), Moldova (23.7% in 2012), Russia (average across regions with data, 19.3% in 2012), Ukraine (24.0% in 2014), and Uzbekistan (23.2% in 2011) (187). Among patients with TB diagnosed previously, the percentages with MDR-TB were the highest in Belarus (69.1% in 2014), Estonia (62.1% in 2014), Kazakhstan (57.8% in 2013), Kyrgyzstan (55.1% in 2013), Moldova (62.3% in 2012), Tajikistan (52.2% in 2014), and Uzbekistan (62.0% in 2011). In Russia, even though the average percentage of patients with previously treated TB who have MDR-TB does not exceed 50%, the percentage is well above 50% in several regions (188).

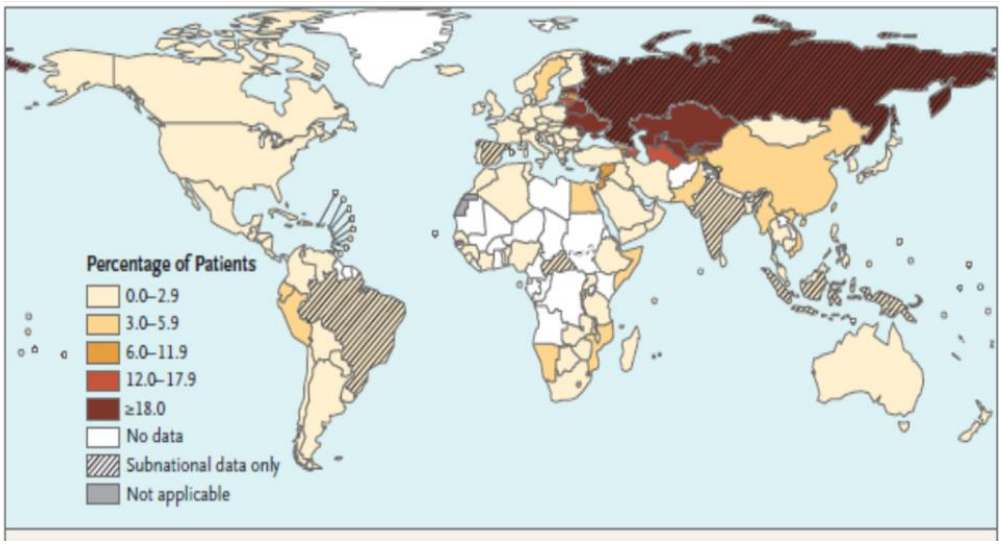
By December 2014, cases of XDR-TB had been reported by 105 countries. A total of 83 countries reported representative data from continuous surveillance or periodic surveys on the percentage of patients with MDR-TB who had XDR-TB. When these data were combined, the average percentage was 9.7% (95% CI, 7.4 to 12.1). Fourteen of these countries reported 10 or more cases of XDR TB in the most recent year for which data were available. Among these countries, the percentage of patients with MDR-TB who had XDR-TB was highest in Belarus (29.3% in 2014), Georgia (15.1% in 2014), Latvia (18.6% in 2014), and Lithuania (24.7% in 2013) (187).

After 20 years of global surveillance of resistance to anti-TB drugs, 3 main challenges remain: i) lack of trend data for many resource-limited countries (mainly in Africa and Asia), owing to insufficient capacity to conduct repeat surveys at regular intervals or to establish continuous surveillance of drug resistance; ii) limited understanding of in-country geographic distribution of drug resistance and limited capacity to detect outbreaks and hot-spot areas; and iii) limited engagement of private health providers in surveys, resulting in an inability to assess the scale of drug resistance outside cases detected in the public sector (189).

Routine testing of all patients with TB is widely recognized as the most appropriate surveillance approach for monitoring trends in drug resistance and detecting outbreaks and hot-spot regions (190). However, in most resource-limited countries in which the burden of TB and MDR-TB is the highest, routine drug-susceptibility testing is not yet accessible to all patients owing to insufficient laboratory capacity, infrastructure (including deficiencies in transportation of specimens and in data collection and management), or resources. Although a surveillance system for TB does exist in most countries, the complexities associated with drug resistance testing do not permit routine drug-susceptibility testing on all patients with newly diagnosed TB. The establishment of continuous surveillance systems for drug resistance can overcome two of the above-mentioned major challenges: a lack of understanding of trends in drug resistance and the

inability to accurately describe the heterogeneity of the epidemic within countries and detect hot-spot areas and disease outbreaks.

Figure 8. Percentage of patients with newly diagnosed Tuberculosis who have Multidrug-Resistant (MDR) Tuberculosis. Source: WHO.



The World Health Organization (WHO) suggested that in settings currently lacking the capacity for exhaustive surveillance based on routine drug susceptibility testing (DST) of all previously treated TB cases, sentinel surveillance based on a sample of previously treated cases, i.e. all first-line failure and relapse cases, should be used as an integral part of programmatic monitoring of MDR-TB. Laboratory-based surveillance should evaluate the susceptibility of *M. tuberculosis* to INH and RMP; and if resistance to RMP is present, then susceptibility to the FQs and second-line injectable agents most often used in the setting should also be tested (183).

1.8 GLOBAL RESPONSE AND TUBERCULOSIS CONTROL

1.8.1 The DOTS strategy

In response to the growing epidemic of TB, WHO introduced the Directly Observed Treatment Short-course (DOTS) in the early 1990s as a cost-effective strategy to control TB (20). Initially, DOTS had five essential components:

- Sustained political commitment;
- Access to quality-assured sputum smears microscopy;
- Standardized short-course chemotherapy for all cases of TB;
- Uninterrupted supply of quality-assured drugs;
- Recording and reporting systems that enable outcome assessment;

The WHO set at the time the global targets to diagnose 70% of new smear-positive cases and to cure 85% of these cases by 2005 and to maintain this therafter. It was expected that if these goals were met, TB incidence should decline by 5–10% annually in countries without endemic HIV. By the end of 2003, roughly 77% of the world's population lived in countries that had officially adopted the DOTS strategy (191) and the large adoption of the DOTS strategy substantially improved TB control. By 2004, more than 20 million patients had been treated in DOTS programmes and more than 16 million of them had been cured. The treatment success rate among new smear-positive TB cases had reached 83% by 2003 (just short of the 2005 target mentioned above), but in 2004 the case detection rate, was only 53% (against the target of 70% by 2005). Meeting the global target for case detection, has proven notoriously difficult, with only 61% of all incident smear-positive cases having been detected in 2006 (1).

After more than a decade of DOTS programme implementation in countries with diverse characteristics, WHO and the Stop TB Partnership recognized that the DOTS strategy is necessary for TB control, but the original five elements of DOTS are by themselves insufficient for global TB control (192).

1.8.2 The Stop TB Strategy

In 2000, the Stop TB partnership, which is a collaborative network of more than 400 organizations from the public and private sectors, was established with the objective of achieving TB control targets by 2015 and ultimately eliminating TB as a public health problem later this century. Since 2001, the Global Fund to fight Aids, Tuberculosis and Malaria (GFATM) disbursed additional resources and increased funding for TB control and research. The first Global Plan to Stop TB, for 2001–2005, achieved the majority of its 2005 targets (with the exception of the case detection rate) . Unfortunately, WHO had to recognize also that rates of progress were

insufficient to allow the targets of halving TB mortality and prevalence by 2015 to be achieved. The TB epidemic was in fact worsening, notably in Africa, mainly because of the HIV-epidemic but also in eastern Europe because of a protracted socio-economic crisis.

Therefore, in 2005, WHO began to work with a wide range of stakeholders to build on the successes of the DOTS strategy while recognizing the additional needs and challenges posed by MDR-TB and HIV-associated TB, and to adapt the innovations in service delivery made in the past five years. A new expanded strategy underpinned the development of the second Global Plan of the Stop TB Partnership and was launched in March 2006 as "**the Stop TB Strategy**".

This new Global Plan re-emphasized bacteriology as the recommended method for TB diagnosis first using smear microscopy and then culture and DST, and incorporates the original DOTS strategy plus five additional elements, as outlined in **Table 12**.

Table 12: WHO's Stop TB strategy*

VISION: A WORLD FREE OF TB
GOAL : To dramatically reduce the global burden of TB by 2015 in line with the
Millennium Development Goals(MDGs) and the StopTB Partnership targets
OBJECTIVES
- Achieve universal access to high quality diagnosis and patient-centered treatment
- Reduce the human suffering and socioeconomic burden associated with TB
- Protect poor and vulnerable populations from TB, TB/HIV and multidrug-resistant TB
- Support development of new tools and enable their timely and effective use
TARGETS
- by 2005: to detect at least 70% of new smear-positive cases and treat successfully at least 85% of these cases
- by 2015: to have halted and begun to reverse the incidence of TB
- by 2015: to have halved TB prevalence and death rates compared to 1990 levels
- by 2050: to have eliminated TB as a public health problem (<1 case per million population)
1. Pursue high-quality DOTS expansion and enhancement
a. Political commitment with increased and sustained financing
b. Case detection through quality-assured bacteriology
c. Standardized treatment with supervision and patient support
d. An effective drug supply and management system
e. Monitoring and evaluation system, and impact measurement
2. Address TB/HIV, MDR-TB and other challenges
• Implement collaborative TB/HIV activities
• Prevent and control multidrug-resistant TB
• Address prisoners, refugees and other high-risk groups and special situations
3. Contribute to health system strengthening
• Actively participate in efforts to improve system-wide policy, human resources,

financing, management, service delivery, and information systems
• Share innovations that strengthen systems, including the Practical Approach to Lung Health (PAL)
• Adapt innovations from other fields
4. Engage all care providers
• Public-Public, and Public-Private Mix (PPM) approaches
• International Standards for TB Care (ISTC)
5. Empower people with TB, and communities
• Advocacy, communication and social mobilization
• Community participation in TB care
• Patients' charter for tuberculosis care
6. Enable and promote research
• Programme-based operational research
• Research to develop new diagnostics, drugs and vaccines

*Adapted from: *WHO_STOP TB STRATEGY_factsheet_2006_eng*

1.8.3 The Sustainable Development Goals

From 2000 to 2015, global efforts to reduce the burden of tuberculosis (TB) disease were focused on achieving targets that were fully embedded within the context of the Millennium Development Goals (MDGs). These MDGs were established by the United Nations (UN) in 2000 and provided a wider framework in the UN system that endorsed the targets set for TB control. Target 6c of MDG 6 was to "halt and reverse" TB incidence. The Stop TB Partnership, described above, adopted this target and set two additional targets. These were to halve TB prevalence and TB mortality rates by 2015 compared with their levels in 1990. The global TB strategy developed by WHO for the decade 2006–2015, the Stop TB Strategy, had the overall goal of reaching all three targets (193). In 2016, the MDGs were succeeded by a new set of development goals, known as the **Sustainable Development Goals** (SDGs). Adopted by the UN in September 2015 following 3 years of consultations, the SDG framework of goals, targets and indicators is for the period 2016–2030 (194) Similarly, WHO initiated work on a new global TB strategy in 2012, which was completed in 2014. The **End TB Strategy** was unanimously endorsed by all WHO Member States at the 2014 World Health Assembly, and is valid for the period 2016–2035 (195).

In this section we provide an overview of the SDGs and in the next we will discuss the End TB Strategy, and introduce the new TB-SDG monitoring framework that has been developed by WHO in 2017. This framework is designed to focus attention on, and encourage analysis of, SDG targets and indicators that will influence the course of the TB epidemic. This is important, because achieving the ambitious targets set in the SDGs and End TB Strategy requires that broader influencing factors on the risks of developing TB and the consequences of TB disease are addressed .

For the first five years of the SDGs and End TB Strategy (2016–2020), WHO has defined three lists of high burden countries (HBCs): for TB, TB/HIV and multidrug-resistant TB (MDR-TB).

The 17 SDGs are shown in **Table 13.**

Table 13: The Sustainable Development Goals

Goal 1. End poverty in all its forms everywhere;
Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture;
Goal 3. Ensure healthy lives and promote well-being for all at all ages;
Goal 4.Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all;
Goal 5. Achieve gender equality and empower all women and girls;
Goal 6. Ensure availability and sustainable management of water and sanitation for all;
Goal 7. Ensure access to affordable, reliable, sustainable and modern energy for all;
Goal 8.Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all;
Goal 9. Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation;
Goal 10. Reduce inequality within and among countries;
Goal 11. Make cities and human settlements inclusive, safe, resilient and sustainable;
Goal 12. Ensure sustainable consumption and production patterns;
Goal 13. Take urgent action to combat climate change and its impacts;
Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development;
Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss;
Goal 16. Promote peaceful and inclusive societies for sustainable development, provide access to justice for all and build effective, accountable and inclusive institutions at all levels;
Goal 17.Strengthen the means of implementation and revitalize the Global Partnership for
Sustainable Development.

The SDG health -related goal is SDG 3. It is defined as “Ensure healthy lives and promote well-being for all at all ages”, and 13 targets have been set for this goal **(**See **Table 14**). One of these targets, Target 3.3, explicitly mentions TB: **“By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water- borne diseases and other communicable diseases”**. The language of “ending epidemics” is also now a prominent element of global health strategies developed by WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS) for the post-2015 era (196) including the End TB Strategy. As the WHO 2016 Annual Tuberculosis Report states, such language is much more ambitious than the MDG language of “halting and reversing” or “stopping” epidemics. SDG 3 also includes a target (Target 3.8) related to universal health coverage (UHC) in which TB is explicitly mentioned. The

WHO/World Bank definition of UHC is that all people receive the health services they need, while at the same time ensuring that the use of these services does not expose the user to financial hardship (197).

Table 14: Sustainable Development Goal 3 and its 13 targets.

SDG3: Ensure healthy lives and promote well being for all at all ages.
Targets
3.1. By 2030, reduce the global maternal mortality ratio to less than 70 per 100 000 live births;
3.2. By 2030, end preventable deaths of newborns and children under 5 years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1000 live births and under-5 mortality to at least as low as 25 per 1000 live births;
3.3. By 2030, end the epidemics of AIDS, tuberculosis, malaria and neglected tropical diseases and combat hepatitis, water-borne diseases and other communicable diseases;
3.4. By 2030, reduce by one third premature mortality from non-communicable diseases through prevention and treatment and promote mental health and well-being;
3.5. Strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol;
3.6. By 2020, halve the number of global deaths and injuries from road traffic accidents;
3.7. By 2030, ensure universal access to sexual and reproductive health-care services, including for family planning, information and education, and the integration of reproductive health into national strategies and programmes;
3.8. Achieve universal health coverage, including financial risk protection, access to quality essential health-care services and access to safe, effective, quality and affordable essential medicines and vaccines for all;
3.9. By 2030, substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination;
3.10. Strengthen the implementation of the World Health Organization Framework Convention on Tobacco Control in all countries, as appropriate;
3.11 Support the research and development of vaccines and medicines for the communicable and non- communicable diseases that primarily affect developing countries, provide access to affordable essential medicines and vaccines, in accordance with the Doha Declaration on the TRIPS* Agreement and Public Health, which affirms the right of developing countries to use to the full the provisions in the Agreement on Trade-Related Aspects of Intellectual Property Rights regarding flexibilities to protect public health, and, in particular, provide access to medicines for all;
3.12. Substantially increase health financing and the recruitment, development, training and retention of the health workforce in developing countries, especially in least developed countries and small island developing States;
3.13. Strengthen the capacity of all countries, in particular developing countries, for early warning, risk reduction and management of national and global health risks.

*TRIPS: Trade-Related Aspects of Intellectual Property Rights.

1.8.4 The End TB Strategy

The End TB Strategy "at a glance" is shown in **Table 15**. With regard to the main topic of this thesis, the strengthening of the diagnostic strategy, the End TB policy calls for the early diagnosis of TB and universal DST and emphasizes the critical role of laboratories. WHO-recommended Rapid Diagnostics (WRD) should be made available to all persons with signs or symptoms of TB, all bacteriologically confirmed TB patients should receive DST at least for rifampicin, and all patients with RR-TB should receive DST at least for FQs and Second-Line Injectable Drugs (SLID). Therefore all NTP need to prioritize the development of a network of TB laboratories that use modern diagnostics, have efficient referral systems, use standard operating procedures (SOPs) and appropriate quality assurance (QA) processes, and have adequate biosafety and sufficient human resources. These priorities should be comprehensively addressed in national strategic plans and adequately funded

The overall goal is to "End the global TB epidemic", and there are three high-level, overarching indicators and related targets (for 2030, linked to the SDGs, and for 2035) and milestones (for 2020 and 2025).

The three indicators are:

- the number of TB deaths per year;
- the TB incidence rate per year; and
- the percentage of TB-affected households that experience catastrophic costs as a result of TB disease.

The **2035 targets** are a 95% reduction in TB deaths and a 90% reduction in the TB incidence rate, compared with levels in 2015. The **2030 targets** are 90% reduction in TB deaths and an 80% reduction in the TB incidence rate, compared with levels in 2015. The most immediate milestones, set for 2020, are a 35% reduction in TB deaths and a 20% reduction in the TB incidence rate, compared with levels in 2015. For the third indicator (the percentage of TB-affected households that experience catastrophic costs as a result of TB disease), the milestone for 2020 is zero, to be sustained thereafter. The Stop TB Partnership has developed a Global Plan to End TB, 2016–2020 (198), which focuses on the actions and funding needed to reach the 2020 milestones of the End TB Strategy.

Progress towards UHC and actions to address health-related risk factors for TB as well as broader social and economic determinants of TB will be fundamental to achieving the targets and milestones for reductions in TB cases and deaths.

Table 15: The End TB Strategy: vision, goal and pillars (Source WHO, 2016).

VISION	**A WORLD FREE OF TB** -zero deaths, disease and suffering due to TB			
GOAL	**END THE GLOBAL TB EPIDEMIC**			
INDICATORS	**MILESTONES**		**TARGETS**	
	2020	**2025**	**SDG 2030***	**END TB 2035**
Percentage reduction in the absolute number of TB deaths (compared with 2015 baseline)	35%	75%	90%	95%
Percentage reduction in the TB incidence rate (compared with 2015 baseline)	20%	50%	80%	90%
Percentage of TB-affected households experiencing catastrophic costs due to TB(level in 2015 unknown)	0%	0%	0%	0%

*Targets linked to the Sustainable Development Goals (SDGs).

1.9 TB CONTROL IN THE DRC

1.9.1 Geography

The Democratic Republic of Congo (DRC) has a surface area of 2,345,409 km² and an estimated population of **92,639,856** in 2018, with a population density of 36 inhb/km^2: 45% of the population is under 15 years of age and 70% of them are living in rural areas. The DRC is a Central African country straddling the Equator between 5 ° 20 'north latitude and 13 ° 28' south latitude and between 12 ° 10 'and 33 ° 27' east longitude. 'somewhere else. It shares 9 165 km of borders with 9 countries, namely, the Republic of Congo and the enclave of Cabinda (Angola) in the west; Central African Republic and South Sudan to the north; Uganda, Rwanda, Burundi and Tanzania to the east; Zambia to the southeast and Angola to the south.

1.9.2 Political and social background

The recent history of the country has been marked by two decades of military and civil unrest which have slowed down the country's development. Since 2016 the country faces again increasing insecurity and forced migration in the central and east provinces. The end of 2016 should have been marked by the 3rd presidential elections. The convening and organization of these elections were not carried out at the beginning of 2016 by the ruling power. The elections were postponed. Protests took place across the country and a national dialogue was launched to find a solution to the political crisis.

Currently the DRC is experiencing mass poverty with large disparities in income level between urban and rural areas and high dependence on informal employment. Life expectancy at birth increased from 49 years in 1990 to 52 years for both sexes in 2012.Despite a decline in the poverty rate from 71% to 64% between 2005 and 2012, the DRC is still among the poorest countries in the world and ranks 176 th out of 187 countries in the latest Human Development Index (2015). Per capita gross domestic product (GDP) in 2015 was $ 442 and was among the lowest in the world (199-200).The 2013 Demographic and Health Survey (DHS) II showed that (201):

- The proportion of households using safe drinking water (from an improved source) increased from 46% in 2007 to 49% in 2013. In rural areas, this proportion has increased from 24% to 32% and urban area from 80% to 85%;
- Access to improved sanitation facilities remains limited in the DRC. Only 18% of households (21% in urban areas and 17% in rural areas) use unshared improved toilets;

- Only 14% of households (42% in urban areas against 0.4% in rural areas) have electricity in their homes;
- An improvement in the index of economic well-being in urban areas. The results show that 85% of the urban population are classified in the first two quintiles of which more than half belong to the highest quintile (57%). This proportion was estimated at 46% in 2007, thus suggesting improved living conditions for urban populations during this period. On the other hand, in rural areas, 57% of the household population is classified in the first two quintiles. This proportion, although declining, is not very different from that observed in 2007 (60%). The index constructed from information on households' possession of certain durable goods (television, radio, car, etc.) and on certain housing characteristics (availability of electricity, type drinking water supply, type of toilet, flooring material, number of rooms used for sleeping, type of fuel for the kitchen, etc)

1.9.3 Health Sector Organization

A recent administrative reform divided the country in 26 provinces (shown on **Figure 9)** and led to the establishment of 26 provincial health divisions that are in charge of managing the health services The peripheral level consists of 516 health zones with 472 hospitals and 8,266 primary health centers. The health system suffers from difficult geographical access, fragmentation, inadequate resources, and weak management. Not surprisingly, there is huge under-utilisation and inequity in the use of basic health services (202).

Figure 9: Map of DRC showing the 26 provinces after the administrative reform of 2014.

1.9.4 TB Services in DRC

The National TB Control Program (NTP) of DRC is largely integrated into the general health system. At central level, the NTP coordinates the implementation of the national TB strategic plans and is responsible for the national TB reference laboratory (NRL) and the medical stores for TB drugs.

The provincial level coordinates the delivery of diagnostic and treatment services. Usually these are medical doctors or nurses with a public health qualification and laboratory supervisors who operate under the name of "*Coordination provinciale de Lutte contre la Lèpre et la Tuberculose,* (CPLT) (Provincial Coordinations for Leprosy and Tuberculosis Control). Each CPLT is responsible for a provincial TB referral laboratory.

At the peripheral level, the health zones are responsible for provision of TB and TB-HIV services through diagnostic and treatment centers (*Centre de Santé de Diagnostic et de Traitement*, CSDT). Each center has at least a TB focal person, usually a nurse, and a laboratory technician. The number of CSDTs has doubled over the last decade to 1,863 CSDTs in 2017 of which half belong to faith-based organizations, 83 are managed by private companies, 84 by private-for-profit institutions, the remaining are public health sector facilities. Of the 472 hospitals at the peripheral level, 393 (83%) provide TB services. WHO recommended TB treatment schemes are implemented for drug-susceptible TB patients and 90% of MDR-TB patients are treated with the short course regimen in the CSDT with trained staff.

Furthermore, several approaches are used in the country to ensure community participation in TB care and control and these include different types of grassroots organizations (patient support groups, networks, non-governmental organizations [NGO] and community based organizations [CBO]).

Currently, the NTP of DRC is implementing the End TB strategy to stop TB in 2035.

1.9.5 TB laboratory network in DRC

Mirroring the health system pyramid, there are laboratory services in the DRC of different levels of complexity: a National Reference Laboratory (NRL) at the central level which is called in french since 2016 "le Laboratoire National de Réference des Mycobactéries", 27 provincial laboratories at the intermediate level called "Laboratoire Provincial de Réference" (henceforward abbreviated as PRL) and a network of 1,863 laboratories located at hospitals and health centers at the peripheral level, called laboratoire de CSD.; on average three in each HZ. The laboratory staff is supervised by the PRL , and these provincial labs are supervised by the NRL in Kinshasa.

There are, in addition, two laboratories that perform *M. tuberculosis* culture and DST using phenotypic and genotypic methods: the NRL and the regional laboratory in Lubumbashi. Plans exist to develop two additional regional laboratories, namely Kisangani and Mbuji-Mayi. A total of 63 GeneXpert platforms are currently installed within the country, able to operate the Xpert® MTB/RIF . We discuss these several levels of the laboratory network in some more detail below.

1.9.5.1 The National Reference Laboratory, NRL

The missions assigned to the NRL are as follows:

- Coordination of all laboratory activities of the network: microscopy (ZN and LED), GenXpert MTB / RIF, culture and DST;
- Elaboration of guidelines and standard operating procedures for AFB microscopy,GenXpert MTB / RIF, culture and DST;
- Technical and logistic support to the 27 PRLs and the 2 Provincial Culture Laboratories;
- Ensure the quality control of microscopy examinations;
- Ensure surveillance of resistance to first- and second-line anti-TB drugs;
- Perform or participate in operational research.

The structure of the NRL was reviewed recently by two external audits. In line with the new strategic vision “END TB” of the WHO as well as the commitment of the Direction of the PNLT for an optimal organization of the work within the NRL, a new structure is proposed, shown in **Figure 10**, that distributes the posts and responsibilities at 3 levels: the Division; the Unit;and the Cell. The six units are:

- Quality assurance;
- Laboratory management information system;
- Culture and DST;
- Molecular biology;
- Biological monitoring and waste management;
- Ziehl-Neelsen / Auramin Microscopy and Quality Control.

However, at the time of writing, there is not yet a normative document from the competent authorities which confirms this new design.

The current staffing of the NRL is 31 skilled agents: 8 medical biologists, 13 biological technicians, 7 laboratory technicians, 1 IT, and 2 lab workers.

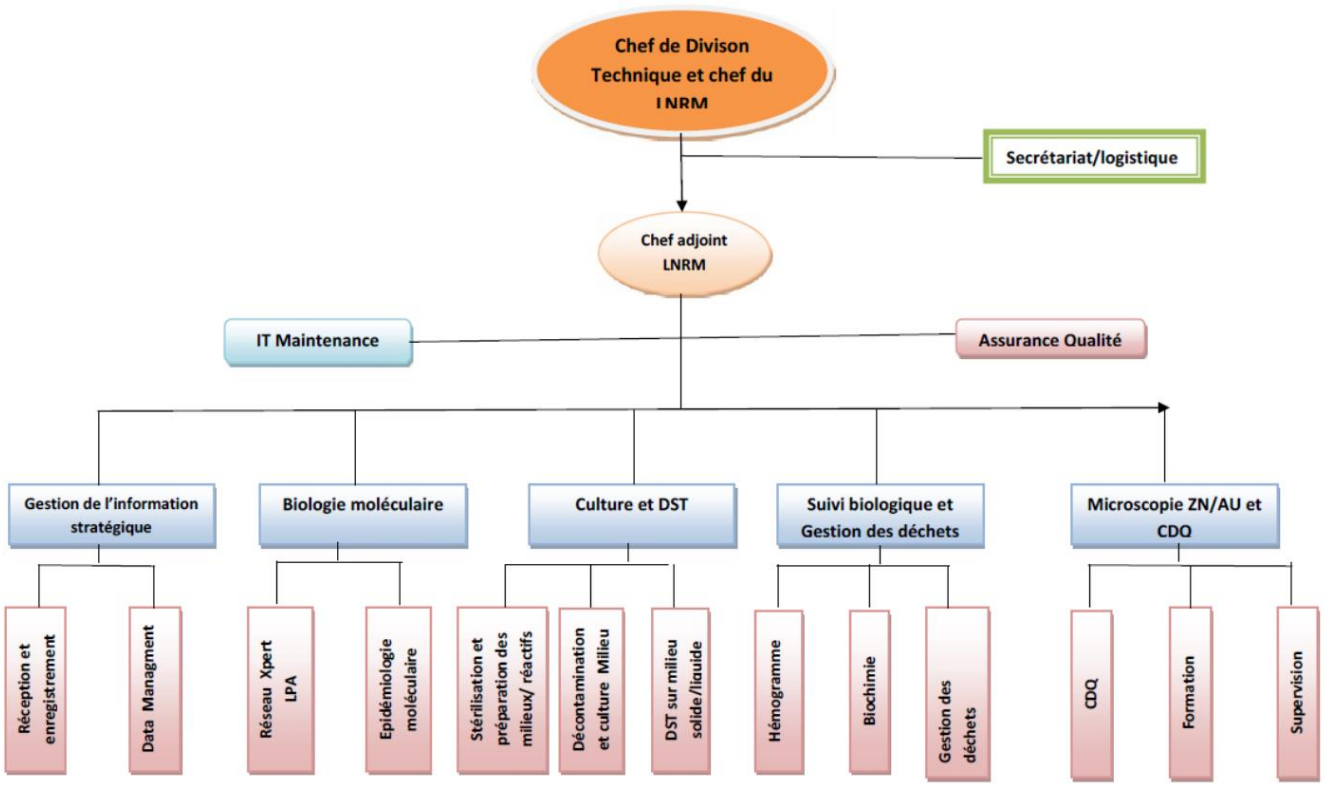


Figure 10: The new organigram of the NRL accounts for 6 units and 13 cells.

1.9.5.2 AFB Laboratories Network

The AFB microscopy network is the backbone of the NTP for the diagnosis and monitoring of patients. As explained above, it is structured in three levels, the NRL, 27 PRL dedicated to external quality assurance (EQA) of microscopy and the operational level with 1863 CSDT of which 10 have been equipped with LED microscopes. The network of labs at the operational level conducts sputum smears with standardized equipment and using the Ziehl-Neelsen and Auramine stains.

The main activities in the microscopy network are related to the planning, execution and evaluation of all laboratory interventions / activities for TB control throughout the Ziehl-Neelsen and Auramine microscopy network for TB control in the DRC.

To ensure the quality of microscopy at the CSDTs, the NTP implements a slide re-readingsystem at the provincial EQA labs to identify problematic CSDTs across the controlled slides. Each CSDT receives regular feedback on this re-reading, the less perfoming ones are followed more closely in the technical supervision in order to take remedial measures.

The analysis of 2014 EQA data in the 23 CPLTs with its 1,621 CSDT shows:

• Of 1,621 CSDT only 1,508 CSDT or 93% participated in the EQA;

• Of 48,825 re checked slides: 12,388 slides were positive (25%) of which 1,000 Highly False Positive or 8% of serious errors, of the 36,437 negative slides (75%) 865 were High False Negative or 2% of serious errors; and 609 minor quantization errors or 5%.

• Of 1,508 CSDTs audited: 833 were judged to be efficient, ie 55% and 675 CSDT less efficient, ie 45%.

The annual analysis of well-performing and underperforming CSDTs is done by means of an excel sheet that includes all CPLT CSDTs, the number of slides rechecked quarterly and annually, the number of major errors made and the number of Ziehl-N examinations.

For the microscopy network, there are problems related to the coverage of health centers, the participation of the CSDTs in the activities of EQA, the presence of a higher error rate than the norms that require an intervention of the LNRM by the training of laboratory technicians in microscopy, supervisions and the setting up of appropriate equipment allowing the realization of an optimal work. It should also be noted that the cart of microscopes has aged (lot of 2003 and before) and becomes less efficient, which requires renewal.

1.9.5.3 Xpert MTB / RIF Laboratory Network

Management of drug resistant TB began first in Kinshasa in the University of Kinshasa's teaching hospital and was only later gradually extended to all provinces. At that time, the organization of MDR-TB diagnosis was coordinated at the national level by the LNRM that conducted phenotypic and genotypic cultures and susceptibility testing. However the introduction of the GeneXpert as a novel diagnostic platform for RR-TB detection allowed the decentralization of diagnosis to the provincial level and this has led to rapid changes.

The management of drug-resistant TB is now being scaled up throughout the country across all provinces. The NTP at central level has revised the policy and made the required organizational adjustments over the last 2 years. TB drug resistance guidelines have been developed at central level for effective and efficient control. These guidelines take into account the latest WHO recommendations. The capacity of the TB staff has been strengthened at all levels of the health pyramid, including community health workers who are now more involved in screening for, diagnosis and treatment of MDR-TB patients. Rapid diagnosis is being extended with the gradual and extensive deployment of GeneXpert equipment nationwide. The optimal deployment and use of this device is a major challenge in DRC. The LNRM now manages the entire network of GeneXpert platforms for the Xpert® MTB/RIF assay as described previously. At the level of each TB provincial office, there is at least one GeneXpert device located at the level of a provincial laboratory or at the TB clinic: these are known as the GeneXpert sites.

Two main strategies are pursued to decrease the burden of MDR-TB in DRC: the scaling up of the Xpert® MTB/RIF test and the implementation of a sample transportation network.

Currently the NTP Xpert MTB / RIF network has identified 93 sites that will be able to do the Xpert MTB / RIF assay. Sixty-five GeneXpert platforms are already installed and 28 are in the process of being deployed and installed in the selected sites. **Figure 11** shows the actual site of deployment of all this equipment throughout the DRC (203).

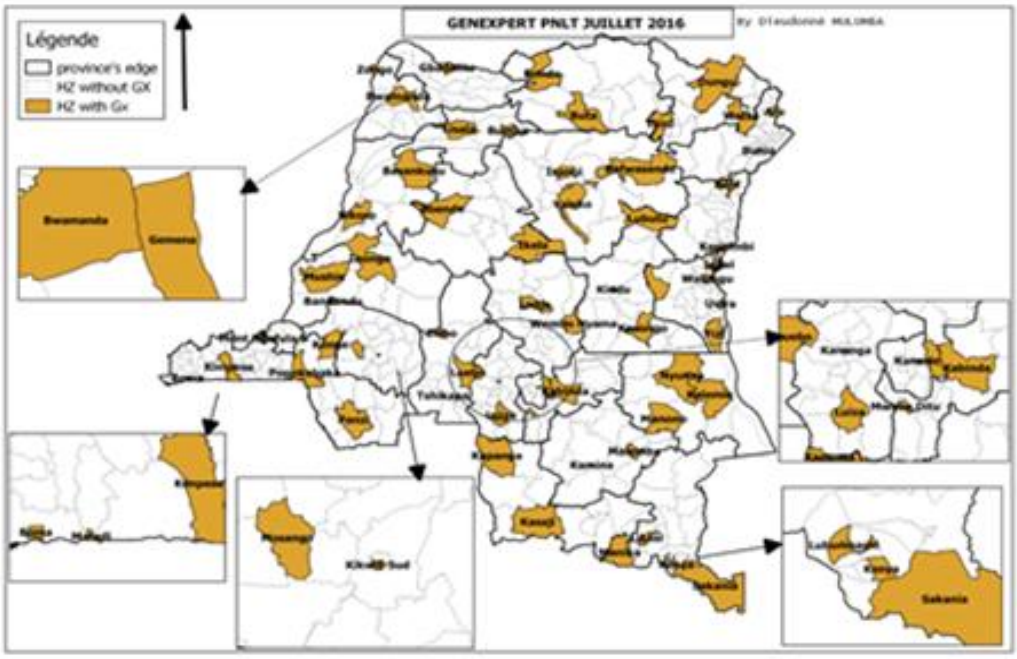


Fig 11: Localization of GeneXpert platforms in DRC (203)

1.9.5.4 Network of culture laboratories

This network currently consists currently of 2 microbiological culture laboratories located in Kinshasa at the NRL and in Lubumbashi at the PRL of Haut Katanga. A third laboratory located in Kisangani at CPLT Tshopo is under development and has not started to operate yet. Only the LNRM performs the 1st and 2nd line sensitivity tests. The NRL also participates in the WHO International Test Panel on 1st and 2nd line DSTs.

Currently, the entire network annually produces more than 5000 primary cultures (4787 in the NRL and nearly 800 in Lubumbashi in 2015) and a hundred sensitivity tests, mainly to 80% of patients in Kinshasa. Samples from other co-ordinations arrive very rarely at the culture laboratories because of the lack of an efficient network of samples transport from the periphery to the culture laboratories.

The current technical platform of the NRL and the PRLs consists essentially of the realization of the microscopy BAAR by Ziehl-Neelsen and Auramine methods, the primary culture in solid media Löwenstein Jensen (LJ), the tests identification and antibiogram on solid media.

Table 16: Summary of *M.tuberculosis* culture results from 2012 to 2017 obtained in the national reference laboratory of the NTP, DRC

Year	CULTURE POSITIVES	CULTURE NEGATIVES	CULTURE CONTAMINATED	TOTAL
2012	574	1761	611(20.7%)	2946
2013	203	922	305(21.3%)	1430
2014	647	1997	363(12.7%)	3007
2015	441	2416	198(6.5%)	3055
2016	366	2710	228(6.9%)	3304
2017	302	2766	429(12.3%)	3497

Table 16 shows that culture contamination rates vary, it was 21% in 2012 and 2013; 13% in 2014 and 2017 and 7% in 2015 and 2016. From 2012 to 2015, the general contamination rate of LJ solid media cultures decreased from 21% to 6.5%, showing the efforts made by the NRL for the reorganization of the NRL (harvesting and culture production, centrifuge problems, environmental quality, reduced number of laboratory personnel, etc.). After its refurbishment and renovation, the NRL is now equipped with a BSL-3 containment level laboratory with the presence of an MGIT 960 machine for culture and sensitivity tests in a liquid medium.

The most important gaps in the network of culture laboratories are still the low coverage of testing presumed drug-resistant patients; the long delay between presumption and bacteriological confirmation of MDR-TB; and the low culture yield due to wastage, poor transport conditions and high contamination rates of cultures.

1.9.5.5 Summary of issues related to the diagnosis of TB and drug resistant TB in DRC

A SWOT analysis carried out and validated during the process of writing the National Strategic Plan in 2005 identified the following priority issues in the domain of TB diagnosis in DRC:

1. Weak institutional, organizational and regulatory framework of the NRL;
2. Insufficient human resource capacity;
3. Insufficient capacity in material resources, equipment and reagents of quality;
4. Low capacity to mobilize financial resources in line with the roles and missions of the laboratory network;
5. Insufficient involvement of public-public and public-private partnerships around the NRL;
6. Decrease of the quality of smear microscopy;

7. Low quality of bacteriological diagnosis and programmatic management of TB and MDR-TB not benefiting from the optimal performance of the sample transport network;

8. Weak implementation of quality assurance throughout the laboratory network;

9. Low management of the laboratory health information;

10. Low capacity of Monitoring & Evaluation of laboratory network activities;

11. Low level of operational research within the laboratory network.

1.9.6 Epidemiological situation of TB in DRC

DRC, with more than 100,000 notified TB cases each year, is one of the 30 high-burden countries for TB, MDR-TB, and TB-HIV (1). According to the national records, the HIV prevalence among the general population is 1.2% and 10% among all TB patients tested. No TB prevalence survey has yet been carried out in DRC, and only estimates with wide confidence intervals are available for the main impact indicators as shown on **Table 17**.

Table 17: TB Impact indicators 2010-2016 for DRC (Source: TB Global Report, WHO 2016)

Impact Indicators	2011	2010	2012	2013	2014	2015
TB incidence rate/100,000/yr (all forms, includes PLHIV)	220 (190-250)	220 (190-250)	327 (282-375)	220 (200–240)	325 (295-356)	324 (210-463)
TB mortality rate/100,000/yr *excluding* PLHIV	54 (24-96)	54 (41-69)	54 (24-97)	68 (33-78)	69 (50-90)	66 (39-99)
TB mortality rate/100,000 /yr *among* PLHIV			9.7 (8.3-12)	9.5 (0.25–5)	8.4 (6.7-10)	21 (17-26)

1.9.6.1 Susceptible TB

a) Detection rate and incidence rate

Figure 12 shows the evolution of the detection rate of TB all forms in the DRC from 2008 to 2017. From 2008 and 2010, there is a slight increase in the detection rate from 162 to 167 per 100,000 inhabitants per year. Between 2010 and 2015 we have a decrease from 167 to 145 per 100,000 inhabitants. Since 2015, there has been an increase again from 145 to 174 per 100,000 inhabitants in 2017. This increase can be explained to a large extent by the extension of the supply of TB care across the country (203).

Figure 12 Trend in TB detection rate per 100'000 inhabitants per year (all forms) in DRC (2008-2017).

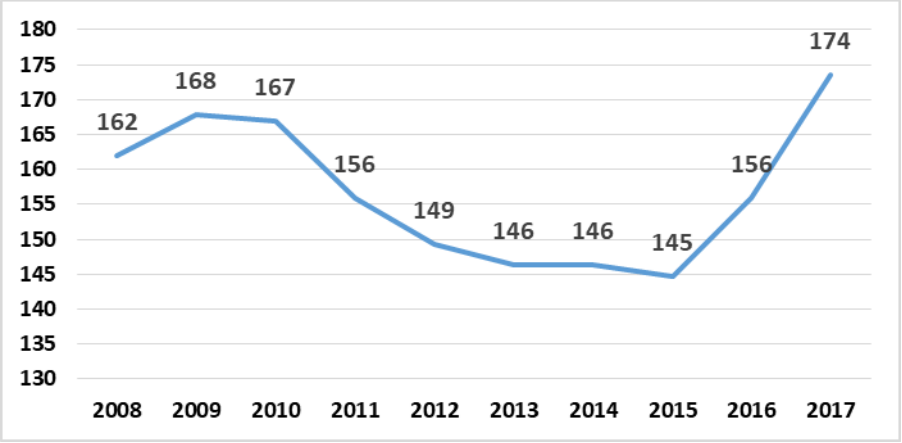


Figure 13 shows the TB notification rates per 100,000 inhabitants per year in 2016 and 2017 by province in the DRC. Between 2016 and 2017, there is an 11% increase in the national notification rate in 22 of the country's 26 provinces. There are disparities in the provinces that may be related to an uneven distribution of TB burden in the country.

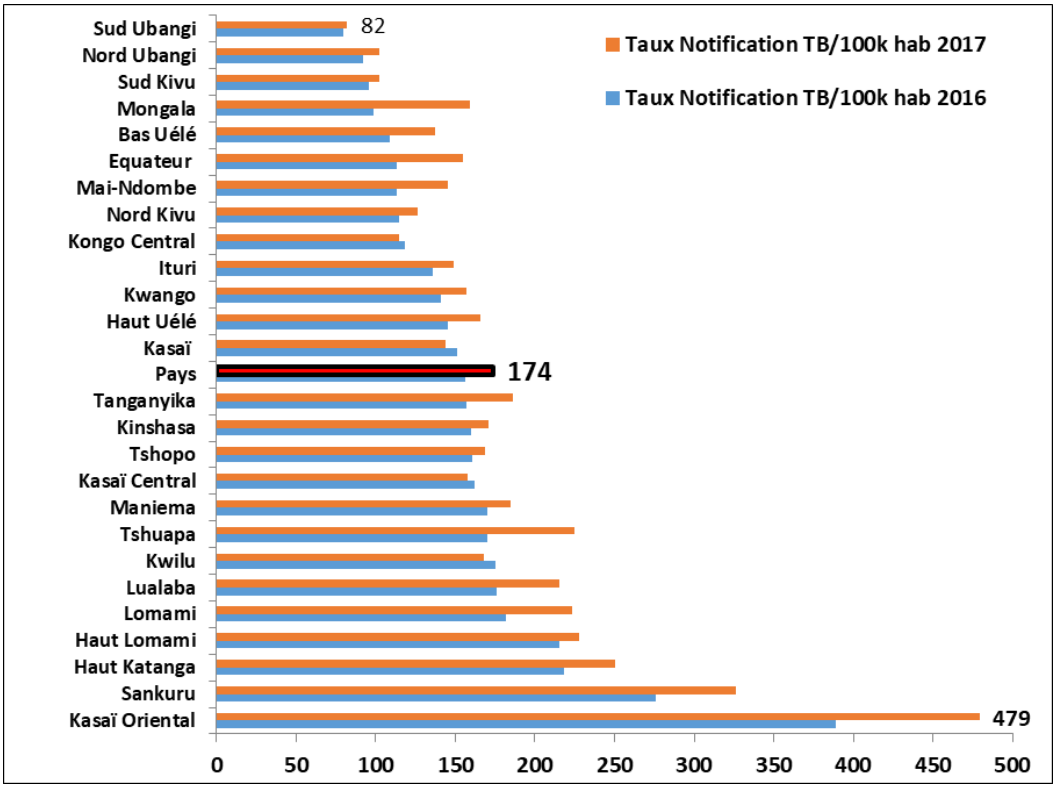


Figure 13: TB notification rate per 100,000 population in 2016, in 2017

Despite the increase observed between 2016 and 2017, the notification rate for 2017 remains below the Stop TB Strategy and MDG targets. Indeed, in 2016, the true incidence rate of tuberculosis in the DRC is estimated by WHO at 323 cases per 100 000 inhabitants. This estimated rate is high and has been stationary since 2000. With a 174 per 100 000 notification rate in 2017, this leads to a detection rate in DRC of 54% only, and shows the challenges ahead.

b) Distribution of new smear-positive pulmonary TB cases by age group

Figure 14 shows the distribution of new cases of bacteriologically confirmed pulmonary tuberculosis by age group in 2008, 2010 and again in 2015. The shape of the curve remains has not substantially changed over those years. The highest number of TB cases are observed in the age group 25 to 34 years, but of course these absolute numbers should be compared in relation the country's demographic pyramid. TB remains a major public health problem in the DRC with high transmission in the young and active population.

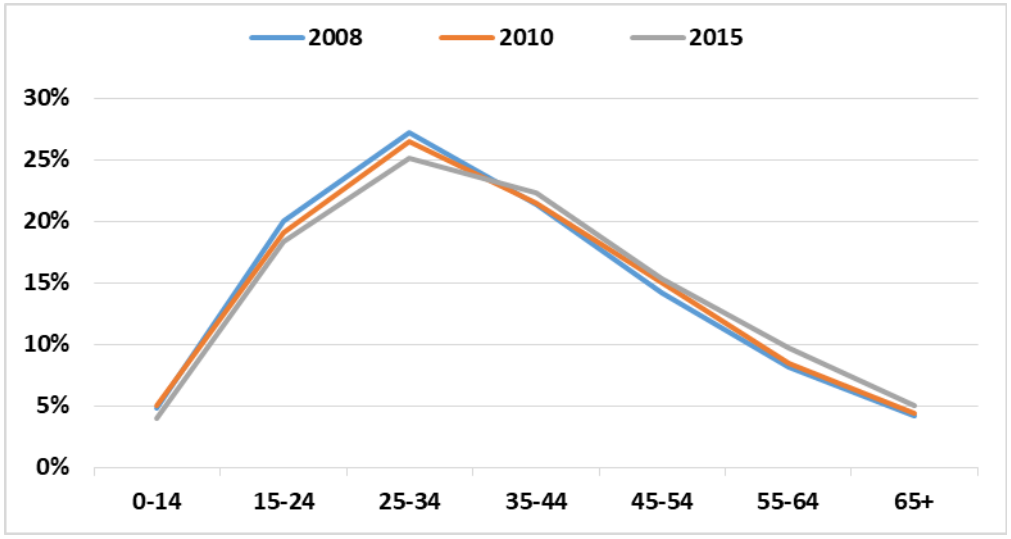


Figure 14: Distribution of new smear-positive pulmonary TB cases by age group, DRC 2008 to 2015

c) TB treatment success rate

The proportion of cases successfully treated for TB in DRC has increased since 2013 as shown on figure 7. The treatment success rate (i.e. the sum of cure rate and completion rate) for the 2016 cohort was 89% and just below the 90% target set by WHO in the End TB strategy - but better indeed than the Stop TB Strategy and MDG targets of 85%. **Table 18** describes the treatment outcomes of bacteriologically confirmed TB cases (new and relapsed) in the NTP from 2008 to 2016. The therapeutic success rate was high over this period, with a slight dip in 2011. From 2013 onwards the outcomes are quite similar, with a sustained high success rate of 89%. However, more detailed tretament outcome data by CPLT in 2016 show that the therapeutic success rate varies from province to province.

Table 18: Treatment Outcomes in bacteriologically confirmed TB patients (new and relapse) enrolled in the NTP, DRC (2008-16). Source: NTP

	Cured	Treatment completed	Death	Failure	LTFU	Not evaluated
2008	83%	4%	5%	1%	3%	4%
2009	85%	3%	4%	1%	3%	4%
2010	86%	4%	4%	1%	3%	3%
2011	81%	5%	4%	1%	4%	5%
2012	82%	5%	4%	1%	4%	4%
2013	84%	5%	4%	1%	3%	3%
2014	84%	5%	4%	1%	3%	3%
2015	85%	4%	4%	1%	3%	3%
2016	84%	5%	4%	1%	4%	2%

LTFU: Lost to Follow-up

d) TB-HIV coinfection

Screening for HIV in TB patients

HIV testing activities conducted from 2009 to 2017 nationwide are reported for all forms of TB in **Table 19** below. At the country level, there is an increase in the proportion of TB patients who have been tested for HIV from 27% in 2009 to 63% in 2017, which is still insufficient compared

to the expected targets of more than 90%. Over the same period, the percentage of HIV-positive TB patients decreased from 20% in 2009 to 10% in 2017. It is difficult to compare the 2009 figures to those of 2017 because in 2009, only 27% of patients were tested, and they may have had different risk profiles. Nevertheless, since 2015, the country has tested about 50% or more of all TB cases and the decrease persists. This decrease in HIV co-infection rates is consistent with the decline in the incidence and prevalence of HIV in the country.

Table 19. HIV Screening in Tuberculosis Patients, DRC 2009 to 2017

Year	Total TB	Tested		HIV-Positive	
		N	%	N	%
2009	115 625	31 312	27%	6 126	20%
2010	118 636	28 997	24%	5 273	18%
2011	114 290	30 636	27%	4 942	16%
2012	112 786	35 097	31%	5 748	16%
2013	113 881	49 125	43%	7 636	16%
2014	117 214	53 285	45%	7 206	14%
2015	120 508	59 027	49%	7 154	12%
2016	132515	71065	54%	8344	12%
2017	151881	96261	63%	9556	10%

The HIV testing activities conducted in 2017 in each of the new provincial TB coordinations are shown in the figure below. The analysis by province in 2017 shows that provinces with a co-infection rate above the national average are Haut Uélelé, Bas Uélélé, Ituri, Kinshasa, Thsuapa, Kasai, Thsopo, Lualaba, Haut Katanga. We note that six provinces have insufficient screening rates <40% (Ituri, Thsuapa, Kasai, Ecuador, North and South Ubangi).

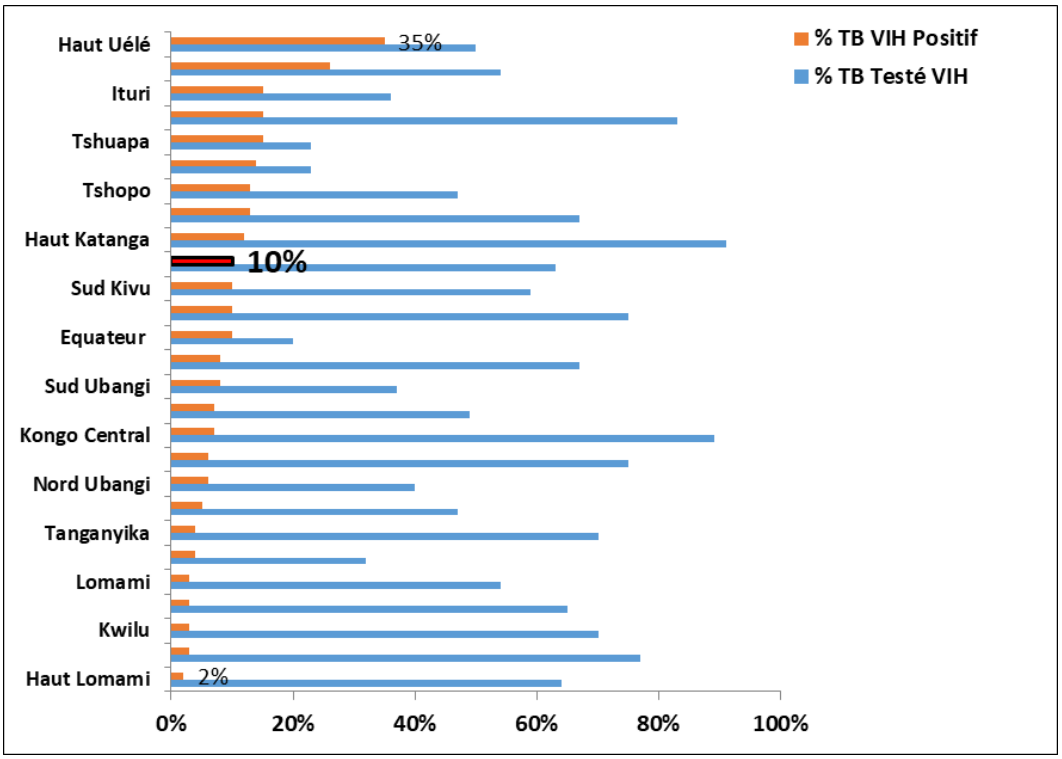


Figure 15: HIV Testing rates in Tuberculous Patients, by province, DRC 2017. Source: NTP

Management of TB / HIV coinfected patients

At the national level, the proportion of patients on cotrimoxazole and / or on ARVs in co-infected patients has increased considerably. In 2009, these rates were 21% for cotrimoxazole and 21% for ARV treatment. In 2017, these rates were 91% and 82% respectively. Sixteen out of 26 Provinces have an ARV treatment rate above 80% and 2 provinces a rate below 50% (Ecuador, Tshupa).

e) Drug-Resistant Tuberculosis (DR TB)

Recently a nation wide drug resistance survey was conducted in DRC, according to standard WHO methodology. The preliminar results show that at the country level, the prevalence of DR TB in new TB cases was 2.2% and the prevalence of DR TB in previously treated patients is 16.7%. As Figure 16 shows, there is quite some variation across provinces though.

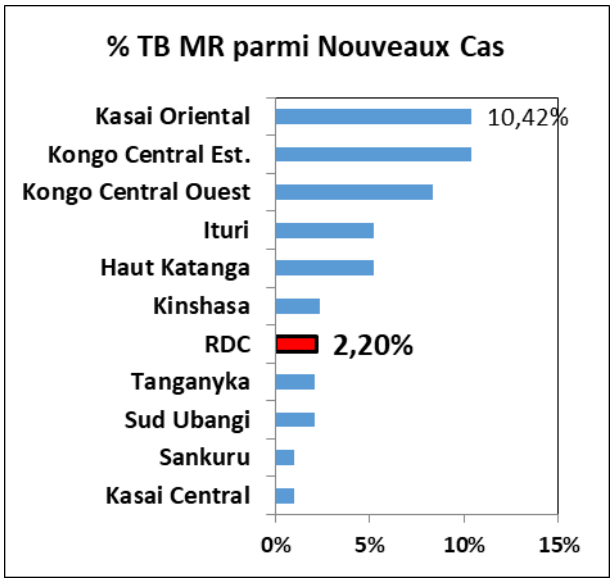


Figure 16: Preliminar results of national survey of drug susceptibility in TB (2017) in DRC: prevalence of resistance at the provincial level

The results of the prevalence survey show that:

- The prevalence of TB DR in new patients is higher than the national prevalence (2.2%) in the following coordinations: Kongo Central (East and West), Kasaï Oriental, Haut Katanga, Ituri and Kinshasa.

- The prevalence of TB DR in previously treated patients exceeds 10% in the following coordinations: Central Kongo (East), Eastern Kasai, Upper Katanga, Tanganyika, Mongala, Sankuru, Upper Lomami, Kwuilu and Kinshasa.

Screening Activities for Drug-Resistant Tuberculosis (DR TB)

The presumed cases of multidrug-resistant tuberculosis (MDR-TB) according to the national definition, are patients eligible for reprocessing (failure, relapse, recovery), contacts of a confirmed MDR case, and patients whose control examination of the 3rd month is positive. The molecular test used for the detection of MR TB is GeneXpert MTB / RIF. If GeneXpert MTB / RIF exhibits resistance to rifampicin, treatment of the patient is initiated and the sample is sent to the NRL for culture and susceptibility testing. Figure 17 shows the evolution of MDR TB screening

in the DRC from 2010 to 2017. There is an increase in TB PR cases detected in the DRC from 189 in 2010 to 506 in 2015. In 2017, 885 patients were screened "Resistant to Rifampicin (RR) nationwide. For the year 2017, a total of 19 patients were diagnosed either ultra resistant or ultra resistant. According to the WHO global report 2017 (1) these notifications are still too low. Indeed, it should be noted that according to WHO, the estimated number of expected MDR-TB cases is 3.2% among new cases and 14% among cases already treated. That is why the NTP carried out a drug resistance survey in DRC in 2015-2016 and was able to establish a new map showing disparities between the provinces. Preliminary results show a crude rate of 2.26% MDR-TB among new patients and 15.65% among previously treated cases. According to these results "5 hot spot provinces' have shown high rates of MDR TB: Kinshasa, Kongo central, Haut Katanga, Kasai oriental and Ituri.

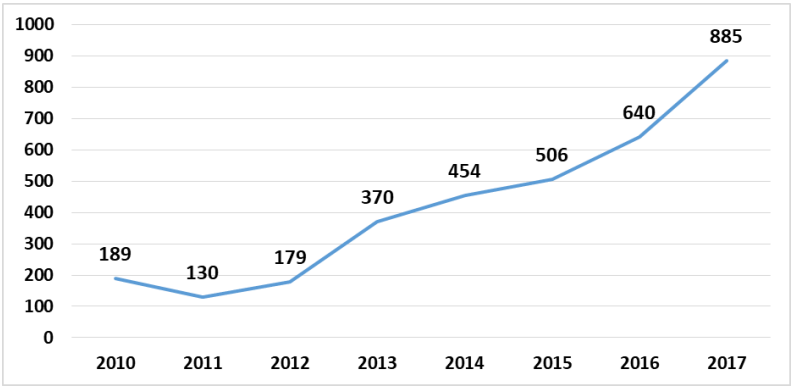


Figure 17: Evolution of the number of MR / RR TB cases detected in the DRC from 2010 to 2017

Management of drug-resistant tuberculosis

Table 20 presents the ratio of patients treated and cases detected for MDR-TB from 2013 to 2017. It is noted that this ratio is on average more than 94% over the last five years. In 2017, it was 95%.

Table 20: Patients Screened and Treated for DR TB in DRC, 2013 to 2017

	# Detected	# Treated	%
2013	374	370	99%
2014	454	443	98%
2015	506	454	90%
2016	709	640	90%
2017	885	839	95%

In 2017, of the 19 patients diagnosed with ultra-resistant pre-TB or ultra-resistant TB, 15 were put on treatment and four died before starting their treatment.

In a large part of the country, the regimen used for the management of TB MR patients is the 9-month short regimen. There are still some coordinations that use the 20-month-long regimen because of the clinical requirement and the patient's profile. The treatment results of the 2015 cohort, presented in Figure 18 below, show good outcomes in these MDR patients with a therapeutic success rate – for both regimens- of more than 85%.

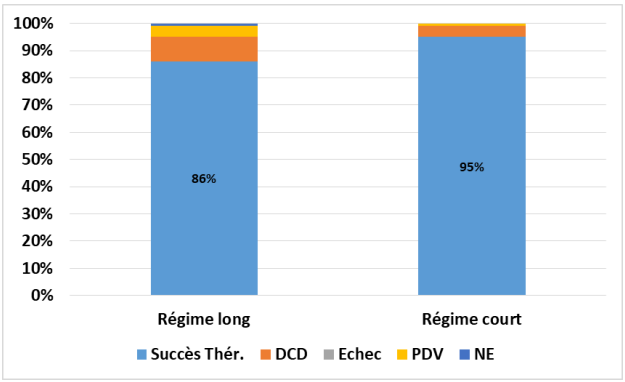


Figure 18: Treatment Outcomes of MDR TB Patients, DRC Cohort 2015

Chapter 2. Thesis outline

2.1 PROBLEM STATEMENT

In Low-Income Countries such as the Democratic Republic of Congo (DRC), the challenge posed by MDR-TB is huge. During the two last decades, the history of the DRC has been rife with civil unrest, which has led to the collapse of the health system and the recrudescence of TB and other infectious diseases. DRC, with an estimated population of 85 million, is ranked 09th among the 30 TB High Burden Countries and has an estimated incidence of TB of 324 per 100 000 inhabitants per year. DRC is classified also among the 27 countries with a high burden of MDR-TB, but the actual data on the magnitude, trends, and the distribution of MDR-TB in DRC are scanty. In 2008, the World Health Organization (WHO) estimated that the total number of MDR-TB cases in DRC was 5600 (95%CI: 530-11 000). However, less than 2% of this estimated number were detected and put on specific treatment during that same year. Kinshasa, the capital city of DRC which accounts for 20% of all TB cases nationwide, is notifying more than 80% of all MDR suspects, and not all of these are laboratory-confirmed (DRC National TB Program data; unpublished). Extremely long turn-around times for laboratory results to reach the treating clinician increase the risk of the spread of resistant strains. The aim of this research project was to provide the National TB Program (NTP) of the DRC with key information on the emergence of drug-resistant TB and its public health impact. We focused on strategies for diagnosing and monitoring drug resistance that were appropriate for the NTP in DRC. More specifically we evaluated new molecular techniques, and developed practical guidance on how, in the specific context of DRC, they could improve the programmatic management of MDR-TB.

2.2 OBJECTIVES OF THE THESIS

The overall objective of this doctoral thesis was to contribute to the reduction of the burden of drug resistant TB in Kinshasa, DRC. We had initially three specific objectives.

Firstly, we documented a cluster of MDR-TB cases in Mosango, DRC which motivated us to evaluate the feasibility of a simplified and more efficient strategy for monitoring of drug resistance in DRC.

Secondly, we aimed to assess the diagnostic accuracy and feasibility of a rapid molecular method for MDR-TB detection, labeled" *Genotype® MTBDR plus assay*" in the Mycobacteriology Unit of the INRB in Kinshasa, DR Congo.

Thirdly, we carried out an epidemiological analysis to determine the burden and the trends of drug resistance, particularly RMP and FQ resistance, in the patients with recurrence (failure and relapse/reinfection) after primary treatment of tuberculosis in Kinshasa, the capital of DRC.

As, in the meantime, new molecular tools became commercially available, and as one of them, the Xpert® MTB/RIF assay was quickly adopted by WHO policy, we used it to document the emergence of TB/MDR-TB cases in a prison setting in DRC in order to guide appropriate treatment and infection control measures.

2.3 OVERVIEW OF THE THESIS BY CHAPTER

2.3.1 MDR in Mosango district

Kaswa MK, Bisuta S, Kabuya G, Lunguya O, Ndongosieme A, Muyembe JJ, Van Deun A, Boelaert M (2014). Multi Drug Resistant Tuberculosis in Mosango, a Rural Area in the Democratic Republic of Congo. PLoS ONE 9(4): e94618. doi:10.1371/journal.pone.0094618.

This is a report of a cluster of MDR-TB cases that we investigated in the Mosango health district, in the Bandundu-South Province, DRC in 2008. From all TB suspects and their contacts identified and included in this investigation, 2 sputum specimens were collected to ensure an adequate recovery of *Mycobacterium tuberculosis* on solid medium Löwenstein Jensen (LJ) and followed by detection of RMP and INH resistance by conventional Drug Susceptibility Testing (DST) on solid medium and further DNA sequencing. The Mosango study is included in the PhD thesis as it provides the rationale for the evaluation of novel tools to assess drug resistance. Phenotypic Drug Sensitivity Testing and DNA sequencing were performed on 18 sputum specimens collected from 4 MDR-TB suspects and 5 household contacts. Sequencing data confirmed that the 4 suspects were indeed Rifampicin resistant cases. Sequencing of the *rpoB* gene showed that 3 cases had a single mutation encoding a substitution to 526Tyr, 531Trp and 526Leu respectively. Patient C had a double mutation encoding a change to 531Leu and 633Leu. Two of the investigated cases died within 4 months of a second-line treatment course.

2.3.2 Validation and feasibility study

Kaswa MK, Aloni M, Nkuku L, Bakoko B, Lebeke R, Nzita A, Muyembe JJ, de Jong B, de Rijk P, Verhaegen, Boelaert M, Ieven M, Armand Van Deun A. Pseudo-outbreak of pre-extensively drug-resistant (Pre-XDR) tuberculosis in Kinshasa: collateral damage caused by false detection of fluoroquinolone resistance by GenoType MTBDR *sl*. J Clin Microbiol. 2014;52:2876-2880.

In 2016, the WHO made a strong recommendation for using the short course MDR TB regimen. LPA MTBDR sl should be used before starting the second line MDR-TB treatment. The double gyrA mutation 80Ala and 90Gly represented 57% of all fluoroquinolone mutations identified from MDR-TB patient sputum samples, as confirmed by DNA sequencing. This double mutation was previously found to be associated with susceptibility to fluoroquinolones, yet leads to absent hybridization of a wildtype band in the MTBDR sl and is thus falsely scored as resistance. Our findings suggest that MTBDR *sl* results should be interpreted with caution when the interpretation is solely based on the absence of a wildtype band without confirmation by visualization of a mutant band.

2.3.3 The burden of MDR TB and Surveillance of drug resistance in Kinshasa, DRC

Kaswa MK, Van Deun A, Hasker E, Aloni M, Nkuku L, de Rijk P, Bola V, Bakoko B, Lebeke R, Nzita A, de Jong B C, Verhaegen J, Muyembe JJ, Bakaswa G., Ieven M, Boelaert M. Surveillance of drug resistance among retreatment cases in Kinshasa: lessons learned?(in preparation).

During the period 2005-2010, among 2 349 retreatment patients sampled, 1 609 (68%) had a valid phenotypic DST result, 282 (17.5%) of those were MDR-TB, and 6 (2.3%) of the MDR-TB also were resistant to FQs. During the period 2011-2013, among 192 retreatment patients, 157 (82%) had valid molecular DST, including 64 (40.7%) with MDR-TB, of whom 4 (7.0 %) also had FQ resistance. The estimated proportion of first failure cases that was screened reached 409 (17%) and 71 (37%) for the periods 2005-2010 and 2011-2013, with 3.8% and 7.0% RMP resistance detected, of whom 2.0 % and 5% also had FQ resistance. Although there seems to be a trend towards increased resistance, this was not confirmed by triangulation of our findings with registrations on recurrence cases.

2.3.4 Emergence of TB/MDR-TB cases in prison documented by using molecular tests.

Kaswa KM, Hasker E, Bakaswa G, Aloni M, Nkuku L, Kazadi M, Kabuya G, Muteteke D, Nkiere N, Nkake V, Bisuta S, Bagazani , Mukadi YD, Muyembe JJ, Ieven M, Boelaert M and De Jong BC. Assessing the burden of Tuberculosis and resistant tuberculosis in prison inmates in Mbuji-Mayi, DR Congo, an outbreak investigation based on GeneXpert® MTB/RIF (submitted to Emerging Infectious Diseases).

In this manuscript an outbreak investigation is reported that signals a high prevalence of undiagnosed TB cases, as well as probable MDR-TB in 2015 in the prison of Mbuji-Mayi, DRC. We conducted active TB case finding operation. We clinically screened all consenting prisoners for presumptive TB and took sputum samples for analysis by the Xpert MDR/RIF assay. Of the 918 inmates found in the prison, 29 were already under TB treatment. We confirmed TB in 170 additional persons, bringing the TB prevalence rate to 21.7 percent. There were 14 TB rifampicin resistant (TB-RR) cases. All TB and TB RR have been put on treatment. The overcrowded conditions, poor ventilation, and malnutrition, were the likely drivers of this huge problem.

2.3.5 References to Chapters 1 and 2

1. World Health Organization. Global Tuberculosis Report 2017. WHO, Geneva. http://www.who.int/tb/publications/global_report/en/.
2. Murray C J, Salomon J A. Modeling the impact of global tuberculosis control strategies. Proc. Natl Acad.Sci.1998; 95: 13881–13886.
3. World Health Organization. Global tuberculosis control. Communicable Diseases report. Geneva: World Health Organization; 2001 [WHO/CDS/TB/2001.287].
4. Elliott A M, Luo N, Tembo G, Halwiindi B, Steenbergen G, Machiels L, Pobee J, Nunn P, Hayes RJ, McAdam KP. Impact of HIV on tuberculosis in Zambia: a cross sectional study.BMJ. 1990; 301:412-415.
5. Crofton J, Horne N, Miller F. Clinical tuberculosis. 2nd Edition. TALC, IUATLD, Mac Milan;1999. 222 p. 135-141.
6. World Health Organization . TB/HIV: research priorities in resource-limited settings. Report of Expert Consultation. Geneva: World Health Organization; 2005 [WHO/HTM/TB/2005.355].

7. World Health Organization . The Stop TB strategy: building on and enhancing DOTS to meet the TB-related Millennium Development Goal. Geneva: World Health Organization; 2006 [WHO/HTM/TB/2006.368].

8. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. Am Rev Respir Dis 1986; 133: 423-30.

9. Centers for Disease Control (CDC). Transmission of multidrug-resistant tuberculosis among immunocompromised persons in a correctional system--New York, 1991. MMWR Morb Mortal Wkly Rep. 1992 Jul 17;41(28):507-9. PubMed PMID: 1630427

10. Centers for Disease Control and Prevention. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second- line drugs-worldwide. Morbidity and Mortality Weekly Report. 2006: 55; 301–305.

11. Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: the effects of chemotherapy. Tubercle. 1976;57:275-299.

12. Rieder HL. Epidemiological Basis of Tuberculosis control. 1st Edition. IUTLD.pp11-15. 1999.

13. Loudon RG, Roberts RM. Droplet expulsion from the respiratory tract. Am Rev Respir Dis. 1966; 95: 435-442.

14. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med. 2016;13(10):e1002152 (https://www.ncbi.nlm.nih.gov/pubmed/27780211, accessed 2 August 2017).

15. Raviglione MC, Harries AD, Msiska R, Wilkinson D, Nunn P. Tuberculosis and HIV: current status in Africa. AIDS. 1997; 11 Suppl B: S115-S123.

16. De Cock KM, Soro B, Coulibaly IM, Lucas SB. Tuberculosis and HIV infection in sub-Saharan Africa. JAMA. 1992; 268: 1581-1587.

17. Toman K. Tuberculosis case-finding and chemotherapy. Questions and answers. 1st Edition. Geneva: World Health Organization, 1979.

18. European Society for Mycobacteriology. Manual of diagnostic and public health mycobacteriology. 2nd Edition. London: Bureau of Hygiene and Tropical Medicine, 1991.

19. Grybowski S, Barnett GD, Styblo K. Contacts of cases of active pulmonary tuberculosis. Bull Int Union Tuberc. 1975; 50: 90-106.

20. Shaw JB, Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. Am Rev Tuberc. 1954; 69: 724-732.

21. Van Geuns HA, Meijer J, Styblo K. Results of contact examination in Rotterdam, 1967-69. Bull Int Union Tuberc. 1975;50: 107-119.

22. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, Small PM. Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet. 1999; 353: 444-449.

23. Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents: recommendations for HIV-prevalent and resource-constrained settings. Geneva, World Health Organization, 2007 (WHO/HTM/TB/2007.379; WHO/HIV/2007.01).

24. TB/HIV: a clinical manual. Geneva, World Health Organization, 2003 (WHO/HTM/TB/2004.329).

25. Guidelines for HIV surveillance among tuberculosis patients, 2nd ed. Geneva, World Health Organization/Joint United Nations Programme on HIV/AIDS, 2004 (WHO/HTM/TB/2004.339, WHO/HIV/2004.06, UNAIDS/04.30E).

26. Interim policy on collaborative TB/HIV activities. Geneva, World Health Organization, 2004 (WHO/HTM/TB/2004.330 and WHO/HTM/HIV/2004).

27. Pablos-Mendez A, Raviglione MC, Laszlo A et al. Global surveillance for antituberculosis-drug resistance, 1994–1997.The New England Journal of Medicine. 1998: 338(23), 1641–1649.

28. Espinal MA, Laszlo A, Simonsen L et al. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease. Working Group on Anti-Tuberculosis Drug Resistance Surveillance. New England Journal of Medicine. 2001: 344(17), 1294–1303.

29. Wright A, Zignol M, Van Deun A et al. Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the Global Project on Anti-Tubercu- losis Drug Resistance Surveillance. 2009. Lancet :373 (9678), 1861–1873.

30. Canetti G, Froman S, Grosset J, et al. Mycobacteria: laboratory methods for testing drug sensitivity and resistance. Bull WHO 1963;29:565-79.

31. Rastogi N, Goth KS, David HL. Drug susceptibility testing in tuberculosis: a comparison of the proportion methods using Löwenstein-Jensen, Middlebrook 7H10 and 7H11 agar media and a radiometric method. Res Microbiol 1989;140:405-17.

32. Walters SB, Hanna BA. Testing of susceptibility of Mycobacterium tuberculosis to isoniazid and rifampin by Mycobacterium growth indicator tube method. J Clin Microbiol 1996;34:1565-7.

33. Palaci M, Yoko Mizuka Ueki S, Nakamura Sato D, et al. Evaluation of Mycobacteria growth indicator tube for recovery and drug susceptibility testing of Mycobacterium tuberculosis isolates from respiratory specimens. J Clin Microbiol 1996;34:762-4.

34. Wanger A, Mills K. Testing of Mycobacterium tuberculosis susceptibility to ethambutol, isoniazid, rifampin, and streptomycin by using Etest. J Clin Microbiol 1996;34:1672-6.

35. Hausdorfer J, Sompek E, Allerberger F, et al. E-test for susceptibility testing of Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 1998;9:7515.

36. Stevens WS, Scott L, Noble L, et al. Impact of the GeneXpert MTB/RIF Technology on Tuberculosis Control. Microbiol Spectr 2017;5(1).

37. Cockerill FR, Uhl JR, Temesgen Z, et al., Rapid identification of a point mutation of the Mycobacterium tuberculosis catalase-peroxydase (katG) gene associated with isoniazid resistance. J Infect Dis 1995;171:240-5.

38. Matsiota-Bernard P, Vrioni G, Marinis E., Characterization of rpoB mutations in rifampin-resistant clinical Mycobacterium tuberculosis isolates from Greece. J Clin Microbiol 1998;36:20-3.

39. Beenhouwer H, Lhiang Z, Jannes W, et al., Rapid detection of rifampin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tubercle Lung Dis 1995;76:425-30.

40. Kapur V, Li LL, Hamrick MR, et al. Rapid Mycobacterium species assignment and unambiguous identification of mutations associated with antimicrobial resistance in Mycobacterium tuberculosis by automated DNA sequencing. Arch Pathol Lab Med 1995;119:131-8.

41. Cade CE, Dlouhy AC, Medzihradszky KF, et al. Isoniazid-resistance conferring mutations in Mycobacterium tuberculosis katG: catalase, peroxidase, and INH-NADH adduct formation activities. Protein Sci 2010;19:458-74.

42. Vilchèze C, Jacobs WR Jr. Resistance to Isoniazid and Ethionamide in Mycobacterium tuberculosis: Genes, Mutations, and Causalities. Microbiol Spectr 2014;2:MGM2-0014-2013.

43. Lee AS, Teo AS, Wong SY. Novel mutations in ndh in isoniazid-resistant Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother 2001;45:2157-9.

44. Gillespie SH. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrob Agents Chemother 2002;46:267-74.

45. Silva MS, Senna SG, Ribeiro MO, et al. Mutations in katG, inhA, and ahpC genes of Brazilian isoniazid-resistant isolates of Mycobacterium tuberculosis. J Clin Microbiol 2003;41:4471-4.

46. Agrawal S, Ashokraj Y, Bharatam PV, et al., Solid-state characterization of rifampicin samples and its biopharmaceutic relevance. Eur J Pharm Sci 2004;22:127-4.

47. Campbell EA, Korzheva N, Mustaev A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 2001;104:90112.

48. Lu J, Jiang S, Ye S, Deng Y, Ma S, Li CP. Sequence analysis of the drug-resistant rpoB gene in the Mycobacterium tuberculosis L-form among patients with pneumoconiosis complicated by tuberculosis. Mol Med Rep. 2014 ;9:1325-30.

49. Poteïko PI, Liashenko AA, Lebed' LV, et al. Analysis of mutation in codon 531 rpoB gene, associated with rifampicin resistance among samples of Mycobacterium tuberculosis that was isolated from the patients. Lik Sprava 2014;7-8:65-9.

50. Barnes P F, Bloch A B, Davidson P T, et al. Tuberculosis in patients with human immunodefi ciency virus infection. N Engl J Med 1991; 324: 1644–1650.

51. Centers for Disease Control and Prevention. Treatment of tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America. MMWR 2003; 52 (RR-11): 1–77.

52. Vernon A, Burman W, Benator D, et al. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. Tuberculosis Trials Consortium. Lancet 1999; 353: 1843–1847.

53. Traore H, Fissette K, Bastian I, et al. Detection of rifampicin resistance in Mycobacterium tuberculosis isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. Int J Tuberc Lung Dis 2000; 4: 481–484.

54. Mitchison D A. The action of anti-tuberculosis drugs in short course chemotherapy. Tubercle 1985; 66: 219–225.

55. Tarshis M S, Weed W A Jr. Lack of signifi cant in vitro sensitivity of Mycobacterium tuberculosis to pyrazinamide on three different solid media. Am Rev Tuberc 1953; 67: 391–395.

56. McDermott W, Tompsett R. Activation of pyrazinamide and nicotinamide in acidic environment in vitro. Am Rev Tuberc 1954; 70: 748–754.

57. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. Int J Tuberc Lung Dis 2003; 7: 6–21.

58. Wade M M, Zhang Y. Anaerobic incubation conditions enhance pyrazinamide activity against Mycobacterium tuberculosis. J Med Microbiol 2004; 53: 769–773.

59. Wade M M, Zhang Y. Effects of weak acids, UV and proton motive force inhibitors on pyrazinamide activity against Mycobacterium tuberculosis in vitro. J Antimicrob Chemother 2006; 58: 936–941.

60. Zhang Y, Wade M M, Scorpio A, et al. Mode of action of pyrazinamide: disruption of Mycobacterium tuberculosis membrane transport and energetics by pyrazinoic acid. J Antimicrob Chemother 2003; 52: 790–795.

61. Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. Nat Med 1996; 2: 662–667.

62. Zhang Y, Scorpio A, Nikaido H, et al. Role of acid pH and defi cient effl ux of pyrazinoic acid in unique susceptibility of Mycobacterium tuberculosis to pyrazinamide. J Bacteriol 1999; 181: 2044–2049.

63. Zimhony O, Cox J S, Welch J T, et al. Pyrazinamide inhibits the eukaryotic-like fatty acid synthetase I (FASI) of Mycobacterium tuberculosis. Nat Med 2000; 6: 1043–1047.

64. Boshoff H I, Mizrahi V, Barry C E III. Effects of pyrazinamide on fatty acid synthesis by whole mycobacterial cells and purifi ed fatty acid synthase I. J Bacteriol 2002; 184: 2167–2172.

65. Konno K, Feldmann F M, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. Am Rev Respir Dis 1967; 95: 461–469.

66. Scorpio A, Lindholm-Levy P, Heifets L, et al. Characterization of pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother 1997; 41: 540– 543.

67. Cheng S J, Thibert L, Sanchez T, et al. pncA mutations as a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis: spread of a monoresistant strain in Quebec, Canada. Antimicrob Agents Chemother 2000; 44: 528–532.

68. Sreevatsan S, Pan X, Zhang Y, et al. Mutations associated with pyrazinamide resistance in pncA of Mycobacterium tuberculosis complex organisms. Antimicrob Agents Chemother 1997; 41: 636–640.

69. Hirano K, Takahashi M, Kazumi Y, et al. Mutation in pncA is a major mechanism of pyrazinamide resistance in Mycobacterium tuberculosis. Tubercle Lung Dis 1997; 78: 117–122.

70. Lemaitre N, Sougakoff W, Truffot-Pernot C, et al. Characterization of new mutations in pyrazinamide-resistant strains of Mycobacterium tuberculosis and identifi cation of conserved regions important for the catalytic activity of the pyrazinamidase PncA. Antimicrob Agents Chemother 1999; 43: 1761–1763.

71. Marttila H J, Marjamaki M, Vyshnevskaya E, et al. pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis isolates from northwestern Russia. Antimicrob Agents Chemother 1999; 43: 1764–1766.

72. Morlock G P, Crawford J T, Butler W R, et al. Phenotypic characterization of pncA mutants of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2000; 44: 2291–2295.

73. Portugal I, Barreiro L, Moniz-Pereira J, et al. pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis isolates in Portugal. Antimicrob Agents Chemother 2004; 48: 2736– 2738.

74. Takayama K, Kilburn J. Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. Antimicrob Agents Chemother 1989; 33: 1493–1499.

75. Mikusov K, Slayden R, Besra G, et al. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. A ntimicrob Agents Chemother 1995; 39: 2484–2489.

76. Wolucka B, McNeil M, de Hoffmann E, et al. Recognition of the lipid intermediate for arabinogalactan/arabinomannan biosynthesis and its relation to the mode of action of ethambutol on mycobacteria. J Biol Chem 1994; 269: 23328–23335.

77. Telenti A, Philipp W J, Sreevatsan S, et al. The emb operon, a unique gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. Nature Med 1997; 3: 567–570.

78. Belanger A, Besra G, Ford M, et al. The embAB genes of Mycobacterium avium encode an arabinosyl transferase involved in cell wall arabinan biosynthesis that is the target for the antimycobacterial drug ethambutol. Proc Natl Acad Sci USA 1996; 93: 11919–11924.

79. Heifets L, Desmond E. Clinical mycobacteriology laboratory. In: Cole S, Eisenach K, McMurray D, Jacobs W Jr, eds. Tuberculosis and the tubercle bacillus. Washington DC, USA: ASM Press, 2005: pp 49–70.

80. Ramaswamy S V, Amin A G, Goksel S, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother 2000; 44: 326–336.

81. Sreevatsan S, Stockbauer K, Pan X, et al. Ethambutol resis tance in Mycobacterium tuberculosis: critical role of embB mutations. Antimicrob Agents Chemother 1997; 41: 1677–1681.

82. Mokrousov I, Otten T, Vyshnevskiy B, et al. Detection of embB306 mutations in ethambutol-susceptible clinical isolates of Mycobacterium tuberculosis from northwestern Russia: implications for genotypic resistance testing. J Clin Microbiol 2002; 40: 3810–3813.

83. Hazbon M H, Bobadilla del Valle M, Guerrero M I, et al. Role of embB codon 306 mutations in Mycobacterium tuberculosis revisited: a novel association with broad drug resis tance and IS6110 clustering rather than ethambutol resistance. A ntimicrob Agents Chemother 2005; 49: 3794–3802.

84. Shi R, Zhang J, Otomo K, et al. Lack of correlation between embB mutation and ethambutol MIC in Mycobacterium tuberculosis clinical isolates from China. Antimicrob Agents Chemother 2007; 51: 4515–4517.

85. Perdigao J, Macedo R, Ribeiro A, et al. Genetic characterisation of the ethambutol resistance-determining region in Mycobacterium tuberculosis: prevalence and signifi cance of embB306 mutations. Int J Antimicrob Agents 2009; 33: 334– 338.

86. Parsons L M, Salfinger M, Clobridge A, et al. Phenotypic and molecular characterization of Mycobacterium tuberculosis isolates resistant to both isoniazid and ethambutol. Antimicrob Agents Chemother 2005; 49: 2218–2225.

87. Safi H, Sayers B, Hazbon M H, et al. Transfer of embB codon 306 mutations into clinical Mycobacterium tuberculosis strains alters susceptibility to ethambutol, isoniazid, and rifampin. Antimicrob Agents Chemother 2008; 52: 2027–2034.

88. Alcaide F, Pfyffer G E, Telenti A. Role of embB in natural and acquired resistance to ethambutol in mycobacteria. Antimicrob Agents Chemother 1997; 41: 2270–2273.

89. Takiff H, Salazar L, Guerrero C, et al. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother 1994; 38: 773–780.

90. Alangaden G J, Manavathu E K, Vakulenko S B, et al. Characterization of fl uoroquinolone-resistant mutant strains of Mycobacterium tuberculosis selected in the laboratory and isolated from patients. Antimicrob Agents Chemother 1995; 39: 1700–1703.

91. Cheng A F, Yew W W, Chan E W, et al. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fl uoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. Antimicrob Agents Chemother 2004; 48: 596–601.

92. Pitaksajjakul P, Wongwit W, Punprasit W, et al. Mutations in the gyrA and gyrB genes of fl uoroquinolone-resistant Mycobacterium tuberculosis from TB patients in Thailand. Southeast Asian J Trop Med Public Health 2005; 36 Suppl 4: 228– 237.

93. Lee A S, Tang L L, Lim I H, et al. Characterization of pyrazinamide and ofl oxacin resistance among drug resistant Mycobacterium tuberculosis isolates from Singapore. Int J Infect Dis 2002; 6: 48–51.

94. Sun Z, Zhang J, Zhang X, et al. Comparison of gyrA gene mutations between laboratory-selected ofl oxacin-resistant Mycobacterium tuberculosis strains and clinical isolates. Int J Antimicrob Agents 2008; 31: 115–121.

95. Sulochana S, Narayanan S, Paramasivan C N, et al. Analysis of fl uoroquinolone resistance in clinical isolates of Mycobacterium tuberculosis from India. J Chemother 2007; 19: 166– 171.

96. Sreevatsan S, Pan X, Stockbauer K E, et al. Restricted structural gene polymorphism in the Mycobacterium tuberculosis complex indicates evolutionarily recent global dissemination. Proc Natl Acad Sci USA 1997; 94: 9869–9874.

97. Matrat S, Veziris N, Mayer C, et al. Functional analysis of DNA gyrase mutant enzymes carrying mutations at position 88 in the A subunit found in clinical strains of Mycobacterium tuberculosis resistant to fl uoroquinolones. Antimicrob Agents Chemother 2006; 50: 4170–4173.

98. Wang J Y, Lee L N, Lai H C, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis isolates: associated genetic mutations and relationship to antimicrobial exposure. J Antimicrob Chemother 2007; 59: 860–865.

99. Kocagoz T, Hackbarth C, Unsal I, et al. Gyrase mutations in laboratory selected, fl uoroquinolone-resistant mutants of Mycobacterium tuberculosis H37Ra. Antimicrob Agents Chemother 1996; 40: 1768–1774.

100. Zhou J, Dong Y, Zhao X, et al. Selection of antibiotic-resistant bacterial mutants: allelic diversity among fl uoroquinoloneresistant mutations. J Infect Dis 2000; 182: 517–525.

101. van Doorn H R, An D D, de Jong M D, et al. Fluoroquinolone resistance detection in Mycobacterium tuberculosis with locked nucleic acid probe real-time PCR. Int J Tuberc Lung Dis 2008; 12: 736–742.

102. Antonova O V, Gryadunov D A, Lapa S A, et al. Detection of mutations in Mycobacterium tuberculosis genome determining resistance to fl uoroquinolones by hybridization on biological microchips. Bull Exp Biol Med 2008; 145: 108–113.

103. Hegde S S, Vetting M W, Roderick S L, et al. A fl uoroquinolone resistance protein from Mycobacterium tuberculosis that mimics DNA. Science 2005; 308: 1480–1483.

104. Davies J, Gorini L, Davis B. Misreading of RNA codewords induced by aminoglycoside antibiotics. Mol Pharmacol 1965; 1: 93–106.

105. Finken M, Kirschner P, Meier A, et al. Molecular basis of streptomycin resistance in Mycobacterium tuberculosis: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. Mol Microbiol 1993; 9: 1239–1246.

106. Honore N, Cole S T. Streptomycin resistance in mycobacteria. Antimicrob Agents Chemother 1994; 38: 238–242.

107. Nair J, Rouse D A, Bai G H, et al. The rpsL gene and streptomycin resistance in single and multiple drug-resistant strains of Mycobacterium tuberculosis. Mol Microbiol 1993; 10: 521–527.

108. Honore N, Marchal G, Cole S T. Novel mutation in 16S rRNA associated with streptomycin dependence in Mycobacterium tuberculosis. Antimicrob Agents Chemother 1995; 39: 769–770.

109. Cooksey R C, Morlock G P, McQueen A, et al. Characterization of streptomycin resistance mechanisms among Mycobacterium tuberculosis isolates from patients in New York City. Antimicrob Agents Chemother 1996; 40: 1186–1188.

110. Okamoto S, Tamaru A, Nakajima C, et al. Loss of a conserved 7-methylguanosine modifi cation in 16S rRNA confers low-level streptomycin resistance in bacteria. Mol Microbiol 2007; 63: 1096–1106.

111. Spies F S, da Silva P E, Ribeiro M O, et al. Identifi cation of mutations related to streptomycin resistance in clinical isolates of Mycobacterium tuberculosis and possible involvement of effl ux mechanism. Antimicrob Agents Chemother 2008; 52: 2947–2949.

112. Alangaden G, Kreiswirth B, Aouad A, et al. Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother 1998; 42: 1295–1297.

113. Suzuki Y, Katsukawa C, Tamaru A, et al. Detection of k anamycin-resistant Mycobacterium tuberculosis by identifying mutations in the 16S rRNA gene. J Clin Microbiol 1998; 36: 1220–1225.

114. Maus C E, Plikaytis B B, Shinnick T M. Mutation of tlyA confers capreomycin resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2005; 49: 571–577.

115. Johansen S, Maus C, Plikaytis B, et al. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2'O-methylations in 16S and 23S rRNAs. Mol Cell 2006; 23: 173–182.

116. Maus C E, Plikaytis B B, Shinnick T M. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. Antimicrob Agents Chemother 2005; 49: 3192–3197.

117. Implementing the WHO Stop TB Strategy. A Handbook for National Tuberculosis Control Programmes Geneva: World Health Organization; 2008. ISBN-13: 978-92-4-154667-6.

118. Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis. 2000; 4:97-107.

119. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis. 2007 Aug 15;196 Suppl 1:S15-27.

120. Mase SR et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systemic review. *International Journal of Tuberculosis and Lung Disease*, 2007, 11:485–495.

121. Getahun et al. Diagnosis of smear-negative pulmonary tuberculosis in people living with HIV/AIDS in resource constrained settings: informing urgent policy changes. Lancet, 2007, 369:2042–2049.

122. Crofton J, Horne N, Miller F. Clinical tuberculosis. 2nd Edition. TALC, IUATLD, Mac Milan;1999. 222 p. 135-141.

123. Pfyffer G E, Welsher HM, Kissling P, Cielslak, Casal M, Gutierrez J, Rush-Gerdes S. Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid media for recovery of acid-fast bacilli. J Clin Microbiol. 1997; 35:364-368.

124. Somoskovi A, Kodmon C, Lantos A, Bartfal Z, Tamasi L, Fuzy J, Magyar P. Comparison or Recoveries of Mycobacterium tuberculosis Using the Automated Bactec MGIT 960 System, the BACTEC 460 TB System and Loweinstein-Jensen Medium.J Clin Microbiol. 2000; 38: 2395-2397.

125. Macondo EA, Ba F, Toure-Kane N C, Kaire O, Gueye-Ndiaye A, Gaye-Diallo, Boye C S, M'boup S. Amélioration du diagnostic de la tuberculose par le Mycobacteria Growth Indicator Tube (MGIT) dans un laboratoire de pays en développement. Bull Soc Path Exot. 2000; 93(2):97-100.

126. Diraa O, Fdany K, Boudouma M, Elmdaghri N, Benachir M. Assessment the Mycobacteria Growth Indicator Tube for the bacteriological diagnosis of tuberculosis. Int J Tuberc Lung Dis. 2003; 7(10): 1010-1012.

127. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C.Meta-analysis of BACTEC MGIT 960 with or without solid media ,for detection on mycobacteria. J Clin Microbiol. 2005;42(5):2321-2325.

128. Morgan MA, Horstmeir CD, De Young DR, Robers G D. Comparison of radiometric method (BACTEC) and conventional culture for recovery of mycobacteria from smear-negative. J Clin Microbiol. 1983; 18:384-388.

129. Rajalahti I, Vuorinen P, Nieminen M M, Miettinen A. Detection of Mycobacterium tuberculosis complex by the automated Roche Cobas Amplicor Mycobacterium tuberculosis test. J Clin Microbiol. 1998; 36:975–978.

130. Reischl, U., N. Lehn, H. Wolf, and L. Naumann. Clinical evaluation of the automated Cobas Amplicor MTB assay for testing respiratory and non respiratory specimens. Clin Microbiol. 1998; 36:2853–2860.

131. Bogard M, Vincelette J, Antinozzi R, Alonso R, Fenner T, Schirm J, Aubert D, Gaudreau C, Sala E, Ruiz-Serrano M J, Petersen H, Oostendorp L A B, Burkardt H. Multicenter study of a commercial, automated polymerase chain reaction system for the rapid detection of Mycobacterium tuberculosis in respiratory specimens in routine clinical practice. Eur J Clin Microbiol Infect Dis. 2001; 20:724–731.

132. Gamboa F, Manterola J M, Lonca J, Matas L, Cardona P J, Padilla E, Vinado B, Dominguez J, Hernandez A, Ausina V. Comparative evaluation of two commercial assays for direct detection of Mycobacterium tuberculosis in respiratory specimens. Eur J Clin Microbiol Infect Dis.1998; 17:151–156.

133. Piersimoni C, Callegaro A, Scarparo C, Penati V, Nista D, Bornigia S, Lacchini C, Scagnelli M, Santini G, De Sio G. Comparative evaluation of the new Gen-Probe Mycobacterium tuberculosis amplified direct test and the semi automated Abbott LCx Mycobacterium tuberculosis assay for direct detection of Mycobacterium tuberculosis complex in respiratory and extra pulmonary specimens. J Clin Microbiol. 1998; 36:3601–3604.

134. Wang S X, Tay L. Evaluation of three nucleic acid amplification methods for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol. 1999; 37:1932–1934.

135. Brown T J, Power E G M, French G L. Evaluation of three commercial detection systems for Mycobacterium tuberculosis where clinical diagnosis is difficult. Clin Pathol. 1999; 52:193–197.

136. Scarparo C, Piccoli P, Rigon A, Ruggiero G, Scagnelli M, Piersimoni C. Comparison of enhanced Mycobacterium tuberculosis amplified direct test with COBAS AMPLICOR Mycobacterium tuberculosis assay for direct detection of Mycobacterium tuberculosis complex in respiratory and extrapulmonary specimens. J Clin Microbiol. 2000; 38:1559–1562.

137. Della-Latta P, Whittier S. Comprehensive evaluation of performance, laboratory application, and clinical usefulness of two direct amplification technologies for the detection of Mycobacterium tuberculosis complex. Am J Clin Pathol.1998; 110:301–310.

138. Piersimoni C, Scarparo C, Piccoli P, Rigon A, Ruggiero G, Nista D, Bornigia S. Performance assessment of two commercial amplification assay for direct detection of Mycobacterium tuberculosis complex from respiratory and extra pulmonary specimens. J Clin Microbiol. 2002; 40:4138–4142.

139. Pfyffer G E, Funke-Kissling P, Rundler E, Weber R. Performance characteristics of the BD Probe Tec system for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol. 1999; 37:137–140.

140. Implementing tuberculosis diagnostics. Policy framework. Geneva, World Health Organization. 2015. (WHO/HTM/TB/2015.11). http://www.who.int/tb/publications/implementing_TB_diagnostics/en/

141. Drlica K, Malik M. Fluoroquinolones: action and resistance. Curr Top Med Chem 2003; 3: 249–282.

142. Rastogi N, Goth KS, David HL. Drug susceptibility testing in tuberculosis: a comparison of the proportion methods using Löwenstein-Jensen, Middlebrook 7H10 and 7H11 agar media and a radiometric method. Res Microbiol 1989;140:405-17.

143. Walters SB, Hanna BA. Testing of susceptibility of Mycobacterium tuberculosis to isoniazid and rifampin by Mycobacterium growth indicator tube method. J Clin Microbiol 1996;34:1565-7.

144. Palaci M, Yoko Mizuka Ueki S, Nakamura Sato D, et al. Evaluation of Mycobacteria growth indicator tube for recovery and drug susceptibility testing of Mycobacterium tuberculosis isolates from respiratory specimens. J Clin Microbiol 1996;34:762-4.

145. Wanger A, Mills K. Testing of Mycobacterium tuberculosis susceptibility to ethambutol, isoniazid, rifampin, and streptomycin by using Etest. J Clin Microbiol 1996;34:1672-6.

146. Hausdorfer J, Sompek E, Allerberger F, et al. E-test for susceptibility testing of Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 1998;9:7515.

147. Guidance on ethics of tuberculosis prevention, care and control. Guidance document. Geneva, World Health Organization, 2010. WHO/HTM/TB/2010.16 (available at http://www.who.int/ethics/publications/9789241500531/en/ accessed 12 February 2015).

148. Updated interim critical concentrations for first-line and second-line DST (as of May 2012). Geneva, World Health Organization, 2012 (http://www.stoptb.org/wg/gli/assets/documents/ Updated%20critical%20concentration%20table_1st%20and%202nd%20line%20drugs.pdf, accessed 5 March 2015).

149. Using the Xpert MTB/RIF assay to detect pulmonary and extrapulmonary tuberculosis and rifampicin resistance in adults and children: expert group meeting report. Geneva, World Health Organization, 2013 (WHO/HTM/TB/2013.14) (available at http://www.who.int/tb/laboratory/expert_group_report.pdf?ua=1).

150. Xpert MTB/RIF implementation manual. Technical and operational 'how-to': practical considerations. Geneva, World Health Organization, 2014 (WHO/HTM/TB/2014.1) (available at http://www.who.int/tb/publications/xpert_implem_manual/en/).

151. Laboratory services in TB control. Part III: culture. Geneva, World Health Organization, 1998 (WHO/TB/98.258) (available at http://whqlibdoc.who.int/hq/1998/WHO_TB_98.258_(part3).pdf?ua=1).

152. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. Geneva, World Health Organization, 2008 (WHO/HTM/TB/2008.392) (available at http://whqlibdoc.who.int/hq/2008/WHO_HTM_TB_2008.392_eng.pdf?ua=1)

153. Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis: policy statement. Geneva, World Health Organization, 2011 (WHO/HTM/TB/2011.9) (available at http://whqlibdoc.who.int/publications/2011/9789241501620_eng.pdf).

154. Van Deun A, Maug AK, Bola V, Lebeke R, Hossain MA, et al. (2013) Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol 51:2633–2640.

155. The use of molecular line probe assay for the detection of resistance to second-line anti-tuberculosis drugs: expert group meeting report. Geneva, World Health Organization, 2013 (WHO/HTM/ TB/2013.01) (available at http://apps.who.int/iris/bitstream/10665/78099/1/WHO_HTM_TB_2013.01.eng.pdf).

156. Stevens WS, Scott L, Noble L, et al. Impact of the GeneXpert MTB/RIF Technology on Tuberculosis Control. Microbiol Spectr 2017;5(1).

157. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert®MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults.Cochrane Database Syst Rev. 2014 Jan 21;(1):CD009593.

158. Cockerill FR, Uhl JR, Temesgen Z, et al., Rapid identification of a point mutation of the Mycobacterium tuberculosis catalase-peroxydase (katG) gene associated with isoniazid resistance. J Infect Dis 1995;171:240-5.

159. Theron G, Peter J, Richardson M, Barnard M, Donegan S, Warren R, Steingart KR,Dheda K. The diagnostic accuracy of the GenoType(®) MTBDRsl assay for the detection of resistance to second-line anti-tuberculosis drugs. Cochrane Database Syst Rev. 2014 Oct 29;(10):CD010705.

160. Matsiota-Bernard P, Vrioni G, Marinis E., Characterization of rpoB mutations in rifampin-resistant clinical Mycobacterium tuberculosis isolates from Greece. J Clin Microbiol 1998;36:20-3.

161. Beenhouwer H, Lhiang Z, Jannes W, et al., Rapid detection of rifampin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tubercle Lung Dis 1995;76:425-30.

162. Kapur V, Li LL, Hamrick MR, et al. Rapid Mycobacterium species assignment and unambiguous identification of mutations associated with antimicrobial resistance in Mycobacterium tuberculosis by automated DNA sequencing. Arch Pathol Lab Med 1995;119:131-8.

163. Zignol M, Cabibbe A M, Dean A S, Glaziou P, Alikhanova N et al. Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. Lancet Infect Dis 2018;18:30073-2.

164. Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis. 2007 Aug 15;196 Suppl 1:S15-27.

165. Implementing the WHO Stop TB Strategy. A Handbook for National Tuberculosis Control Programmes Geneva: World Health Organization; 2008. ISBN-13: 978-92-4-154667-6.

166. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Recommendations for a public health approach – Second edition. Geneva, World Health Organization. 2016. http://www.who.int/hiv/pub/arv/arv-2016/

167. Guidelines for treatment of drug-susceptible tuberculosis and patient care, 2017 update. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.

168. International standards for tuberculosis care. The Hague, Tuberculosis Coalition for Technical Assistance, 2006.

169. Fixed-dose combination of tablets for the treatment of tuberculosis. Geneva, World Health Organization, 1999 (WHO/CDS/CPC/TB/99.267).

170. Abdool Karim SS, Naidoo K, Grobler A, et al. Timing of initiation of antiretroviral drugs during tuberculosis therapy. N Engl J Med. Feb 25 2010;362(8):697-706. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20181971..

171. Blanc FX, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med. Oct 20 2011;365(16):1471-1481. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22010913.

172. Havlir DV, Kendall MA, Ive P, et al. Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. N Engl J Med. Oct 20 2011;365(16):1482-1491. Available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=22010914.

173. Scaling up antiretroviral therapy in resource-limited settings. Updated guidelines for a public health approach. 2006 revision. Geneva, World Health Organization, 2006.

174. World Health Organization. Definitions and reporting framework for tuberculosis–2013 revision. Geneva, World Health Organization; 2013:1–40 WHO/HTM/TB/2013.2 (Available from: http://apps.who.int/iris/handle/10665/79199).

175. World Health Organization. Guidelines for treatment of tuberculosis, 4th ed. Geneva, World Health Organization, 2009 (WHO/HTM/TB/2009.420; available at http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf).)

176. World Health Organization. Antimicrobial resistance: Global report on surveillance 2014. Geneva: World Health Organization, 2014.

177. Laszlo A, Rahman M, Raviglione M, Bustreo F. Quality assurance programme for drug susceptibility testing of Mycobacterium tuberculosis in the WHO/IUATLD Supranational Laboratory Network: first round of proficiency testing. Int J Tuberc Lung Dis 1997; 1: 231-8.

178. Laszlo A, Rahman M, Espinal M, Raviglione M. Quality assurance programme for drug susceptibility testing of Mycobacterium tuberculosis in the WHO/IUATLD Supranational Reference Laboratory Network: five rounds of proficiency testing, 1994-1998. Int J Tuberc Lung Dis 2002; 6: 748-56.

179. Van Deun A, Wright A, Zignol M, Weyer K, Rieder HL. Drug susceptibility testing proficiency in the network of supranational tuberculosis reference laboratories. Int J Tuberc Lung Dis 2011; 15: 116-24

180. Guidelines for surveillance of drug resistance in tuberculosis: WHO/TB/94.178. Geneva: World Health Organization, 1994.

181. Guidelines for surveillance of drug resistance in tuberculosis: WHO/TB/96.216. Geneva: World Health Organization, 1997.

182. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. October 2016 revision. Geneva. World Health Organization, 2016. WHO/HTM/TB/2016.04 (http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/ resources/en/, accessed 16 August 2017).

183. Guidelines for surveillance of drug resistance in tuberculosis. 4th ed.: WHO/TB/2009.422. Geneva: World Health Organization, 2009.

184. Guidelines for surveillance of drug resistance in tuberculosis. 5th ed.: WHO/HTM/TB.13.2015. Geneva: World Health Organization, 2015

185. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs — worldwide, 2000–2004. MMWR Morb Mortal Wkly Rep 2006; 55: 301-5.

186. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 2006; 368: 1575-80

187. Global tuberculosis report 2015: WHO/HTM/TB/2015.22. Geneva: World Health Organization, 2015.).

188. South African tuberculosis drug resistance survey 20122014. Sandringham, South Africa: National Institute for Communicable Diseases, 2016 (http://www .nicd .ac .za).

189. Zignol M, Dean A S, Falzon D, van Gemert W, Wright A, van Deun A et al. Twenty Years of Global Surveillance of Antituberculosis-Drug Resistance.The special report. N Engl J Med 2016:375;11;1081-1089. Accessed at www. nejm.org September 15, 2016).

190. Resolution WHA62/15: prevention and control of multidrugresistant tuberculosis and extensively drug-resistant tuberculosis. Presented at the 62nd World Health Assembly, Geneva, May 18–22, 2009.

191. World Health Organization. Stop TB Partnership and World Health Organization. Global Plan to Stop TB. Phase 1 2001 to 2005. Geneva: World Health Organization; 2001 [WHO/CDS/STB/2001.16].

192. World Health Organization. Stop TB Partnership and World Health Organization. Second Global Plan to Stop TB 2006–2015. Geneva: World Health Organization; 2006 [WHO/HTM/STB/2006.35].

193. World Health Organization. Global tuberculosis report 2015. Geneva: WHO; 2015 (http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_ eng.pdf, accessed 2 August 2017).

194. United Nations. Sustainable Development Goals (https:// sustainabledevelopment.un.org/topics/sustainabledevelopmentgoals, accessed 2 August 2017).

195. Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias HM, et al. WHO's new End TB Strategy. Lancet. 2015;385(9979):1799–1801 (http:// www.sciencedirect.com/science/article/pii/ S0140673615605700?via%3Dihub, accessed 2 August 2017).

196. World Health Organization. Accelerating progress on HIV, tuberculosis, malaria, hepatitis and neglected tropical diseases: A new agenda for 2016–2030. Geneva: WHO; 2015 (http://www.who.int/about/structure/ organigram/htm/progress-hiv-tb-malaria-ntd/en/, accessed 2 August 2017).

197. World Health Organization/World Bank Group. Tracking universal health coverage: first global monitoring report. Geneva: WHO; 2015 (http://apps. who.int/iris/bitstream/10665/174536/1/9789241564977_eng.pdf?ua=1, accessed 2 August 2017).

198. The Global Plan to End TB, 2016–2020. Geneva: Stop TB Partnership; 2015 (http://www.stoptb.org/global/plan/, accessed 2 August 2017).

199. Ministère du plan et de suivi de la mise en œuvre de la révolution de la modernité, Ministère de la Santé, ICF International et al. Deuxième enquête démographique et de de santé 2013-2014. Septembre 2014.

200. Programme des Nations Unies pour le Développement. Rapport sur le développement humain 2015.

201. Enquete démographique de santé . 2 é édition : 2013-2014. Ecole de Santé publique, Kinshasa. RDC.

202. Plan National Development Sanitaire 2016-2020. Ministère de la Santé Publique RDC. 2017.

203. DRC National Tuberculosis Annual Report 2017

Chapter 3. Thesis results

3.1 Multi Drug Resistant Tuberculosis in Mosango, a Rural Area in the Democratic Republic of Congo [3]

Michel Kayomo Kaswa[1,2,3], Serge Bisuta[1], Georges Kabuya[1], Octavie Lunguya[2], André Ndongosieme[1], Jean Jacques Muyembe[2], Armand Van Deun[3] and Marleen Boelaert[3].

[1]National Tuberculosis Program, Kinshasa, Democratic Republic of Congo;

[2]Institut National de Recherche Bio-Médicale, Kinshasa, Democratic Republic of Congo;

[3]Institute of Tropical Medicine, Antwerp, Belgium.

Corresponding Author

Michel Kaswa Kayomo

Meckkay2002@yahoo.fr

Abstract

Multidrug Resistant Tuberculosis (MDR-TB) is a serious threat which jeopardizes the worldwide efforts to control TB. The Democratic Republic of Congo (DRC) is one of 27 countries with a high burden of MDR-TB. Data on the magnitude, trends, and the distribution of MDR-TB in DRC are scanty. Kinshasa, the capital city of DRC which accounts for 20% of all TB cases nationwide, is notifying more than 80% of all MDR suspects. We report here a cluster of MDR-TB cases that was investigated in the Mosango health district, in the Bandundu south Province, DRC in 2008. Phenotypic Drug Sensitivity Testing and DNA sequencing were performed on 18 sputum specimens collected from 4 MDR-TB suspects and 5 household contacts. Sequencing data

[3] Kaswa MK, Bisuta S, Kabuya G, Lunguya O, Ndongosieme A, Muyembe JJ, Van Deun A, Boelaert M (2014). Multi Drug Resistant Tuberculosis in Mosango, a Rural Area in the Democratic Republic of Congo. PLoS ONE 9(4): e94618. doi:10.1371/journal.pone.0094618.

confirmed that the 4 suspects were indeed Rifampicin resistant cases. Sequencing of the *rpoB* gene showed that 3 cases (patients A, B and D) had a single mutation encoding a substitution to 526Tyr, 531Trp and 526Leu respectively. Patient C had a double mutation encoding a change to 531Leu and 633Leu. Two of the investigated cases died within 4 months of a second-line treatment course. Results highlight the need to enhance adequate laboratory services within the country for both clinical as well as surveillance purposes.

Introduction

Multidrug Resistant Tuberculosis (MDR-TB) defined as the resistance of clinical isolates of *Mycobacterium tuberculosis* strains against Rifampicin (RMP) and Isoniazid (INH) is considered as a serious threat which jeopardizes the worldwide efforts to control Tuberculosis [1]. Conventional methods for diagnosing MDR-TB are slow and cumbersome requiring at least 2 months for test execution and the treatment is complex.

In Low-Income Countries such as the Democratic Republic of Congo (DRC), the challenge posed by MDR-TB is huge. During the two last decades, the history of the DRC has been rife with civil unrest, which has led to the collapse of the health system and the recrudescence of Tuberculosis (TB) and other infectious diseases. DRC, with an estimated population of 66 million, is ranked 11th among the 22 TB High Burden Countries and has an estimated incidence of TB of 327 per 100 000 inhabitants per year [2, 3]. DRC is classified also among the 27 countries with a high burden of MDR-TB [4], but the actual data on the magnitude, trends, and the distribution of MDR-TB in DRC are scanty. In 2008 the World Health Organization (WHO) estimated that the total number of MDR-TB cases in DRC was 5 600 (95%CI: 530-11 000) [4]. However, less than 2% of this estimated number were detected and put on specific treatment during that same year. Kinshasa, the capital city of DRC which accounts for 20% of all TB cases nationwide, is notifying more than 80% of all MDR suspects, and not all of these are laboratory-confirmed (DRC National TB Program data; unpublished). The burden of MDR-TB in the rest of the country is even less well defined as a result of the scarce laboratory infrastructure and some logistical limitations. Extremely long turn-around times for laboratory results to reach the treating clinician increase the risk of the spread of resistant strains.

Because of the need to closely monitor the spread of this problem across DRC and the scarcity of data beyond the capital Kinshasa, we report here a cluster of MDR-TB cases that occurred in the Mosango health district, in the Bandundu south Province, DRC in 2008.

Methods

Ethics statement

This study used specimens and data collected in the course of routine patient care and resistance surveillance, performed without ethics review or informed consent. The study was approved by the health authorities of DR Congo. To ensure confidentiality, the data were completely delinked from any personal identifiers before data analysis.

Settings

During September 2008, the National TB Program (NTP) of DRC was notified by the Chief Medical Officer of Mosango health district about the admission of 2 laboratory-confirmed cases of MDR-TB in the inpatient wards of the Mosango Hospital. Mosango hospital is the third largest tertiary hospital in DRC with a capacity of 541 beds of which 121 are fully dedicated to TB patients. It is located at 420 Km from Kinshasa. In response to this alert, the NTP launched an investigation and sent a team of 1 microbiologist and public health officer (MKK), 1 MDR program officer (SB) and 1 laboratory technician (GK) to Mosango to review the 2 case histories and actively search for other MDR suspects amongst TB cases and contacts. The team reached the hospital in October 2008 and reviewed all available NTP records as well as the hospital admission register and patient files. Patients in retreatment for tuberculosis with Acid-Fast Bacilli (AFB) smear-positive at month 3 or 5 were considered as MDR-TB suspects. A contact was defined as a first degree relative of a MDR-TB suspect living in the same household for at least 3 months. All retrieved retreatment cases positive for AFB from Mosango Hospital area were listed, their demographic data, clinical TB history and household contacts information were collected and a list of their close contacts was then established. Individual demographic, clinical and laboratory data were captured in an Excel sheet (version 5.0) and later analyzed by the software Epi-Info 3.5.4 (Centers for Disease Control and Prevention, Atlanta, GA).

Laboratory procedures

From each suspect and those contacts identified and included in this investigation, 2 sputum specimens were collected to ensure an adequate recovery of *Mycobacterium tuberculosis* on solid medium Löwenstein Jensen (LJ) and followed by detection of RMP and INH resistance by conventional Drug Susceptibility Testing (DST) on solid medium and further DNA sequencing. Firstly, sputum specimens were collected in a Falcon tube containing 5 ml of cetyl-pyridinium chloride (CPC) at 1% and were transported to the National Reference Laboratory (NRL) in Kinshasa for culture and DST on LJ medium. For primary culture, sputum samples were processed according to standard methods previously described [5]: decontamination and processing with NaOH according to a modified Petroff technique [6], with a contact time shortened to 10 min.

First Line Drugs (FLD) DST was performed on Löwenstein-Jensen according to the indirect proportion method as described by Canetti et al. [7] with final readings at 6 weeks. Strains appearing to belong to the *Mycobacterium tuberculosis* complex (because of their macroscopic appearance, acid fast staining and slow growth) were tested at the critical concentrations of INH 0.2 µg/ml and RMP 40 µg/ml, besides ρ-nitrobenzoic acid (PNB) for differentiation from non-TB mycobacteria according to international guidelines [8]. Secondly, for each sputum specimen collected, an aliquot was preserved in 50% alcohol to permit additional genetic testing in the Supra National Reference Laboratory (SRL) in Antwerp/Belgium. RMP mutations were defined later and independently of DST result by sequencing of the *rpoB* gene. DNA extracts from clinical specimens were prepared using the automated Boom extraction method as described elsewhere [9]. Detection of *rpoB* mutations, targeting a 1,674-bp region from codon 176 to 672, from which RMP resistance-conferring mutations have been reported, was performed as described previously.

A nested PCR with primers *rpoB*geneSAnew (5'-GCAAAACAGCCGCTAGTCCTAGTCCGA-3') and *rpoB*geneRA (5'-GCGCCATCTCGCCGTCGTCAGTACAG-3') for the first run, and *rpoB*geneSA and *rpoB*geneRB as inner primers, was used for amplification [10]. All Rifampicin resistance-determining region (RRDR) mutations, plus others previously reported for the *rpoB* gene, were considered potentially relevant for Rifampicin resistance [11, 12]. Mutations were identified by *rpoB* codon number (Escherichia coli numbering) and amino acid substitution [13]. INH resistance was not determined genetically.

Results

The two index cases mentioned above could not be included in this investigation: one had died before the arrival of the team, and no clinical specimens were available for the other case. The Mosango district TB register listed a total of 24 cases as being in retreatment in October 2008, including the two index cases. We could retrieve 16 (73%) of the 22 suspects within a perimeter of 50 Km around the hospital (the others left an incorrect address, had left the area or lived beyond 50 Km from the hospital). Ten (42%) had clinical symptoms and we collected their sputum for AFB testing. Four of them were AFB positive and were considered as "MDR suspects". Three of them were on their third TB treatment course, and one patient was taking the second course of first-line drugs. None of them had ever interrupted treatment. The team listed 28 household contacts for these 4 patients. Twenty (71%) contacts were retrieved and screened for TB symptoms. Five (25%) had at least one symptom suggesting TB. Culture, phenotypic DST and DNA sequencing were performed on 18 sputum specimens collected from 4 MDR-TB suspects and 5 household contacts. Table I summarizes demographic, clinical and laboratory results of the 9 suspects included in the final analysis. Median age was 28 years (IQR 24-38), 5 (56%) were female. All patient samples were positive in culture (4/4) but none of the samples of their household contacts was positive. Conventional DST showed RMP and INH resistance (MDR) in 2

out of the 4 positive patients and RMP resistance in one. However, sequencing data confirmed that the 4 suspects were indeed RMP-resistant cases. Sequencing of the *rpoB* gene showed that 3 MDR cases (patients A, B and D) had a single mutation encoding a substitution to 526Tyr, 531Trp and 526Leu respectively. Patient C had double mutations encoding a change to 531Leu and 633Leu. Patients B and D died within 4 months of second line drugs (SLD) treatment with Kanamycin, Ofloxacin, Prothionamide, Cycloserine, Pyrazinamide and Ethambutol. Their HIV status was not known. Patients A and C had favorable treatment outcomes under SLD. Recently we reviewed again the TB registers of Mosango health district to assess clinical outcomes of the 36 retreatment cases enrolled in 2008 (not including the 6 MDR patients described above). Treatment outcomes were available for thirty three of the 36: 25 were cured, 4 died, 3 failed and 1 was transferred out.

Discussion

The cluster of MDR-TB cases reported here is to our knowledge the first published account of confirmed MDR-TB from a rural area in DRC. Our findings suggest that MDR-TB is present in Mosango Health district since at least 2008. The DNA sequencing documented molecular mutations of *rpoB* genes conferring resistance in MTBC strains in all 4 suspect patients investigated, but in none of their contacts. Conventional DST performed poorly and missed 2 of these 4 cases of MDR-TB. This is partly explained by contamination of DST but also by highly variable results for RMP sensitivity in the proficiency testing at the Kinshasa NRL. Unfortunately, the delay in diagnosis and appropriate management of the cases led to a high case fatality rate: two of the 4 investigated cases and 1 of the two index cases died. This high case fatality is most likely due to the inadequate retreatment regimen used during several months, as the risk of death with standard short-course chemotherapy is highest when there is resistance to both INH and RMP [14, 15].

The sequencing data did show a set of 4 different drug resistant patterns in the 4 cases suggesting no evidence of a link between these MDR-TB cases. Most likely, the MDR-TB was due to acquired resistance in patients previously treated. Three MDR patients had a strain with a single *rpoB* mutation (526Tyr, 531Trp and 526Leu) and one patient had a strain with a double mutation 531Leu and 633Leu. This double mutation observed in a patient who had been previously treated 3 times with First-Line Drug treatment, is probably due to acquired drug resistance [16]. The mutation 531Leu has been described recently as the most frequent in Kinshasa among relapse and treatment failure TB cases. In a prospective cohort study of TB patients starting retreatment, Van Deun found that this mutation accounts for 63% of all *rpoB* mutations from first recurrence sputum specimens from Kinshasa [17]. Contrastingly, our series consisted of multiple retreatment cases. The MDR-TB patient D had a strain with 526Leu, a mutation that is considered rare by some authors [18]. However the *rpoB* 526Leu, which is part of the group called “disputed“ mutations described previously by Van Deun, is rather common in DRC and elsewhere [17, 18].

Of note, conventional DST at Kinshasa NRL failed to detect it [19, 20]. DNA sequencing performed in our study showed the *rpoB* 633Leu in a double mutation, and to the best of our knowledge, this was never described before. Current molecular DST targeting only the RRDR (codons 507-533) is missing it. We doubt its clinical significance, as it has been found only once and accompanying other, more common mutations. If more frequently seen and also in isolation it might be assumed to have significance.

This study has some limitations. The case definition for a suspect case used in the outbreak investigation was rather restrictive and probably allowed to select only a part of all the people at risk of MDR-TB. Secondly, INH resistance was not determined genetically. Even if RMP resistance is widely recognized as a proxy for MDR-TB, the genotypic pattern of INH is important for surveillance and molecular epidemiology of MDR-TB.

Culture of sputum on a solid agar medium could provide more reliable DST results but is extremely lengthy and significantly delays the initiation of adequate therapy [21]. Liquid-growth based methods and Nucleic Acid Amplifications techniques have demonstrated superior performance in many settings [22, 23, 24, 25, 26]. However under field conditions in high TB settings and low income countries, such as DRC, these methods have high technical and logistical requirements that are not easy to meet in a sustainable and accessible way. The latest genotypic techniques might be definitely much easier to set up and run [27, 28]. From a public health perspective, rapid and timely detection of TB cases and strengthened capacity to diagnose cases of drug-resistant TB remain thus global priorities for TB care and control.

Conclusion

Results observed during this investigation highlight the need to enhance adequate laboratory services in countries confronted with MDR-TB. Fragile states as DRC are facing huge barriers requiring significant investment in laboratory infrastructures and strengthening of human resources. MDR-TB is a serious concern to be considered in all rural settings in Africa and elsewhere, and surveillance of DR-TB should be strengthened globally. A Point-of-Care diagnostic tool, one that can be more readily deployed at the district level would greatly facilitate the timely detection of resistant TB. Its introduction will not only serve diagnosis but also routine surveillance. Sustained SLD provision and adequate case management of MDR-TB patients are moreover essential.

References

1. The worldwide epidemic of multidrug-resistant tuberculosis. Editorial. Lancet Infect Dis 11(5):333.

2. World Health Organization. Global TB report 2012. (WHO/HTM/TB/2012.6). Geneva, WHO, 2012. Available at: http://www.who.int/tb/publications/global_report/gtbr12_main.pdf Accessed 25 November 2013

3. World Health Organization: Global TB Report 2013. Annex 2 Country Profiles: DR of Congo. P.120. Available at : http://www.who.int/tb/publications/global_report/gtbr13_annex_2_country_profiles.pdf

4. Accessed 25 November 2013. World Health Organization 2013.

5. World Health Organization. Anti-tuberculosis drug resistance in the world. Fourth Global Report Geneva. The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance. Fourth Global Report. Geneva, WHO, 2008. WHO/HTM/TB/2008.394 Available at: http://whqlibdoc.who.int/hq/2008/who_htm_tb_2008.394_eng.pdf Accessed 25 November 2013.

6. Van Deun A, Salim AH, Daru P, Das APK, Aung KJM, et al. (2004) Drug resistance monitoring: combined rates may be the best indicator of programme performance. Int J Tuberc Lung Dis 8:23–30.

7. Petroff SA (1915) A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J Exp Med 21: 38–42.

8. Canetti G, Fox W, Khomenko A, Mitchison DA, Rist N, et al. (1969) Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 41: 21– 43.

9. Rieder HL, Van Deun A, Kam KM, Kim SJ, Chonde TM, et al. (2007) Priorities for tuberculosis bacteriology services in low-income countries. Second edition. Paris: International Union Against Tuberculosis and Lung Disease. 120p.

10. Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, et al. (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503.

11. Rigouts L, Nolasco O, de Rijk P, Nduwamahoro E, Van Deun A, et al. (2007) Newly developed primers for comprehensive amplification of the rpoB gene and detection of rifampin resistance in Mycobacterium tuberculosis. J Clin Microbiol 45:252–254.

12. Ramaswamy S, Musser JM (1998) Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis. Tuber Lung Dis 79:3–29.

13. Sandgren A, Strong M, Muthukrishnan P, Weiner BK, Church GM, et al. (2009) Tuberculosis drug resistance mutation database. PLoS Med 6(2):e1000002. doi:10.1371/journal.pmed.1000002.

14. Van Der Zanden AGEM, Te Koppele-Vije N, Vijaya Bhanu D, Van Soolingen, Schouls LM (2003) Use of DNA extracts from Ziehl-Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of Mycobacterium tuberculosis. J Clin Microbiol 41:1101– 1108.

15. Schreiber YS, Herrera AF, Wilson D, Wallengren K, Draper R, et al. (2009) Tuberculosis retreatment category predicts resistance in hospitalized retreatment patients in a high HIV prevalence area. Int J Tuberc Lung Dis 13(10):1274-80.

16. Lan NTN, Lademarco MF, Binkin NJ, Tung LB, Quy HT, et al. (2001) A case series: initial outcome of persons with multidrug-resistant tuberculosis after treatment with the WHO standard retreatment regimen in Ho Chi Minh City, Vietnam. Int J Tuberc Lung Dis 5(6):575-8.

17. Lipsitch M, Levin BR (1998) Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. Int J Tuberc Lung Dis 2:187–199.

18. Van Deun A, Maug AK, Bola V, Lebeke R, Hossain MA, et al. (2013) Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol 51:2633–2640.

19. Yip CW, Leung KL, Wong D, Cheung DTL, Chu MY, et al. (2006) Denaturing HPLC for high-throughput screening of rifampicin-resistant Mycobacterium tuberculosis isolates. Int J Tuberc Lung Dis 10:625–630.

20. Ohno H, Koga H, Kohno S, Tashiro T, Hara K (1996) Relationship between rifampin MICs and rpoB mutations of Mycobacterium tuberculosis strains isolated in Japan. Antimicrob Agents Chemother 40:1053–1056.

21. Van Deun A, Barrera L, Bastian I, Fattorini L, Hoffmann H, et al. (2009) Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results. J Clin Microbiol 47:3501–3506.

22. Quezada C M, Kamanzi E, Mukamutara J, De Rijk P, Rigouts L, et al. (2007) Implementation validation performed in Rwanda to determine whether the INNO-LiPA Rif.TB Line Probe Assay

can be used for detection of Multidrug-Resistant *Mycobacterium tuberculosis* in low-resource countries. J Clin Microbiol 45:3111–3114.

23. Pfyffer GE, Welsher HM, Kissling P, Cielslak, Casal M, et al. (1997) Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid media for recovery of acid-fast bacilli. J Clin Microbiol 35:364-368.

24. Somoskovi A, Kodmon C, Lantos A, Bartfal Z, Tamasi L, et al. (2000) Comparison or Recoveries of Mycobacterium tuberculosis Using the Automated Bactec MGIT 960 System, the BACTEC 460 TB System and Lowenstein-Jensen Medium. J Clin Microbiol 38: 2395-2397.

25. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, et al (2005) Meta-analysis of BACTEC MGIT 960 with or without solid media ,for detection on mycobacteria. J Clin Microbiol 42(5):2321-2325.

26. Bogard M, Vincelette J, Antinozzi R, Alonso R, Fenner T, et al. (2001) Multicenter study of a commercial, automated polymerase chain reaction system for the rapid detection of Mycobacterium tuberculosis in respiratory specimens in routine clinical practice. Eur J Clin Microbiol Infect Dis 20:724–731.

27. Piersimoni C, Scarparo C, Piccoli P, Rigon A, Ruggiero G, et al. (2002) Performance assessment of two commercial amplification assay for direct detection of Mycobacterium tuberculosis complex from respiratory and extra pulmonary specimens. J Clin Microbiol 40:4138–4142.

28. Hillemann D, Rüsch-Gerdes S, Richter E (2007) Evaluation of the Geno-Type MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J Clin Microbiol 45:2635–2640.

29. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, et al. (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert® MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377(9776):1495–505.

Table 1. Demographic, clinical and laboratory results of MDR suspects (n=9).

N°	Status	Age	Sex e	Clinical History	N° of FLD course	Culture and DST on LJ in NRL Kinshasa					*RpoB* Sequencing at SRL in Antwerp	Treatment outcomes of SLD assessed in 2011
						SM	Culture	ID	RMP	INH		
A	Case	20	F	F2	3	+	+	MTBC	R	C	His526Tyr(TAC)	Treatment completed
B	Case	29	M	F1	2	++	+	MTBC	R	R	Ser531Trp(TGG)	Died
C	Case	31	F	R2	3	++	+	MTBC	R	R	Ser531Leu(TTG), Arg633Leu (CTC)	Cured
D	Case	38	M	F2	3	++	+	MTBC	S	R	His526Leu(CTC)	Died
A1	Contact	6	F	NA	NA	0	NG	NT				
A2	Contact	22	M	NA	NA	0	NG	NT				
B1	Contact	59	F	NA	NA	0	NG	NT				
C1	Contact	24	F	NA	NA	0	NG	NT				
D1	Contact	41	M	NA	NA	0	NG	NT				

A1: First contact of case A; **A2**: Second contact of case A; **B1**: First contact of case B; **C1**: First contact of case C; **D1**: First contact of case D. **M**: male; **F**: female. **F1**: Failure in WHO standard first line treatment regimen for category 1 for new cases; **F2**: Failure in WHO standard first line treatment regimen for category 2 retreatment cases; **R2**: Relapse in WHO standard first line treatment regimen for category 2 retreatment cases. **NA**: Not applicable. **N° of FLD**: Number of first-line drugs. **SM**: smear microscopy results. **NG**: no growth. **ID**: identification. **MTBC**: *Mycobacterium tuberculosis* complex. **NT**: not tested. **R**: Resistant; **S**: sensitive; **C**: contaminated. **SLD**: second-line drugs.

3.2 A PSEUDO-OUTBREAK OF PRE-XDR TB IN KINSHASA: A COLLATERAL DAMAGE OF FALSE FLUOROQUINOLONE RESISTANT DETECTION BY GENOTYPE® MTBDRSL [4]

Michel K. Kaswa,[a,b,c]# Muriel Aloni,[b] Léontine Nkuku,[b] Brian Bakoko,[a] Rossin Lebeke,[a] Albert Nzita, [a] Jean Jacques Muyembe, [b] Bouke C. de Jong,[c,d] Pim de Rijk, [c] Jan Verhaegen,[e] Marleen Boelaert,[c] Margareta Ieven,[f] Armand Van Deun[c]

National Tuberculosis Program, Kinshasa, Democratic Republic of Congo [a]; Institut National de Recherche Bio-Médicale, Kinshasa, Democratic Republic of Congo [b]; Institute of Tropical Medicine, Antwerp, Belgium [c]; New York University, New York, USA [d]; KU Leuven, Leuven, Belgium [e]; University of Antwerp, Antwerp, Belgium[f]

Running Head: MTBDR*sl* yields false resistance to fluoroquinolones

address correspondence to Michel K. Kaswa, meckkay2002@yahoo.fr

Abstract

Fluoroquinolones are the core drugs for management of multidrug-resistant tuberculosis (MDR-TB). Molecular drug susceptibility testing methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB. We describe misidentification of fluoroquinolone resistance by the GenoType®MTBDR*sl* (MTBDR*sl,* Hain Lifescience GmbH, Nehren, Germany) Line Probe Assay (LPA) encountered during a feasibility and validation study for the introduction of this rapid drug susceptibility test in Kinshasa, Democratic Republic of Congo. The double *gyrA* mutation 80Ala and 90Gly represented 57% of all fluoroquinolone mutations identified from MDR-TB patient sputum samples, as confirmed by DNA sequencing. This double mutation was previously found to be associated with susceptibility to fluoroquinolones, yet leads to absent hybridization of a wildtype band in the MTBDR*sl* and is thus falsely scored as resistance. Our findings suggest to interpret MTBDR*sl* results with caution when the interpretation is solely based on the absence of a wildtype band without confirmation by visualization of a mutant band. Performance of the MTBDR*sl* LPA could be improved replacing the *gyrA* wildtype probes by additional probes specific for well documented *gyrA* mutations that confer clinically relevant resistance.

[4] Kaswa MK, Aloni M, Nkuku L, Bakoko B, Lebeke R, Nzita A, Muyembe JJ, de Jong B, de Rijk P, Verhaegen, Boelaert M, Ieven M, Armand Van Deun A. Pseudo-outbreak of pre-extensively drug-resistant (Pre-XDR) tuberculosis in Kinshasa: collateral damage caused by false detection of fluoroquinolone resistance by GenoType MTBDR sl. J Clin Microbiol. 2014;52:2876-2880.

INTRODUCTION

Fluoroquinolones (FQ) are essential drugs for the management of multidrug-resistant tuberculosis (MDR-TB) (1). Resistance to FQs is associated with poor treatment outcome in MDR-TB and is also one of the defining conditions of extensively drug resistant tuberculosis (XDR- TB, i.e. a case of MDR-TB with additional resistance to any FQ and a second line injectable drug such as kanamycin, amikacin and capreomycin; pre-XDR-TB is defined as MDR-TB associated with FQ resistance or a second line injectable, but not both) (2-5). In areas with high rates of TB and MDR-TB, it is extremely important to monitor resistance to these drugs, especially where FQs are widely used for treatment of other bacterial infections. Molecular drug susceptibility testing (DST) methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB. Currently, they are offering speed of diagnosis, standardized testing, potential for high burden settings, and lower level for laboratory biosafety compared to conventional DST (6). In 2008, the World Health Organization (WHO) has endorsed molecular line probe assay (LPA) technology for rapid detection of MDR-TB that brings results within 2 days –even from clinical specimens (7). The GenoType® MTBDR*plus* assay (MTBDR*plus*, Hain LifeScience GmbH, Nehren, Germany) was one of the first commercially available LPAs. Because of its accuracy and rapidity, genotypic detection of Rifampicin (RMP) and Isoniazid resistance the MTBDR*plus* has emerged as an essential tool for the diagnosis of MDR-TB (8, 9). It has also been suggested as an alternative approach for conducting Drug Resistance Surveys (DRS) in settings with limited capacity to perform phenotypic DST (10). In 2009, the company Hain Lifescience introduced a new format of the LPA, the GenoType® MTBDR*sl* test (MTBDR*sl*), for the rapid determination of genetic mutations associated with resistance to FQs, second line injectable drugs and ethambutol. The main mechanism of resistance to FQs in *Mycobacterium tuberculosis* is caused by mutations affecting DNA gyrase, which consists of the GyrA and GyrB subunits, encoded by the *gyrA* and *gyrB* genes, respectively (11). Most mutations conferring bacterial resistance to FQs occur in a short segment termed the quinolone resistance determining region (QRDR) in the *gyrA* gene (12, 13). Analysis of the QRDR alone by genotypic tests has been suggested as sufficient for rapid identification of the vast majority of FQ-resistant *M. tuberculosis* strains with an estimated sensitivity around 85% for FQ resistance (14, 15). The identification of resistance to FQs by the MTBDR*sl* is based on this principle. The format of the MTBDR*sl* is similar to that of the MTBDR*plus* and it also has a turn-around-time of 48 hours. Compared to phenotypic DST, its sensitivity to detect FQ resistance by identification of the best known *gyrA* mutations (but for instance not the *gyrB* mutations) has been generally evaluated as sub-optimal and for this reason it was not yet endorsed by WHO, but its specificity is considered to be very high (15,16). Here we report that also the specificity of MTBDR*sl* LPA was inadequate for FQ-resistance during a validation study of the assay in Kinshasa.

MATERIALS AND METHODS

Patients and specimens. From March 2011 to June 2013, a feasibility and validation study with both the MTBDR*plus* and MTBDR*sl* LPAs was carried out at the National Public Health Laboratory of the Democratic Republic of Congo (DRC)-the Institut National de Recherche Biomédicale (INRB)-in Kinshasa. Kinshasa is the capital city of DRC with an estimated 10 million inhabitants. With a total of 137 TB clinics, the city of Kinshasa is notifying more than 80% of all MDR-TB suspects in the country (DRC National TB Program, unpublished data). We prospectively collected sputum specimens of 587 MDR-TB suspects in 50 out of the 137 TB clinics which were purposefully selected as sentinel sites in Kinshasa for rapid detection of drug resistant TB. Consenting MDR-TB suspects defined according to the WHO categories of treatment (17) have been consecutively included in the study, in addition to smear-positive contacts of known MDR-TB cases.

MTBDR*sl*. Sputa were transported from the TB clinics without any additive. Patient information related to their demographic data and clinical TB history was collected. Sputa were processed according to standard methods previously described: decontamination and processing with NaOH according to a modified Petroff technique, with a final concentration of 2% (18). The sediment obtained was tested by the MTBDR*plus* according to the manufacturer's instructions (19). All specimens showing resistance to RMP and isoniazid or to RMP alone by the MTBDR*plus* were concurrently tested by the MTBDR*sl* from the same DNA extract. Strips were interpreted according to the manufacturer's instructions (20). For each gene, the test evaluates the presence of wildtype (WT) and/or mutant (MUT) sequences, thus covering all high-confidence resistance mutations. For *gyrA* these are 90Val, 91Pro, and the codon 94 mutations Ala, Asn, Gly, His and Tyr. When all the WT probes of a specific gene appear as bands on the strip, there is no detectable mutation within the region examined and the strain is considered sensitive to the corresponding drugs. In case of a mutation, the amplicon cannot bind to the corresponding WT probe, but it may bind to one of the MUT probes provided this specific mutation is represented on the strip. The absence of a WT band or appearance of a MUT band at least as strong as the amplification control band must be interpreted as resistance to the respective drugs.

The INRB laboratory follows a strict unidirectional work flow for all molecular testing. For Quality Control, each test batch included a known pan-susceptible TB strain (H37Rv). Negative controls (water) were included during all steps of the procedure. The strips were interpreted on a regular schedule by two different readers (MKK and MA) who were blinded to the result of genetic sequencing. Discrepancies between both readers were uncommon (less than 10%), they were resolved by consensus.

Genetic sequencing. Sequencing of the *gyrA* was performed at the Supra National Reference Laboratory (SRL) in Antwerp/Belgium on all available DNA extracts from Kinshasa harboring FQ

resistant patterns on LPA. The methodology for PCR amplification and sequencing of genes encoding Gyrase A and B has been published elsewhere (21).

Ethical considerations. The study was approved by the Ethics Committee of the University of Antwerp, Belgium and the National Tuberculosis Program DRC. All data and sputum were collected in the context of routine care and no additional data collection or contact with patients occurred for this study. The data were completely delinked from any personal identifiers before analysis.

RESULTS

MTBDR*sl* test. Of 587 consecutive individual MDR-TB suspects who did submit their sputum at TB clinics during the study period, a total of 211 MDR-TB and 28 RMP mono-resistant results were obtained from DNA extracts tested with MTBDR*plus*. All 239 extracts with RMP resistance were also tested by MTBDR*sl.* Of those, 87% (209/239) yielded an interpretable test for FQ *gyrA*, with the remainder invalid due to absence of the *gyrA* control band. As shown in Table 1, of 209 samples with an interpretable result, 177 (85%) were identified as FQ-susceptible and 32 (15%) as FQ-resistant. Out of 32 FQ-resistant samples, 20 (63%) were identified as resistant only based on the lack of hybridization with WT probe number 2 (WT2), while for the other 12 one or two specific mutation bands appeared.

The distribution of the 14 gene mutations found in the 12 FQ-resistant samples with a MUT band on the MTBDR*sl* strips is shown in Table 1. The predominant mutations identified as conferring FQ-resistance were *gyrA* MUT3A (94Ala) (6/14: 43%) followed by MUT3C (94Gly) (5/14: 36%), MUT1 (90Val) (2/14: 14%) and MUT2 (91Pro) (1/14: 7%), with Arg94Ala & Arg94Gly found twice as a double mutation. No MUT3B (94Asn or Tyr) or MUT3D (94His) mutations were found in our study.

DNA sequencing results. *gyrA* sequencing was performed on 25 (25/32:78%) DNA extracts identified by MTBDR*sl* as FQ-resistant. DNA sequencing confirmed mutations in the *gyrA* QRDR for 23/25 (92%), but two contained only WT DNA. Table 2 shows the type of mutations detected by DNA sequencing and their frequencies, stratified by WHO patient category and compared to MTBDR*sl* results. There were 4 patients in WHO category 4 (recurrence after 2nd-line TB treatment) versus 12 in category 2 (recurrence after retreatment with 1st-line drugs) and 9 in category 1 (recurrence after primo-treatment with 1st-line drugs). The 94Gly substitution was detected in three of the four DNA extracts from category 4 patients, once as a triple mutation (80Ala & 90Gly & 94Gly), while the fourth showed a 94Ala mutation. Mutations in this group were detected directly with MTBDR*sl* by hybridization with the *gyrA* probes in MUT3C or MUT3A and missing corresponding WT bands, except for the 94Ala which showed as MUT3A and MUT3C bands without loss of WT3. Eleven samples from WHO category 2 patients were confirmed to

contain multiple mutations, but one showed only wildtype DNA on sequencing. The two multiple mutations, 94Tyr & 94Ala and 80Ala & 90Gly & 94Ala, showed only a *gyrA* MUT3A band on the MTBDR*sl* strip. The expected MUT3B was missing for the first, and for both all wildtype bands were still present. The nine remaining with an 80Ala and 90Gly mutation on sequencing only showed as an absent WT2 band in the MTBDR*sl*. From WHO category 1 patient samples, two single and six double mutations were identified by DNA sequencing, but only wildtype DNA could be found in one sample. Both single mutations (90Val and 91Pro) and one of the 6 double mutations (80Ala & 90Val) were detected with the MTBDR*sl* by hybridization with the *gyrA* probe in MUT1 or MUT2 and absence of the WT2 band. The 5 remaining double mutations (80Ala & 90Gly and 80Ala & 90Arg) were all associated with lack of hybridization with WT2 in MTBDR*sl* without a MUT band appearing. Overall, results by sequencing showed that the double mutations 80Ala & 90Gly represent 57% of all 23 confirmed *gyrA* mutations among MDR-TB patients in Kinshasa, DRC.

DISCUSSION

This is the first assessment of the performance of LPA technology under routine diagnostic conditions in the capital city of DRC, one of the 22 TB high burden countries. According to the MTBDR*sl* results, the proportion of FQ resistance among MDR-TB (i.e. RMP-resistant) samples was alarming at 15%. Of these *gyrA* mutants, 63% were indirectly detected by MTBDR*sl* through lack of hybridization with the WT2 nor any mutant *gyrA* probes. However, besides a few cases without any mutation detected, DNA sequencing showed for more than half of those a combination of mutations 80Ala and 90Gly, which has previously been demonstrated to confer FQ hyper-susceptibility (22). Studies by the same group suggest that this hyper-susceptibility may be caused by a stronger covalent bond and resulting enzyme blockage if the WT 90Ala is replaced by 90Gly because of its smaller side-chain, while a bulkier side-chain, as with the 90Val mutation, has the opposite effect and causes resistance (23). 80Ala is not detected by MTBDR*sl* LPA since codon 80 is not covered by the test. The *gyrA* codons analyzed range from 89-93, including codon 90. Our sequence results revealed a mutation in 90Gly (GGG), while the only mutation probe included on the strip for position 90 is 90Val (GTG), explaining why only the absence of WT2 was found in these samples but no confirmation by appearance of a mutant probe. The MTBDR*sl,* which contains mutant DNA probes only for the most frequent *gyrA* QRDR mutations observed (12, 24) at codons 90, 91 and 94, has previously been assessed as highly specific in several countries worldwide (25-31). In our series in Kinshasa, the majority of the QRDR mutations observed were not associated with true FQ resistance. Mutation prevalence might differ by geographical areas and by pre-selection of patients (26). Negative controls, included in DNA extraction, PCR amplification, and hybridization, never showed evidence of contamination. Moreover, 14/20 profiles with a lacking WT2 were single occurrences found in as many runs, and the remaining six were found per two in three runs. For these reasons we believe that

contamination or cross-contamination of the tests is highly unlikely to explain the high frequency of this unusual pattern.

Fundamentally, the problem is that interpretation of the MTBDR*sl* is based on absence of WT bands, which is equated with resistance (20). Errors are known to occur because of silent mutations with a change of nucleotide resulting in a different code but for the same amino-acid (26). The systematic error we report here was hitherto hardly known, i.e. a polymorphism not associated with resistance that appears to be more common in Kinshasa, DRC. In contrast to what was reported elsewhere (27-30), the predictive value for demonstration of FQ resistance of the assay in Kinshasa is therefore low, since the proportion of test results falsely indicating resistance to FQs was high. We erroneously alarmed the DRC National TB Program that FQ resistance had taken a big leap, based on these LPA results, before finding out through sequencing that the combination of 80Ala (GCC) and 90Gly (GGG) mutations were in fact not associated with true FQ resistance. After this correction, the proportion of FQ resistance did drop to 5%. Considering sequencing as the gold standard, two samples had been wrongly classified as resistant because of a missing WT2 band. This may have been due to poor amplification compared to the control, leading to erroneous interpretation. Aberrant results were seen also for two samples with multiple mutations on DNA sequencing, but only a MUT3A band appearing on the LPA, and no wildtype band disappearing. Another sample with only a 94Ala mutation detected by sequencing showed two mutation bands on LPA, MUT3A and MUT3C. These differences may have been caused by different proportions of the alleles present in the aliquots used for the different tests, possibly together with some remaining wildtype DNA. At a too low proportion, the alleles would not be reliably detected by either test. DNA sequencing performed in our study showed the *gyrA* 80Ala (GCC) & 90Arg (AGG) in a double mutation, and to the best of our knowledge, this was never described previously.

In low-income countries with high TB burden, resistance to FQ is not routinely tested because of the very limited laboratory infrastructure. New molecular techniques that do not have the same biosafety requirements as conventional techniques have the potential to overcome this problem and are an alternative to periodic or continuous surveillance of resistance against this important class of drugs. Local validation of a novel molecular assay will require assessing its accuracy compared to a reference, ideally composed of standard pheno- and genotypic techniques. For MDR-TB management, knowledge on FQ susceptibility is crucial as FQs represent the core drugs in all the second-line drugs regimens. Failure to detect mutations conferring resistance (i.e. poor sensitivity) or over-detection of false resistance (i.e. poor specificity) results in poor programmatic management of MDR-TB cases. FQ-resistance in *M. tuberculosis* has a major impact on MDR-TB patient outcome, and removal of FQ seriously jeopardizes the strength of the second-line regimen, so false positive results should be avoided. Molecular differentiation of the *gyrA* mutations 80Ala and 90Gly has important clinical consequences, since these mutations are

not associated with FQ resistance. In Kinshasa, managing MDR-TB cases based on results of the MTBDR*sl* LPA only would thus have been detrimental for patient prognosis.

In this study, a significant proportion of DNA extracts from MDR patients had mutations observed in the QRDR, suggesting a rapid increase or even an outbreak of pre-XDR-TB in Kinshasa. Although not confirmed as pre-XDR, the high frequency of the unusual combination of 80Ala and 90Gly mutations still suggests clonal expansion of an MDR-TB strain in Kinshasa. These mutations do not confer any advantage due to FQ resistance and were also not observed among category 4 recurrences. Their high prevalence may thus point to continued MDR transmission due to delayed or absent detection and treatment of MDR-TB. Further studies using genotyping techniques with higher resolution should clarify the proportion of MDR-TB due to recent transmission.

This study has some limitations. First, we could not correlate the mutations with FQ resistance (level) in our population, since only few strains were still available and local phenotypic DST appeared unreliable. In turn, this was caused by extremely high culture contamination rates besides numerous operational problems, illustrating the higher feasibility of genotypic compared to conventional DST, even at the capital. Second, DNA sequencing was not performed on susceptible RMP and on susceptible FQ to verify if this polymorphism is frequent in Kinshasa also in absence of MDR-TB. Moreover, fingerprinting of these isolates was not done to identify whether the 80Ala (GCC) and 90Gly (GGG) mutations are characteristic of a single MTBc lineage that is common in Kinshasa. Further studies are necessary to characterize the biological significance and potential selective advantage of these mutations not conferring resistance.

Our results warrant caution in the interpretation of the MTBDR*sl* when the only sign of resistance is the absence of WT2 band hybridization, without the presence of confirmatory mutation bands. Such instances may lead to the false interpretation as FQ resistance in settings with high prevalence of the 80Ala and 90Gly polymorphisms that do not confer resistance. Performance of the MTBDR*sl* LPA could thus be improved by omitting all *gyrA* WT probes and adding the few missing mutant probes well documented to confer FQ resistance, so that all clinically relevant mutations are confirmed by a mutant band.

ACKNOWLEDGMENTS

This work was funded by a grant from the Directorate General of Development Cooperation of the Belgian Government through the Institutional Collaboration program between of ITM with INRB and a grant by the Vlaamse Inter universitaire Raad (VLIR), both institutions from Belgium.

REFERENCES

1. Yew W W, Chan C K, Chau C H, Tam CM, Leung CC, Wong PC, Lee J. 2000. Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin/levofl oxacin-containing regimens. Chest. 117: 744–751.
2. Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP, Gandhi NR, Galvani AP. 2009. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect. Dis. 9:153-161.
3. Mitnick CD, Shin SS, Seung GY, Rich ML, Atwood SS, Furin JJ, Fitzmaurice GM, Alcantara Viru FA, Appleton SC, Bayona JN, Bonilla CA, Chalco K, Choi S, Franke MF, Fraser HS, Guerra D, Hurtado RM, Jazayeri D, Joseph K, Llaro K, Mestanza L, Mukherjee JS, Muñoz M, Palacios E, Sanchez E, Sloutsky A, Becerra MC. 2008. Comprehensive treatment of extensively drug-resistant tuberculosis. N. Engl. J. Med. 359:563-574.
4. Jacobson KR, Tierney DB, Jeon CY, Mitnick CD, Murray MB. 2010. Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. Clin. Infect. Dis. 51:6-14.
5. Banerjee R, Allen J, Westenhouse J, Oh P, Elms W, Desmond E, Nitta A, Royce S, Flood J. 2008. Extensively Drug-Resistant Tuberculosis in California, 1993-2006. Clin. Infect. Dis. 47:450-457.
6. World Health Organization. 2013. The use of molecular line probe assay for the detection of resistance to second-line anti tuberculosis drugs: expert group meeting report. (WHO/HTM/TB/2013.01). World Health Organization, Geneva, Switzerland.
7. World Health Organization.2008. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement. World Health Organization, Geneva, Switzerland.
8. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. 2008. Rapid molecular screening for multidrug resistant tuberculosis in a high volume public health laboratory in South Africa. Am. J. Respir. Crit. Care Med. 177:787–792.
9. Hillemann D, Rüsch-Gerdes S, Richter E. 2007. Evaluation of the GenoType MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J. Clin. Microbiol. 45:2635–2640.
10. Rigouts L, Hoza A S, De Rijk P, Torrea G, Chonde T M, Basra D, Zignol M, van Leth F, Egwaga S M, Van Deun A. 2011. Evaluation of the Genotype® MTBDR*plus* assay as a tool for drug resistance surveys. Int. J. Tuberc. Lung Dis. 15:959–965.
11. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J,

Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG.1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393: 537–44.

12. Cheng AFB, Yew WW, Chan EWC, Chin ML, Hui MM, Chan RCY. 2004. Multiplex PCR amplimer conformation analysis for rapid detection of *gyrA* mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob. Agents Chemother. 48:596–601.
13. Matrat S, Veziris N, Mayer C, Jarlier V, Truffot-Pernot C, Camuset J, Bouvet E, Cambau E, Aubry A. 2006. Functional analysis of DNA gyrase mutant enzymes carrying mutations at position 88 in the A subunit found in clinical strains of *Mycobacterium tuberculosis* resistant to fluoroquinolones. Antimicrob. Agents Chemother. 50:4170–4173.
14. Chang KC, Yew WW, Chan RCY. 2010. Rapid assays for fluoroquinolone resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. J. Antimicrob. Chemother. 65:1551-1561.
15. Hillemann D, Rüsch-Gerdes S, Richter E. 2009. Feasibility of the GenoType MTBDR*sl* assay for fluoroquinolone, amikacin-capreomycin and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. J. Clin. Microbiol. 47:1767-1772.
16. Barnard M, Warren R, Gey Van Pittius N, van Helden P, Bosman M, Streicher E, Coetzee G, O'Brien R. 2012. Genotype MTBDR*sl* line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory. Am. J. Respir. Crit. Care Med. 186:1298-305.
17. World Health Organization. 2003. Treatment of tuberculosis: guidelines for national programmes, 3rd ed. World Health Organization document WHO/ CDS/TB/2003.313. World Health Organization, Geneva, Switzerland.
18. Petroff SA. 1915. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J. Exp. Med. 21: 38–42.
19. Hain Lifescience GmbH. 2009. GenoType MTBDR*Plus* Ver2.0: instruction manual. Hain Lifescience, GmbH, Nehren, Germany.
20. Hain Lifescience GmbH. 2009. GenoType MTBDR*sl* Ver1.0: instruction manual. Hain Lifescience, GmbH, Nehren, Germany.
21. Von Groll A, Martin A, Jureen P, Hoffner S, Vandamme P, Portaels F, Palomino JC, Almeida da Silva P. 2009. Fluoroquinolone resistance in *Mycobacterium tuberculosis* and mutations in *gyrA* and *gyrB*. Antimicrob. Agents Chemother. 53:4498-4500.
22. Aubry A, Veziris N, Cambau E, Truffot-Pernot C, Jarlier V, Fisher L M. 2006. Novel gyrase mutations in quinolone-resistant and –hypersusceptible clinical isolates of *Mycobacterium*

tuberculosis: functional analysis of mutant enzymes. Antimicrob. Agents Chemother. 50:104–112.

23. Piton J, Petrella S, Delarue M, André-Leroux G, Jarlier V, Aubry A, and Mayer C. 2010. Structural insights into the quinolone resistance mechanism of *Mycobacterium tuberculosis* DNA gyrase. PLoS One 5: e12245.doi:10.1371/journal.pone.0012245.

24. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs WR Jr, Telenti A. 1994. Cloning and nucleotide sequence of *Mycobacterium tuberculosis* gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother. 38: 773–80.

25. Miotto P, Cabibbe AM, Mantegani P, Borroni E, Fattorini L, Tortoli E, Migliori GB, Cirillo DM. 2012. Genotype MTBDR*sl* performance on clinical samples with diverse genetic background. Eur. Res. J. 40: 690-698.

26. Lacoma A, Garcia-Sierra N, Part C, Maldonado J, Ruiz-Manzano J, Haba L, Gavin P,S. Samper S, Ausina V, Domíngueza J. 2012. Genotype MTBDR*sl* for molecular detection of second-line-drug and Ethambutol resistance in *Mycobacterium tuberculosis*. J. Clin. Microbiol.50: 30–36.

27. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. 2010. Detection by genotype MTBDR*sl* test of complex mechanisms of resistance to second-line drugs and Ethambutol in multidrug-resistant *Mycobacterium tuberculosis* Complex isolate. J. Clin. Microbiol.48: 1683–1689.

28. Huang WL, Chi TL, Wu MH, Jou R. 2011. Performance Assessment of the GenoType MTBDR*sl* test and DNA Sequencing for detection of Second-Line and Ethambutol Drug Resistance among patients infected with multidrug-resistant *Mycobacterium tuberculosis*. J. Clin. Microbiol. 49:2502–2508.

29. Kiet VS, Lan NT, An DD, Dung NH, Hoa DV, van Vinh Chau N, Chinh NT, Farrar J, Caws M. 2010. Evaluation of the MTBDR*sl* test for detection of second-line-drug resistance in *Mycobacterium Tuberculosis*. J. Clin. Microbiol. 48: 2934–2939.

30. Ajbani K, Nikam C, Kazi M, Gray C, Boehme C,Balan K, Shetty A, Rodriguez C. 2012. Evaluation of Genotype MTBDR*sl* assay to detect drug Resistance associated with Fluoroquinolones, Aminoglycosides and Ethambutol on clinical sediments. PLoS One 7(11): e49433. doi:10.1371/journal.pone.0049433.

31. Maruri F , Sterling TR, Kaiga AW, Blackman A, van der Heijden Y F, Mayer C, Cambau E, Aubry A. 2012. A systematic review of gyrase mutations associated with fluoroquinolone-resistant *Mycobacterium tuberculosis* and a proposed gyrase numbering system. J. Antimicrob. Chemother. 67: 819–831.

Table 1. Genotypic pattern obtained by MTBDR*sl* line-probe assay on 209 sputum DNA extracts from documented multi-drug resistant tuberculosis in Kinshasa

MTBDR*sl* results	*gyrA* pattern	No. of strains	%
Resistant (N=32)	MUT3A	4	12.5
	MUT3C	3	9.3
	MUT1	2	6.2
	MUT3A+MUT3C	2	6.2
	MUT2	1	3.1
	ΔWT2	20	62.5
Sensitive (N=177)	WT1+WT2+WT3	177	84.7

MUT1, MUT2, MUT3A, MUT3C: mutation bands appearing

WT1, WT2, WT3: wildtype DNA bands appearing

ΔWT2: wildtype 2 band disappearing

Table 2. MTBDR*sl* patterns versus *gyrA* mutations detected by DNA sequencing among 25 of the 32 DNA extracts, by WHO patient category

Type of patient	*gyrA* MTBDR*sl* pattern	*gyrA* sequencing data, codons and amino acid (nucleotide) substitutions	N (%)
Cat.4			
	ΔWT3+MUT3C	94Gly (GGC)	2 (8)
	ΔWT2+ΔWT3+MUT3C	80Ala(GCC),90Gly(GGG),94Gly(GGC)	1 (4)
	MUT3A+MUT3C	94Ala (GCC)	1 (4)
Cat.2			
	MUT3A	94Tyr (TAC) & 94Ala (GCC)	1 (4)
	MUT3A	80Ala(GCC),90Gly(GGG),94Ala(GCC)	1 (4)
	ΔWT2	80Ala (GCC), 90Gly (GGG)	9 (36)
	ΔWT2	WT	1 (4)
Cat.1			
	ΔWT2+MUT1	90Val(GTG)	1 (4)
	ΔWT2+MUT1	80Ala (GCC), 90Val (GTG)	1 (4)
	ΔWT2+MUT2	91Pro (CCG)	1 (4)
	ΔWT2	80Ala (GCC), 90Gly (GGG)	4 (16)
	ΔWT2	80Ala (GCC), 90Arg (AGG)	1 (4)
	ΔWT2	WT	1 (4)

WT: only wildtype DNA detected; ΔWT: omission of the respective wildtype band; Cat. 1, 2 and 4: WHO patient categories of recurrences after respectively 1st-line primo-treatment, 1st-line retreatment and 2nd-line treatment.

3.3 SURVEILLANCE OF DRUG RESISTANCE AMONG RETREATMENT CASES IN KINSHASA: LESSONS LEARNED?[5]

Michel Kaswa Kayomo[1,2,3,4], Armand Van Deun[4], Epco Hasker[4], M. Aloni[2,3], L. Nkuku[3], P. de Rijk[4], V. Bola[1], B. Bakoko[1], R. Lebeke[1], A. Nzita[1], Bouke C. de Jong[4,5], J. Verhaegen[6], Jean-Jacques Muyembe Tamfum[2,3], G. Bakaswa[1], Margareta Ieven[7] and Marleen Boelaert[4]

1 National Tuberculosis Program, Kinshasa, Democratic Republic of Congo;
2 Université de Kinshasa, Kinshasa, Democratic Republic of Congo;
3 Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo;
4 Institute of Tropical Medicine, Antwerp, Belgium;
5 New York University, New York, USA;
6 KU Leuven, Leuven, Belgium;
7 University of Antwerp, Antwerp, Belgium.

CORRESPONDING AUTHOR
Michel Kaswa Kayomo
National Tuberculosis Program, Kinshasa, Democratic Republic of Congo.
Meckkay2002@yahoo.fr

Running head: RMP and MDR-TB resistance in Kinshasa, DRC.

[5] Manuscript in preparation

ABSTRACT

SETTINGS: Kinshasa, Democratic Republic of Congo (DRC).

BACKGROUND: To document rifampicin (RMP) and fluoroquinolone (FQ) resistance among recurrent cases (i.e. failure or relapse) after first treatment, and to evaluate the feasibility of Multi Drug Resistant Tuberculosis (MDR-TB) surveillance in DRC context.

METHODS: Sputum samples of recurrent cases were collected by the diagnostic units of Kinshasa's TB program and sent for phenotypic drug susceptibility testing (DST) to the Supra-National Reference Laboratory in Antwerp (2005 to 2010), or for genotypic DST by line probe assay to the National Public Health Laboratory INRB in Kinshasa (since 2011).

RESULTS: For the period 2005-2010, among 2 349 retreatment patients sampled, 1 609 (68.0%) had a valid phenotypic DST result, 282 (17.5%) of those were MDR-TB, and 6 (2.3%) of the MDR-TB also were resistant to FQs. For the period 2011-2013, among 192 retreatment patients, 157 (82.0%) had valid molecular DST, including 64 (40.7%) with MDR-TB, of whom 4 (7.0 %) also had FQ resistance. Although there seemed to be a trend towards increased resistance, this was not confirmed by triangulation of our findings with trends in number of recurrent cases.

CONCLUSION: Drug resistance monitoring combined with routine MDR case management proved challenging, in particular transport of the samples to the laboratory was a major bottleneck. Trends were hard to analyze because of the change to a more selective drug regimen, and from pheno- to genotypic DST. A robust surveillance system for all previously treated cases requires addressing the gaps in DST coverage.

KEYWORDS: Main first and second-line drugs; resistance; failure and relapse cases, DST coverage.

BACKGROUND

Any problem with treatment of multi-drug resistant tuberculosis (MDR-TB) represents a source of risk for Fluoroquinolones (FQs)-resistant TB. Resistance of MDR-TB strains to FQs is difficult to manage and is one of the two defining criteria of extensively drug resistant tuberculosis (XDR-TB) [1]. XDR-TB is defined as TB caused by *Mycobacterium tuberculosis* complex (MTBC) strains resistant to both Rifampicin (RMP) and Isoniazid (INH) with additional resistance to any FQ and a second line injectable drug such as kanamycin, amikacin or capreomycin. Emergence of XDR-

TB has been reported from an increasing number of settings [2]. With an estimated TB incidence of 326 per 100 000 inhabitants per year, the Democratic Republic of Congo (DRC) is classified among the 22 and the 27 countries with a high burden of TB and MDR-TB respectively [3, 4]. However, the evidence on the magnitude, distribution, and trends of MDR-TB in DRC is scanty, since data on drug resistance are only generated at specific sites at the subnational level or in the framework of research projects. In 1998, during the Kinshasa survey performed by the Antwerp Supra-National TB Reference Laboratory (SRL), the proportion of MDR-TB was 2.2% among new cases of TB and 22.1% among retreatment cases. FQ resistance was not tested in those days [Van Deun A; unpublished data]. The proportion of MDR-TB with FQ resistance in DRC is not well documented due to the scarce laboratory infrastructure [5, 6]. Surveillance of FQ resistance is essential to track the emergence of XDR-TB, especially in areas such as the DRC where they are widely used for treatment of other bacterial infections. In the DRC, logistical constraints have so far prevented the implementation of a nationwide survey for this purpose [2], with the first such survey scheduled to commence in 2015. The World Health Organization (WHO) suggested that in settings currently lacking the capacity for exhaustive surveillance based on routine drug susceptibility testing (DST) of all previously treated TB cases, sentinel surveillance based on a sample of previously treated cases, i.e. all first-line failure and relapse cases, should be used as an integral part of programmatic monitoring of MDR-TB. Laboratory-based surveillance should evaluate the susceptibility of *M. tuberculosis* to INH and RMP; and if resistance to RMP is present, then susceptibility to the FQs and second-line injectable agents most often used in the setting should also be tested [7]. Recently the molecular line probe assay (LPA) that was endorsed by the WHO in 2008 has been suggested as an alternative approach for drug resistance surveys in settings with limited capacity to perform phenotypic DST, by targeting primarily rifampicin resistance [8].

Since 1996, the National TB Program (NTP) DRC introduced the directly observed treatment, short course programme (DOTS). Up to 2006 the first-line regimen for WHO category 1 patients in Kinshasa was 2RHEZ/6HE[6]. From 2007 onwards this regimen was replaced by 2RHEZ/4HR. In 2006, the Green Light Committee (GLC) approved a 5 years project to treat 1 100 MDR-TB patients in DRC with second-line drugs. The prevention of the emergence of drug resistance relies on the strict use of treatment regimens, individualized or standardized as long as the regimen provides sufficient coverage for the patients' resistance pattern and regularity of drug intake. Unfortunately, the NTP of the DRC was confronted in recent years with shortages in several TB drugs (first- as well as second-line) and logistical challenges related to the drug supply chain. This has resulted in treatment interruptions and patients being placed on waiting lists.

[6] E, EMB =ethambutol; H, INH =isoniazid; R, RMP =rifampicin; Z, PZA =pyrazinamide. Numbers preceding the letters indicate the duration in months of the phase of treatment.

Such drug stock outs are of major concern. The conditions for emergence of drug resistance are undoubtedly present in DRC.

The primary objective of this analysis was to determine the magnitude of drug resistance particularly RMP, MDR-TB and FQ resistance, in the patients with recurrence (failure or relapse/reinfection) after primary treatment of tuberculosis in Kinshasa, the capital of DRC with about 10 million inhabitants. In parallel, we evaluated the feasibility of a simplified and more efficient strategy for monitoring of drug resistance in DRC.

METHODS

Ethical aspects

From 2005 to 2010 named "early period", all data and sputum were collected in the context of routine care and no additional data collection or contact with patients occurred for this study. The data were delinked from any personal identifiers before data analysis. This study was approved by the health authorities of DR Congo. From 2011 to 2013 called "late period", an approval from the Ethical Committee of the University of Antwerp, Belgium and of the Ministry of Health in DRC was obtained for this study.

Study patients and specimens.

During the early period, the 30 largest diagnostic centers of Kinshasa were instructed to send sputum samples of smear-defined recurrences (first failures and first relapses) after primary treatments [9] for DST. The final inclusion was limited to first failure and first relapse cases. Sputa mixed with cetylpyridinium chloride (CPC) were sent to the SRL at the Institute of Tropical Medicine (ITM) in Antwerp for DST on solid media until early 2010, when this scheme was stopped by the NTP. During the late period, fresh sputum from the same type of patients suspect for drug resistance had to be sent for phenotypic DST to the Kinshasa National Reference Laboratory (NRL), from where it was further referred for genotypic testing to the Institut National de Recherche Biomédicale (INRB), the National Public Health Laboratory in Kinshasa. During this second period, 20 additional diagnostic centers were included in the network, bringing the total number to 50.

Reference laboratory tests

During the early period, primary culture and first-line DST on Löwenstein-Jensen medium (LJ) with final reading at 6 weeks were performed at the SRL in Antwerp/Belgium using standard methods, as previously described [10]. Ofloxacin susceptibility testing by the proportion method

was performed at 2 µg/ml on Middlebrook 7H11 agar [11]. Internal quality control was performed using a reference susceptible and an MDR–TB strain.

During the late period, the GenoType® MTBDR*plus* and MTBDR*sl* Line Probe Assays (LPA, Hain LifeScience GmbH, Nehren, Germany) were used for DST at INRB. Plain sputa were decontaminated with NaOH according to a modified Petroff technique [12]. The sediment obtained was tested by the MTBDR*plus* and MTBDR*sl* according to the manufacturer's instructions [13]. Only specimens showing resistance to RMP by the MTBDR*plus* were concurrently tested by the MTBDR*sl* from the same DNA extract.

For all available DNA extracts harboring FQ resistant patterns on LPA, sequencing of the *gyrA* was performed later and independently of LPA results in the SRL in Antwerp/Belgium, as MTBDR*sl* was not endorsed by WHO. The methodology for PCR amplification and sequencing of genes encoding Gyrase A and B has been published elsewhere [14].

Data collection, cleaning and analysis

Routine data were recorded from the request forms. For this analysis, we double-checked the recorded treatment antecedents with the TB nurses to reach the most correct patient category classification based on previous treatment status (i.e. failure, relapse or other retreatment type after first treatment, after retreatment with first-line drugs or after second-line treatment).

To assess the coverage of drug resistance monitoring, we collected the annual numbers of smear-positive retreatment cases reported by the NTP for the Kinshasa province during the study period. To triangulate our findings on drug resistance among isolates tested, we also assessed the proportion of treatment after failure and relapse cases among all smear-positive cases registered each year, as well as their treatment outcomes.

The main outcomes of interest analyzed for this study were the proportion of retreatment cases with valid DST results for isoniazid, rifampicin and ofloxacin (or fluoroquinolones) available and the prevalence of drug resistance computed as the proportion of confirmed drug resistant TB cases over the number of cases with valid DST results. Valid DST results were defined as a result showing the detection of TB and susceptibility or resistance to RMP, INH and FQ. LPA FQ resistant samples were reassessed at SRL using DNA sequencing and corrected if required [15].

All data related to drug resistance were entered in Excel sheet (version 2010) and verified by one of the authors (MKK). These data were later linked with DNA sequencing results from the SRL. Both data sets were compared using Epi info (version 3.5.4) and in case of discrepancies we consulted the source document: questionnaires, laboratory forms and LPA results. Comparisons between groups were made using Fisher's exact method (for two data points) presented with

95% confidence intervals (CIs). Trends over longer periods were assessed with chi square for trend.

RESULTS

NTP registrations 2005-2013

According to the NTP notification routine data, the total number of smear-positive cases registered from 2005-2013 in Kinshasa was 97 680. As shown in figure 1, the proportion of failures and relapses among all smear-positive cases registered remained fairly stable (X^2=0.06). Out of 97 680 patients reported over the study period, treatment outcomes of 84 963 were evaluated (figure 2). The proportion of failure as treatment outcome among new smear-positive cases fluctuated between 1.4 and 1.9%. There was overall a small increase that was statistically significant (P=0.04). Proportions of failure as treatment outcome among patients registered as treatment after failure or treatment after relapse did not show any clear trends (P= 0.40 and P=0.46 respectively).

DST coverage

During the study period (2005-2013) among the 97 680 smear-positive cases, 14 156 retreatment cases were registered. As observed in figure 3, of this number 2 541 (17.9 %) provided a sputum sample for a DST and 1 766 (69.5%) had a valid DST result. Table 1 presents for the two periods numbers of first failure and first relapse cases as registered by the NTP versus the number of sputum samples collected from these cases. Their number among smear-positive TB cases registered did not fluctuate much over the study period. However the proportion of first failure cases sampled per year fluctuated from 44.0 % in the early period to 23.0 % in the late period. The sample coverage of first relapse cases dropped even further, from 26.0% in the early period to 4.0 % in the late period.

Overall laboratory results

During the early period, of 2 349 sputum samples enrolled in this study 409 (17.0%) were from first failure cases and 1 940 (83.0%) from first relapse cases (Table 2). Of those, 1 609 (68.0%) yielded a MTBC strain with valid DST result, 672 (29.0%) remained culture-negative, 46 (2.0%) were contaminated and 22 (1.0%) contained mycobacteria other than *M. tuberculosis*.During the late period, 192 patients tested by MTBDR*plus* performed at INRB were enrolled in this study: 71 (40.0 %) were first failure cases and 121 (60.0%) were first relapse cases. Of their sputa, 157 (82.0%) yielded a TB-specific amplicon, 33 (17.0%) failed to amplify and 2 (1.0%) contained DNA from mycobacteria other than *M. tuberculosis*. Overall MTBC recovery by conventional DST

(68.5%, 95% CI 67-70) was significantly lower than by LPA (81.8%, 95% CI 75-87). The proportion of first failure cases among retreatment cases enrolled had increased significantly from 17.0% until 2010 to 37.0% after 2010 (P=0.001).

First-line DST results

The total number of valid DST for the whole study period was 1 766. Six hundred forty nine (36.8%) of the 1 766 *M. tuberculosis* isolates were resistant to RMP or to RMP and INH. Overall, the prevalence of mono-resistance to RMP was 3.5 % vs. 19.5% for MDR. Prevalence of MDR resistance was much higher among first failure cases than among first relapse cases (61.5% vs.11.3%). Table 3 presents the results of first-line DST performed during the study period. When comparing between the two periods we observed an increase in resistance in both categories of patients for mono RMP resistance (from 3.2% to 5.0% among first relapses [P=0.05] and from 3.8% to 7.0% among first failures [P= 0.03]) as well as for MDR (from 10.6% to 20.0% among first relapses [P=0.02] and from 57.6% to 77.1% among first failures [P=0.001]).

Fluoroquinolone DST results

Table 4 reports the results of FQ DST performed for RMP resistant and MDR-TB cases enrolled during the study period. The proportion of specimens resistant to FQ among MDR-TB cases was 3.0 % overall. We observed an increase in the proportion of FQ resistant isolates from the first period to the second, i.e. 6/269 (2.3%) vs 4/57 (7.0 %). However, this difference was not statistically significant (P=0.14).

DISCUSSION

Summary of findings

We have spent substantial time and effort to describe changing rates of tuberculosis drug-resistance in Kinshasa, Congo. This represents an interest for policy makers, public health programs and clinicians since data from the DRC on MDR-TB are sparse. Unfortunately, the changes in populations sampled and methods used over the study periods require interpreting our findings with caution. This study provides the first report on monitoring of MDR-TB and FQ resistance in Kinshasa, DRC. From 2005 to 2013, among failure and relapse cases with valid DST results, 346 (19.5%) showed resistance to RMP and INH. This proportion of MDR-TB amongst recurrent cases is similar to the one observed in the survey of retreatment cases done in 1998 [Van Deun A; unpublished data] but is 1.5 fold higher than the current DRC estimates (13%, 95% CI 0.2-28) [3]. This proportion is also higher than the MDR-TB prevalence reported in the remaining 8 African TB high burden countries in 2013 [3]. Our data suggest that the overall

frequency of MDR-TB remained at the same level in Kinshasa between 2005 to 2009. An apparent increase of MDR-TB occurred in our series after 2010 but this finding is biased by drastic changes in population sampled and DST method. Several other factors than a genuine increase in MDR resistance may explain the higher proportions of MDR-TB in our second study period. First of all, laboratory procedures differed between the two study periods. Overall recovery in culture among smear-defined recurrences was rather low in the 2005-2010 periods (68 %), which is partly explained by often very long transportation delays between Kinshasa and the SRL in Belgium. Second, patient spectrum did differ substantially between the 2 periods. During the early period, failure cases represented only 17% of the sample. During the late period, they represented 37%. Failure cases are more often associated with MDR-TB than relapse cases [16-18], as it is indeed seen in stratified analysis in the present study (Table 3). In 2012, the cure rate of failure cases after primary treatment was 52% versus 79% among relapse cases [Kinshasa Office, DRC NTP data; unpublished]. In support of a true increase in MDR is the lower sensitivity of the LPA molecular test for rifampicin resistance relative to the proportion method on Löwenstein-Jensen. Last but not least, several programmatic weaknesses of the control program in Kinshasa (DRC) may indeed have caused increasing numbers of acquired resistance. Though this phenomenon can only be proven if the original strain – before any treatment - is available for comparison, the apparent increase in total RMP resistance should be a warning to the NTP that the acquired component may be on the increase and the program thus dysfunctional.

We found FQ resistance to be uncommon among MDR-TB in our cohort in Kinshasa (3.0%). The proportion of FQ resistance among MDR-TB cases tested fluctuated particularly during the second period due to the small denominators for the annual number of samples. However, the absolute number of FQ resistance detected each year remained low. Comparing the early and the late periods, there was no significant difference in the proportion of FQ resistance among MDR or among RMP-resistant cases, though the denominator for the second period is small and one should consider that the LPA used during this second period is detecting only about 90% of phenotypically detected FQ resistance [19, 20]. Resistance to FQ is usually considered as substantially less prevalent in countries with Green Light Committee (GLC) approved projects relative to those without [21]. FQ resistance prevalence in Kinshasa, DRC remains lower than that observed in Rwanda (9.4%) in 2005 [22] and in South Africa (12.6%) in 2012 [21]. According to Dalton et al., the prevalence of FQ resistance correlates well with the time that these drugs have been available within the country [21]. Contrastingly, in Kinshasa, where FQ drugs are prescribed since the mid-1990s the level of resistance apparently still remains low, probably because the access to FQ treatment is limited by its cost. Huang et al. suggested that exposure to FQ for treatment of other bacterial diseases in the community did not appear to be responsible for the increasing trend of resistance in the context of Taiwan [23].

Strengths of the study

Few NTPs in low-income countries with a high TB burden reported data so far about their monitoring of drug resistance mainly because of inadequate laboratory infrastructure which leads to a low level of DST coverage. This study exploited all available laboratory data even those collected in the context of externally funded studies to evaluate the magnitude and trends in anti-tuberculosis drug resistance in Kinshasa since 2005. The data will help to quantify the burden of MDR-TB in DRC and document the emergence of pre-XDR-TB. These findings will facilitate the planning and the design of a rational surveillance system for the country by the NTP, and serve as comparator for the systematically sampled DRS currently underway. With the implementation of LPA in Kinshasa, the proportion of valid DST has significantly been raised (82.0 % versus 68.0 % with Conventional DST). Moreover, molecular DST does not require viable bacilli to detect drug resistance. In a vast country such as DRC (2 345 409 Km^2), faced with numerous logistical problems, the implementation of molecular DST methods with fewer requirements for laboratory biosafety, could improve significantly the scaling up of programmatic management and surveillance of TB drug resistance.

Study limitations

The study pulls data together over the period 2005-2013, yet there were major changes in the surveillance methods between the early and the late periods. During the late period, phenotypic DST results, performed in the NRL, were not used in the final analysis due to limited capacity to perform continuously high quality testing. Only genotypic DST results were available. Twenty-eight percent of retreatment cases had a DST in the early period of analysis and 6 % in the late period. There is an over-representation of failure cases in the late period due to changed referral indications, focusing on the group with the highest MDR-TB prevalence. The changing proportion of failures versus relapses sampled, in combination with the change in DST technique between the two study periods; make a straightforward interpretation of trends impossible. The proportion of retreatment cases who had access to DST was declining over time, causing selection bias, so that the level of drug resistance suggested in this report cannot be considered as representative of all previously treated TB cases in Kinshasa. However, the data could still be used to monitor rifampicin resistance among failure cases, as their proportion sampled remained fairly constant and sufficiently high to limit the impact of fluctuations.

Policy implications

Firstly, the Global Plan to STOP TB (2006-2015) foresees that by 2015 all countries should carry out DST for all retreatment TB patients [24]. In Kinshasa, during the process of implementing continuous surveillance of resistance, several logistic and operational challenges occurred such as sample collection, transportation delays, DST operations and a hiatus in funding leading to

reduced culture performed since 2009, which resulted in a small number of samples collected per year and hence limited the accuracy of the estimates. However, the main obstacles that need to be addressed urgently are collection and transport of the samples to the reference laboratory, which requires prompt payment to the health care worker or to a volunteer who brings the samples. Alternative approaches, such as a dedicated vehicle or motorcycle passing along the TB program diagnostic units, reporting a suspect by phone, and the sustainability and cost of such a system should be explored. These important issues need to be addressed in the specific context of the country. In settings currently lacking capacity for surveillance based on routine DST of all previously treated TB cases, separate sampling of previously treated cases should be considered in the design of the surveillance system. Key issues to consider include training of health care workers, implementation of an efficient sputum transportation network and the establishment of a surveillance system that is representative for the patient population and best fits the country needs, as recommended by the WHO [4]. Under program conditions, enrolment criteria might be kept very simple, and more focused on comparison of rates between subgroups of previously treated TB cases to monitor trends of drug resistance and the efficiency of the control program. The sustainable implementation of such surveillance system will require careful planning and the involvement of all stakeholders around the NTP in order to produce reliable data.

CONCLUSION

Drug resistance monitoring combined with routine case management proved challenging in Kinshasa. Analysis of these trends has however shown that MDR/XDR-TB should be recognized as a major public health concern in Kinshasa, DRC, a country already facing a large epidemic of TB and HIV in the context of poverty and insufficient access to public health services. To establish a routine and comprehensive surveillance system for all previously treated cases, which is the first step towards routine drug susceptibility testing for all TB patients, requires not only the implementation of a new molecular technique but also to address a number of programmatic issues related to DST coverage. All barriers limiting access to DST should be removed urgently. NTPs should grasp the opportunities created by the roll-out of the new generation molecular tools to improve and to support the surveillance of drug resistance. At the same time that emphasis is put on their implementation, more attention and funding should be dedicated to strengthening the entire DOTS-plus program to achieve global TB control and avoid treatment stock-outs for all patients, including the increasing number of patients recognized to have resistant tuberculosis.

ACKNOWLEDGEMENTS

This work was funded by a grant from the Directorate General of Development Cooperation of the Belgian Government through the Institutional Collaboration program between of ITM with INRB and a grant by the Vlaamse Interuniversitaire Raad (VLIR) in the project, both institutions from Belgium. Data collection in the first part of the study was funded by the Damien Foundation, USAID, and l'Agence Française de Développement.

List of abbreviations

CPC: Cetylpyridinium chloride;
DRC: Democratic Republic of Congo;
DST: Drug resistance testing;
GLC: Green Light Committee;
FQ : Fluoroquinolone;
INH: Isoniazid;
INRB : Institut National de Recherche Biomédicale ;
ITM: Institute of Tropical Medicine;
LJ: Löwenstein-Jensen medium;
LPA: Line Probe Assay;
MDR: Multi drug resistant;
NRL: National Reference Laboratory;
NTP: National TB Program;
RMP: Rifampicin;
SRL: Supra National Reference Laboratory;
WHO: World Health Organization;
XDR: Extensively drug resistant.

Authors' contributions

Conceived and designed the study: MKK AVD JV MB. Performed the experiments: MKK MA LN PR. Acquisition of the data: MKK AVD VB BB RL AN. Analyzed and interpreted the data: MKK AVD EH. Wrote the paper: MKK. Reviewed the manuscript: AVD EH BJ JV JJM GB MI MB.

The authors have declared to have no conflicts of interest.

REFERENCES

1. Jacobson KR, Tierney DB, Jeon CY, Mitnick CD, Murray MB. Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. Clin Infect Dis. 2010;51:6-14.
2. World Health Organization. Global tuberculosis report 2013 WHO/HTM/TB/2013.11. 2013. http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf. Accessed 6 Oct 2015.
3. World Health Organization. Global tuberculosis report 2014. Annex 2 Country Profiles: Democratic Republic of the Congo - p.130. 2014. http://www.who.int/tb/publications/global_report/gtbr14_annex2_country_profiles.pdf?ua=1. Accessed 6 Oct 2015.
4. World Health Organization. Anti-tuberculosis drug resistance in the world. Fourth Global Report. The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance. WHO/HTM/TB/2008.394. 2008. http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf. Accessed 6 Oct 2015.
5. Richter E, Rüsch-Gerdes S, Hillemann D. Drug-susceptibility testing in TB: current status and future prospects. Expert Rev Respir Med. 2009;3:497–510.
6. Kim SJ, Espinal MA, Abe C, Bai GH, Boulahbal F, Fattorin L, et al. Is second-line antituberculosis drug susceptibility testing reliable? Int J Tuberc Lung Dis. 2004;8:1157–1158.
7. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 4th ed. WHO/HTM/TB/2009.422. 2009. http://apps.who.int/iris/bitstream/10665/44206/1/9789241598675_eng.pdf. Accessed 6 Oct 2015.
8. Rigouts L, Hoza AS, De Rijk P, Torrea G, Chonde TM, Basra D, et al. Evaluation of the Genotype® MTBDR plus assay as a tool for drug resistance surveys. Int J Tuberc Lung Dis. 2011;15(7):959–965.
9. World Health Organization. Le traitement de la tuberculose: Principes à l'intention des programmes nationaux. Troisième edition. WHO/CDS/TB/2003.313. 2003. http://apps.who.int/iris/bitstream/10665/69518/1/WHO_CDS_TB_2003.313_fre.pdf. Accessed 6 Oct 2015.
10. Van Deun A, Salim AH, Daru P, Das AP, Aung KJ, Hossain MA, et al. Drug resistance monitoring: combined rates may be the best indicator of programme performance. Int J Tuberc Lung Dis. 2004;8:23–30.
11. Bozeman L, Burman W, Metchock B, Welch L, Weiner M. Fluoroquinolone susceptibility among Mycobacterium tuberculosis isolates from the United States and Canada. Clin Infect Dis. 2005;40:386–91.

12. Petroff SA. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J Exp Med. 1915;21:38–42.

13. Hain Lifescience GmbH. GenoType MTBDRsl Ver1.0: instruction manual. Hain Lifescience, GmbH, Nehren, Germany, 2009.

14. Von Groll A, Martin A, Jureen P, Hoffner S, Vandamme P, Portaels F, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis and mutations in gyrA and gyrB. Antimicrob. Agents Chemother. 2009;53:4498-4500.

15. Kaswa MK, Aloni M, Nkuku L, Bakoko B, Lebeke R, Nzita A, et al. Pseudo-outbreak of pre-extensively drug-resistant (Pre-XDR) tuberculosis in Kinshasa: collateral damage caused by false detection of fluoroquinolone resistance by GenoType MTBDRsl. J Clin Microbiol. 2014;52:2876-2880.

16. Quy H T, Lan N T, Borgdorff M W, Grosset J, Linh PD, Tung LB, et al. Drug resistance among failure and relapse cases of tuberculosis: is the standard re-treatment regimen adequate? Int J Tuberc Lung Dis. 2003;7:631–636.

17. Becerra MC, Freeman J, Bayona J, Shin SS, Kim JY, Furin JJ, et al. Using treatment failure under effective directly observed short-course chemotherapy programs to identify patients with multidrug-resistant tuberculosis. Int J Tuberc Lung Dis. 2000;4:108–114.

18. Noeske J, Voelz N, Fon E, Abena Foe JL. Early results of systematic drug susceptibility testing in pulmonary tuberculosis retreatment cases in Cameroon. BMC Research Notes. 2012;5:160.

19. Hillemann D, Rüsch-Gerdes S, and Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol. 2009;47:1767-1772.

20. Barnard M, Warren R, Gey van Pittius N, van Helden P, Bosman M, Streicher E, et al. GenoType MTBDRsl line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory. Am J Respir Crit Care Med. 2012;186:1298-1305.

21. Dalton T, Cegielski P, Akksilp S, Asencios L, Campos Caoili J, Cho SN, et al. Prevalence of and risk factors for resistance to second-line drugs in people with multidrug-resistant tuberculosis in eight countries. a prospective cohort study. Lancet. 2012;380(9851):1406-17.

22. Umubyeyi AN, Rigouts L, Shamputa IC, Fissette K, Elkrim Y, de Rijk PW, et al. Limited fluoroquinolone resistance among Mycobacterium tuberculosis isolates from Rwanda: results of a national survey. J Antimicrob Chemother. 2007;59:1031–3.

23. Huang TS, Kunin CM, Shin-Jung Lee S, Chen YS, Tu HZ, Liu YC. Trends in fluoroquinolone resistance of Mycobacterium tuberculosis complex in a Taiwanese medical centre: 1995-2003. J Antimicrob Chemother. 2005;56(6):1058-62.

24. World Health Organization - StopTB Partnership. The global plan to stop TB, 2006-2015. WHO/HTM/STB/2006.35. 2006. http://www.stoptb.org/assets/documents/global/plan/GlobalPlanFinal.pdf. Accessed 6 Oct 2015

Table 1. Drug susceptibility testing coverage during the early period (2005-2010) versus the late period (2011-2013), Kinshasa first failures and relapses.

Years	Number of first failures among retreatment TB cases registered	Number of first relapses among retreatment TB cases registered	Number of first failures among retreatment TB cases sampled (%)	Number of first relapses among retreatment TB cases sampled (%)
2005-2010	934	7549	409 (43.7)	1940 (25.6)
2011-2013*	315	3120	71 (22.5)	121 (3.9)
Total	1 249	10669	480 (38.4)	2061 (19.3)

*****In 2013, data from January to June only.

Table 2. Type of patients and test performance during the early and late period, using LJ DST respectively MDRTBPlus LPA.

Parameters	Conventional First line LJ DST, early period (%)	MDRTB *plus,* late period (%)
1. No. of TB patients tested	2 349	192
2. Type of TB patients		
2.1. First failure (%)	409 (17)	71 (37)
2.2. First relapse (%)	1 940 (83)	121 (63)
3. No. of valid tests (MTBC recovery rate)	1 609 (68)	157 (82)

The increased proportion of first failure cases was due to a change in referral instructions, focusing this group.

Table 3. Proportion of valid DST showing resistance to RMP, INH or both for the early and late surveillance periods, first-time failure versus first-time relapse cases

	Conventional first-line LJ DST from 2005-2010			MDRTB *plus* from 2011-2013*		
Resistance patterns	First failure cases (%)	First relapse cases (%)	Total %	First failure cases (%)	First relapse cases (%)	Total %
No. of valid tests performed	234	1 375	1 609	57	100	157
Mono RMP resistance (%)	9 (3.8)	45 (3.2)	54 (3.3)	4 (7.0)	5 (5.0)	9 (5.7)
Resistance to INH and RMP (%)	135 (57.6)	147 (10.6)	282 (17.5)	44 (77.1)	20 (20.0)	64 (40.7)

*In 2013, data from January to June only.

INH: Isoniazid; RMP: Rifampicin; Mono resistance to RMP: only or with other drugs but not with isoniazid.

Table 4. Fluoroquinolone resistance by period among MDR-TB cases in Kinshasa, DRC and DST method

	First-line failures and relapses combined		
Parameters	Conventional First line LJ DST, early period	MDRTB *plus*, late period	Total
No. of valid tests performed	269	57	326
Resistance to FQ (%)	6 (2.3)	4 (7.0)	10 (3.1)

FQ: Fluoroquinolone.

Figure 1. Evolution of proportion of failure and relapse cases among all smear-positive cases registered by NTP from 2005 to 2013 (n= 97 680).

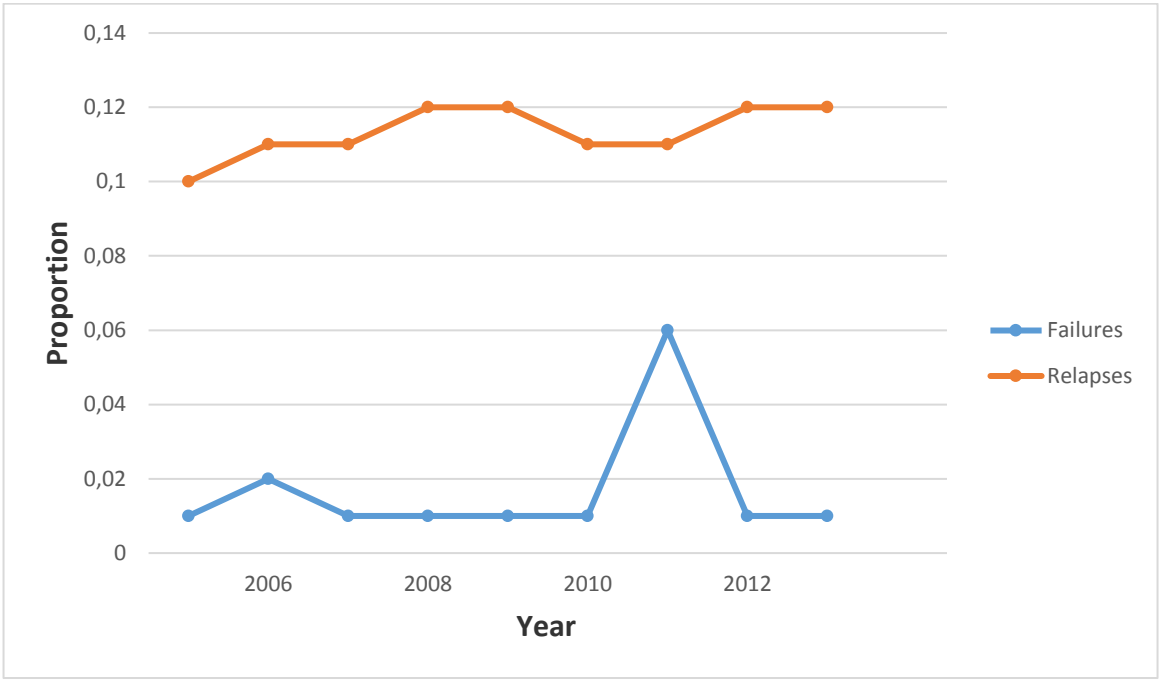


Figure 1 shows evolution of the proportion of relapse and failure cases among all smear-positive cases presenting for first-line treatment. The total number of patients registered were 97 680.

Figure 2. Evolution of proportion of failure as treatment outcome among smear-positive cases after first line treatment from 2005 to 2013 (n=84 963).

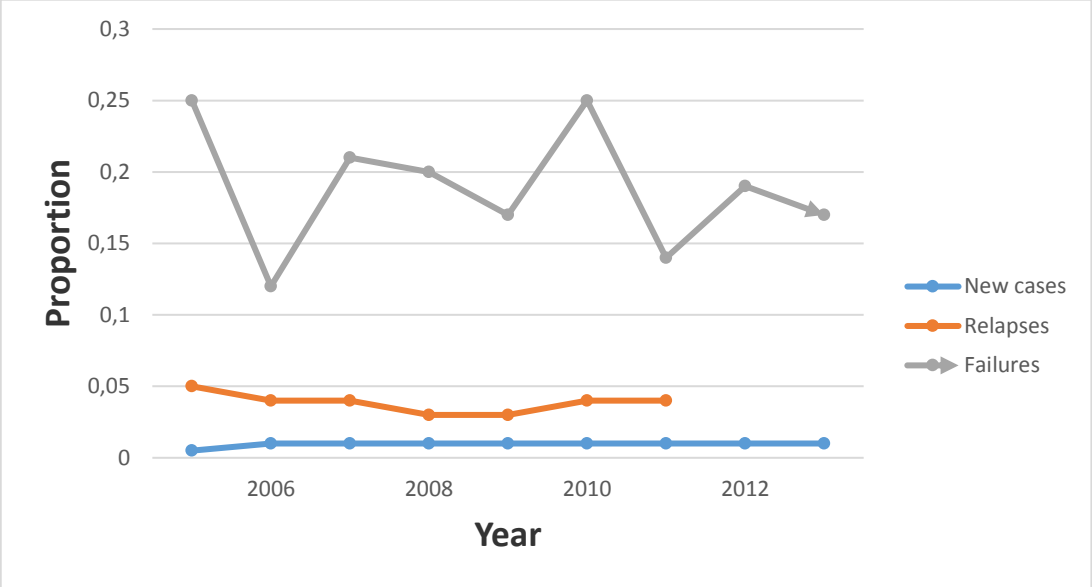


Figure 2 shows the evolution of the proportion of failures among new cases, relapse and failure cases by year after first line treatment. Years 2012 and 2013 have been excluded of the final analysis for relapses.

3.4 EMERGENCE OF TB/MDR-TB IN THE MBUJI-MAYI PRISON IN THE DEMOCRATIC REPUBLIC OF CONGO, AN OUTBREAK INVESTIGATION

Kaswa Kayomo Michel[1,2,3,4], Hasker Epco[4], Aloni Muriel[1,2,3], Nkuku Léontine[3], Kazadi Marcel[1], Kabengele Thierry[1], Muteteke Dorcas[1], Kapita François[1], Lufulwabo Alphonse[1], Mukadi Ya Diul [5], Muyembe-Tamfum Jean-Jacques[2,3], Ieven Margareta[6], de Jong Bouke C.[4] , Boelaert Marleen[4].

AUTHOR AFFILIATION

1) National Tuberculosis Program, Kinshasa, the Democratic Republic of the Congo
2) Université de Kinshasa, Kinshasa, the Democratic Republic of the Congo
3) Institut National de Recherche Biomédicale, Kinshasa, the Democratic Republic of the Congo
4) Institute of Tropical Medicine, Antwerp, Belgium
5) Bureau for Global Health, US Agency for International Development, Washington, DC, USA*
6) University of Antwerp, Antwerp, Belgium

**The views and opinions expressed in this paper are those of the author and not necessarily the views and opinions of the United States Agency for International Development.*

WORD COUNT:

Abstract: 138

Text: 3424

CORRESPONDING AUTHOR

Michel Kaswa Kayomo

National Tuberculosis Program, Kinshasa, Democratic Republic of Congo.

Meckkay2002@yahoo.fr

Tel: +243999928694

RUNNING HEAD: MDR-TB in the Mbuji-Mayi prison, DRC.

KEYWORDS: TB incidence, DR Congo prisons

ARTICLE SUMMARY LINE:

The high frequency of MDR-TB in a Congolese prison is linked with extreme overcrowding and malnutrition and requires urgent medical attention and a more humane prison policy.

BIOGRAPHICAL SKETCH:

Dr. Michel KASWA KAYOMO is the Director of the national TB Program in the Democratic Republic of the Congo. He worked for more than 15 years in TB control and programmatic management of TB nationwide. His research focuses on drug-resistant TB, in collaboration with ITM, Antwerp, Belgium.

ABSTRACT

After an alert about 28 TB and three rifampicin -resistant TB (TB-RR) cases in the prison of Mbuji-Mayi, the diamond capital of DRC, we conducted an outbreak investigation early 2015. We documented the burden of TB in prison by analyzing sputum of presumptive patients by the Xpert® MTB/RIF assay. We further analyzed the *M. tuberculosis* drug susceptibility patterns and the associated risk factors. In a prison population of 918 inmates, there were 29 TB patients already on treatment, and we found - additionally- 475 presumptive TB patients and confirmed 170 of them. The prevalence rate of confirmed TB in March 2015 was 21.7% (199/918). We detected 14 additional TB-RR cases and initiated treatment in all of them. Overcrowded living conditions and poor nutritional status appeared to be the driving factors behind the high TB incidence in this prison.

Introduction

The incidence rate of Tuberculosis (TB) in the Democratic Republic of the Congo (DRC) is estimated at 326 per 100,000 inhabitants per year (1). The DRC, with an estimated population of 68 million, is ranked ninth among 22 TB high-burden countries and is one of 27 countries with a high burden of rifampicin-resistant TB (RR-TB), (2). The magnitude of RR-TB, a marker for multi-drug resistant TB (MDR-TB), is not well known in the DRC (3,4). The first Drug Resistance Survey (DRS) was completed in 2018, and preliminary data show a nation-wide prevalence of RR-TB of 2.2% (95% CI:1.0-3.5) among new patients and 16.7% (95% CI:9.6-23.7) among previously treated cases (unpublished data). In the capital Kinshasa, 64 (40.7%) among 192 patients who were retreated in the period 2011-2013, had MDR-TB, of whom 4 (7.0%) also had fluoroquinolone (FQ) resistance (Kaswa MK; forthcoming). MDR-TB is a major concern for public health in DRC, a country already facing a high burden of TB and HIV in a context of poverty and insufficient access to public health services.

Prisons are high-risk environments for TB and MDR-TB (5). Globally, the prevalence of TB in prisons is much higher than in the general population, both in high- and low-income countries

(7). In Sub Saharan Africa, the spread of TB in prisons has been fueled by the HIV/AIDS epidemic (6), resulting in high rates of progression to active TB. However, the actual TB burden in those prisons is not so well documented. Reports from Malawi, Ivory Coast, and Botswana showed a higher prevalence of smear-positive pulmonary TB in prisons compared to the general population (8–10). Several factors such as poor ventilation, HIV infection, overcrowding, malnutrition, lack of sunshine, stress, prolonged incarceration, and inadequate access to care contribute to the rapid spread and high prevalence of (MDR-)TB in prisons (7). Limited access to high-quality TB diagnosis, due to limited screening procedures, inaccuracy of diagnostic algorithms and lack of adequate laboratory facilities, has been recognized as one of the critical barriers to TB control in prisons (12, 13). All those factors are rampant in the current context of DRC with its protracted socio-economic problems and widespread poverty. The National TB Strategic Plan 2014-2017 identified prison inmates as a high-risk group for TB.

The introduction of the GeneXpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) assay is an important breakthrough in the fight against TB and MDR-TB (14-16). The World Health Organization (WHO) recommends that people suspected of having pulmonary TB and considered to be at risk of harboring MDR-TB bacilli should receive the Xpert® MTB/RIF tests as a first-line diagnostic test (17). At the end of 2013, the National TB Program (NTP) of DRC introduced the Xpert® MTB/RIF technology in each province, mainly motivated by the lack of data on the MDR-TB burden and the extremely long turn-around times with conventional methods. However, in the DRC, as in many low-income countries, the roll-out of Xpert® MTB/RIF machines to date is for financial and logistical reasons still restricted to national- or provincial reference laboratories and certain general hospitals. The Xpert® MTB/RIF is mostly used for the detection of RR in persons at high risk, and not for routine TB detection.

In November 2014, the Provincial TB Program of Kasai Oriental province decided to try out the Xpert® MTB/RIF TB for TB detection in the Mbuji-Mayi Central Prison instead of conventional microscopy- motivated a.o. by a large stock of cartridges that were due to expire soon. By the end of 2014, they had confirmed 28 TB cases among 57 sputum specimens from prisoners with presumptive TB, and, for the first time, three patients with TB-RR were detected, bringing the total TB notification of the prison to 72 patients that year - almost double the 2013 figure. On January 5^{th,} 2015 the NTP country office in Kinshasa was informed about a potential outbreak of TB in the prison and launched an investigation in response to this alert. A clinical microbiologist/public health officer (MKK) and the MDR program Advisor (DM) were sent to review the patient histories, ascertain the emergence of TB/RR-TB, and to implement infection control measures. We report here the outcomes of this assessment, including the burden of TB, the drug susceptibility patterns of the circulating *M. tuberculosis* isolates and the associated risk factors.

Methods

Ethical aspects

The competent authorities of DRC approved the study. The national ethical review board of DRC (Comité National d'éthique de la Santé) gave ethical clearance for the study (N° 09/CNES/BN/PNMMF/2015/). We only used specimens and data collected in the course of routine clinical patient care and drug susceptibility surveillance. Data were delinked from any personal identifiers before data analysis and reporting. All individuals diagnosed with TB or TB-RR received respectively the recommended 6- or 9 month-long treatment. TB-RR patients were isolated at Dipumba General Hospital in a dedicated ward.

Setting

Mbuji-Mayi, the capital of the Kasai Oriental province, is located about 1000 km east of Kinshasa. In 2013, the province, with an estimated population of 6.7 million, had an estimated incidence of TB of 229 per 100 000 per year (Source: DRC NTP; unpublished). The Central Prison of Mbuji-Mayi is a medium-security correctional facility built in 1950 with a capacity of 150 inmates. It is surrounded by schools, houses, and local government offices. It hosts on average 900 inmates (i.e., six times its capacity), in 9 cells: 7 for males, 1 for females, and 1 for children. The number of prisoners per cell varies from approximately 130–160 in the large (36 m^2) to 20–30 in the small (28 m^2) cells, also called 'VIP cells. On arrival, each prisoner is assigned a fixed spot, which in the regular cells is no larger than about 0.25m^2 (see **Figure 2)**. Each cell has at least one window, but prisoners' clothes and other possessions usually cover these. Inmates receive about 5 hours of sunshine exposure per day in a courtyard of 375 m^2. They eat with the inmates of the same cell but meet those of other cells during morning sessions, gym, and vocational training. They also have close contact with prison staff, judges, and their own families. The duration of incarceration ranges from one month to more than 15 years. In the prison, there is a clinic run by one medical doctor and two healthcare workers. Close to the prison, there is an NTP diagnostic- and treatment unit, performing direct smear microscopy (no radiology) and providing TB treatment to prisoners. Prisoners are not routinely screened for TB on entry, due to a critical shortage of health personnel.

Study population

The outbreak investigation team reached Mbuji-Mayi mid-January 2015 and reviewed all available NTP records as well as the prison admission register and patient files. They then screened the entire prison population for TB symptoms to identify those with presumptive TB, with assistance from the TB- and HIV provincial team and prison medical personnel as well as by sensitizing inmates to the signs and symptoms of TB. The team also trained the "chief" inmate of each cell as well as ten peer educators, all inmates, to recognize the major symptoms of TB

and to list those individuals. Inmates with a previous history of TB and/or clinical symptoms such as coughing for more than two weeks, fever, night sweats, loss of weight, and/or hemoptysis were considered as presumptive TB patients.

Prisoners with presumptive pulmonary TB were asked to submit one early morning sputum sample. A healthcare worker supervised the sputum collection, which was done outside the cell in a well-ventilated area. Samples were transported to the provincial reference laboratory (PRL) located at a 3-km distance from the prison. For assessing malnutrition, we calculated the Body Mass Index (BMI = weight in kg/(height in meter)^2) as a measure of adult nutritional status and used the cut-offs proposed by Bailey and Ferro-Luzzi (22) to define three categories of adult malnutrition: severe, moderate and mild.

Laboratory procedures

Xpert® MTB/RIF

Fresh sputa- without any additive- were transported in standardized containers from the prison to the PRL to be examined with the Xpert/MTB/RIF assay. We also recorded demographic data of patients and their TB history. An aliquot of 2ml of each sputum sample was processed in Xpert according to standard methods (14, 18). From each specimen showing resistance to RMP, an aliquot was preserved in 70% alcohol in a Falcon tube to permit additional molecular testing in Kinshasa as well as in the Supra-National Reference Laboratory (SRL) in Antwerp/Belgium. One aliquot without additive was transported to Kinshasa for culture.

MTBDRsl

At the INRB in Kinshasa, GenoType®MTBDR*sl* (Hain LifeScience GmbH, Nehren, Germany) was performed on specimens showing resistance to RMP by Xpert® MTB/RIF . Sputa were decontaminated with NaOH according to a modified Petroff technique (19). The sediment obtained was inoculated on Lowenstein Jenssen and tested by the MTBDR*sl* according to the manufacturer's instructions (20).

Genetic sequencing

For all available DNA extracts harboring second-line resistance patterns on MTBDR *sl*, sequencing of the *gyrA* and *rrs* was performed independently of line probe assay (LPA) results in the SRL in Antwerp/Belgium. The methodology for PCR amplification and sequencing of genes encoding Gyrase A and B has been published elsewhere (21).

HIV testing

During our investigation, the National HIV Program organized a mass screening campaign of HIV, with the support of community health committees in the neighborhood who organized awareness raising activities for the inmates. Skilled counselors performed pretest counseling sessions and testing over a period of 3 weeks. Post-test counseling was carried out in a private one-on-one setting by the same counselors. Individuals who tested positive were referred to the nearby NTP clinic for TB and HIV treatment.

Data collection and analysis

A short standard form was used to collect data related to the previous history of TB, symptoms, duration of stay and location in the cell. We mapped the spatial distribution of TB in the cells on a sketch showing the location of each prisoner. We also extracted data on the TB notification rate in this prison for the seven years before our investigation from the NTP registers.

All data were entered into an EXCEL 2007 worksheet and verified by a second person. Both datasets were compared using Epi Info (version 7.1.4), and in case of discrepancies, we consulted the source document: laboratory forms and LPA results. The primary outcomes of interest for this study were the prevalence rates of TB and TB-RR computed as the proportion of confirmed TB/TB-RR patients over the total number of prisoners at the time of our visit. These data were later linked with DNA sequencing results from the SRL. For this analysis, we divided the prison into three areas: **Area 1**: cell 1-3, **Area 2**: cells 4-7 and **Area 3**: cells 8 and 9. This division corresponds to social stratification in the prison system, as **Area 1** comprises the "VIP" or "first-class" cells, where inmates get better conditions in exchange for payment, **Area 2** corresponds to "second-class," and **Area 3** is the area for women and children. We calculated means (SD) and medians (range) for continuous variables. Comparisons between groups were made using Fisher's exact method, and 95% Confidence Intervals (CI) were calculated when appropriate.

Results

Pulmonary TB among prisoners

In November 2014, 31 inmates had been found positive for TB by GeneXpert, 4 in Area 1 and 25 in Area 2. For two, no information on location was recorded, and they were not present any longer during our outbreak investigation. In January 2015, there were 918 inmates housed in the Mbuji-Mayi Central Prison: 863 (94.0%) males, 26 (2.8%) females and 29 (3.1%) children**.** The majority, 716/918 (78%) were pre-trial detainees, and only 202 (22.0%) were sentenced to imprisonment. **Area 1** housed 206 (22.4 %), **Area 2** 657 (71.6 %) and **Area 3** 55 (6.0 %) inmates.

The median age was 30 years (IQR 25-42), and 29 were already on TB treatment. Out of the remaining 889, 45 were absent for various reasons or declined to be screened. We clinically examined 844 of the 918 inmates (91.9%) and collected sputum of the 475 presumptive TB patients (51.7 %, n=918). The mean age of inmates with presumptive TB was 32 years (median, 31 years), and their mean duration of incarceration was 72 months (median, 42 months; range, 1- 437 months). One Xpert assay was performed per patient: 460 valid tests were included in the final analysis, of which 170 were MTB positive and RIF sensitive and 14 MTB positive and RIF resistant. The remaining 276 were negative. Using the Xpert® MTB/RIF systematically, we raised, therefore, the total number of TB patients detected by this method since November 2014 to 201 (=31+170). The overall prevalence rate of TB among the 918 prisoners present in March 2015 was 21.7% (199/918).

HIV testing

Most (85.6%; 753/879) inmates agreed to attend a pre-test counseling session for HIV **(Table I)**, but only 539 (71.5%) were tested for HIV infection because of a stock-out in tests. The overall proportion of HIV infection was 1.5% (8/539) among inmates tested, 2.6% (5/196) among inmates with bacteriologically confirmed TB and 0.9% (3/343) individuals without TB 5 (p=0.12).

Trend of TB in the prison

From the beginning of 2008 until March 2015 a total of 301 TB patients had been registered at the Central Prison of Mbuji-Mayi. Until the third quarter of 2014, the numbers of TB patients registered remained relatively stable with an average of 2 new cases per quarter (range 0-9). There was a steep increase from the fourth quarter of 2014 onward with 212 new TB cases registered or more than 70 percent of the caseload since 2008 as shown in **Figure 1**.

Drug resistance among TB patients

Using the Xpert® MTB/RIF assay, 199 individuals were found bacteriologically positive for TB from November 2014 to March 2015. Among them, 17 (=3+ 14 additional) (8.5 %) had TB-RR, which is almost three times the expected number of TB-RR in new cases according to WHO estimates for DRC. The overall prevalence of TB-RR in March 2015 was 1,852 per 100,000 inhabitants. **Table II** shows details from the 14 TB-RR cases documented in March 2015. All were new cases except for one patient. For the three first detected TB-RR patients, documented in 2014, the delay between diagnosis to start of treatment was 21 days. This delay decreased to 48 hours for the rest of the RR-TB patients. No second-line drug resistance was identified among any RR-TB patient.

Xpert® MTB/RIF results showed that all TB-RR was based on the absence of probe E, supporting the possibility of clonal spread of one strain containing a mutation in the *rpoB* 531 codon or, less likely, the 533 codon.

Risk factors associated with TB and TB -RR

Overcrowding living conditions

The majority of TB patients were located in the back of the cell where ventilation is poor, and sunshine is lacking more **(Figure 2**). Area 2 (or cells 4-7) of the Mbuji-Mayi Central prison was the most overcrowded of the three areas with a total of 657/918 (71.5%) inmates. In cell 4 and cell 7, the available surface per person was no more than 0.22 m^2. The frequency of TB and TB-RR increased significantly with the number of inmates per area. Of the 199 confirmed TB patients, 19 resided in Area 1, 177 in Area 2 and 3 in area 3. The prevalence of confirmed TB was 2.75 times higher in Area 2 & 3 compared to Area 1 (25.3% versus 9.2% p<0.001). Out of the 14 TB-RR cases detected in Jan-March 2015, only one stayed in Area 1 (7.1%), the others all belonged to Area 2**.**

Nutritional status

Of the 918 inmates, 752 (82%) were screened for nutritional status; and 370 (49.2%) were malnourished, i.e., had a BMI lower than 18.5 kg/m^2 **(Table III).** In the subgroup of 170 confirmed TB patients, 142(83.5%) were screened, and 110 (77.5%) were malnourished. Malnutrition was significantly higher among TB patients than in the other inmates, OR 4.63 (95%CI 3.03-7.08).

DISCUSSION

Our findings provide a first account of the high burden of TB and drug-resistant TB in a large prison in DRC, where living conditions and degree of overcrowding were appalling. The prevalence of TB in this prison was 39 times higher than the estimated 549 cases per 100,000 in the general Congolese population (4) and is 3.5 times greater than the prevalence reported from Zambian prisons (23). The TB prevalence had probably been high for years in the Mbuji-Mayi prison but remained undetected for lack of screening and the insufficient sensitivity of smear microscopy**.** During the outbreak investigation, the high sensitivity of the Xpert® MTB/RIF assay proved very useful.

Although some prison studies in Sub Saharan Africa have reported higher rates of TB (5,6,12) in association with a higher HIV prevalence (13,23), in Mbuji-Mayi central prison, we found a relatively low HIV prevalence in the prison population. One limitation of our study is that not all consenting prisoners could be tested for HIV because of stock-outs of tests. Several factors such as the lack of screening for TB upon arrival, and the characteristics of the prisons, with overcrowding and poor ventilation, further enhance the risk of spread of TB (6, 24-28). These risk factors were also documented in other prison outbreaks (6, 25, 29-34), but the degree of overcrowding in the Central Prison of Mbuji-Mayi was a staggering six times higher than the capacity. Very poor ventilation, lack of sunshine, and high malnutrition rates enhanced the risk. Some prisoners stated that they did not eat during three days before our investigation. Malnutrition is a known problem in the Mbuji-Mayi region, with a 45 % adult malnutrition rate and more than 1 million people requiring nutritional assistance according to a World Food Program report in 2014. Malnutrition tends to amplify TB infection as well as the progress from infection to TB disease (35-36). Nutritional deficits have a negative impact on the cell-mediated immune system and are associated with an increased risk of reactivation of latent to active TB and increased risk of re-infection (37). The duration of incarceration in this prison was long, and a majority of detainees were awaiting sentence, due to lack of judges and other delays in the prosecution. The longer the time spent in prison, the more persons are exposed to overcrowding, malnutrition, and TB. As a result of our investigation and recommendations, the judicial proceedings were sped up, resulting in decongestion of the prison. We aimed to interrupt ongoing TB transmission in prison by prompt initiation of effective TB therapy for confirmed patients, and isolation of the TB-RR. Sustained control of this TB outbreak nevertheless requires sustained efforts (**Table IV)**, including improved ventilation and sustained active case finding efforts as well as screening of inmates at prison entry.

Mbuji-Mayi region is classified among the “5 hot-spot” regions for TB and TB-RR (DRC First Drug Resistance Survey 2017, unpublished data). Delay in the diagnosis of TB in Mbuji-Mayi prison was recognized as a major limitation for TB control efforts in this correctional setting. Prison-based TB control measures in DRC are limited, as so far only passive case detection based on microscopy is deployed in prisons nationwide in DRC. Overall, the active case finding we deployed using the more sensitive Xpert® MTB/RIF technology increased TB case detection by 19-fold over passive case detection. In prisons, passive and active case-finding should be carried out simultaneously and systematically. The Xpert® MTB/RIF offers rapid and accurate diagnostic results from biological specimens with minimal staff training requirements (14, 15, 16), and is a useful tool for active case finding in congregate settings. Although testing with Xpert® MTB/RIF does not require additional laboratory equipment, the sophisticated nature of the device requires care in handling. A stable electrical power supply is required to avoid interruption of the procedure and subsequent loss of results, as well as adequate storage space for the cartridges, and dedicated staff to perform testing. Based on our experience, we do recommend Xpert® MTB/RIF for use at point-of-care in prison clinics.

Importantly, as summarized in **Table IV**, other key public health actions and strategies should be implemented by the NTP to reduce the burden of TB in prison. Overcrowding in prisons is a strong risk factor and should be avoided. Inmates should have access to better nutrition and more sunlight exposure. Given the high risk of TB in prison settings, there is an urgent need for effective TB control to protect not only the health of prison inmates but also the health of the wider community. We recommend that living conditions in prisons should be improved to avoid outbreaks of MDR-TB in similar contexts.

References

1. World Health Organization: Global TB Report 2014. Annex 2 Country Profiles: DR of Congo. P.130. 2014. http://www.who.int/tb/publications/global_report/gtbr14_annex_2_country_profiles.pdf
2. World Health Organization. Anti-tuberculosis drug resistance in the world. Fourth Global Report Geneva. The WHO /IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance. Fourth Global Report. WHO/HTM/TB/2008.394. Geneva, Switzerland: WHO. 2008. http://whqlibdoc.who.int/hq/2008/who_htm_tb_2008.394_eng.pdf.
3. Kaswa MK, Bisuta S, Kabuya G, Lunguya O, Ndongosieme A, et al. (Multi-Drug Resistant Tuberculosis in Mosango, a Rural Area in the Democratic Republic of Congo. PLoS ONE. 2014; 9(4): e94618. doi:10.1371/journal.pone.0094618.
4. Kaswa MK, Aloni M, Nkuku L, Bakoko B, Lebeke R, Nzita A, et al. A pseudo-outbreak of pre-XDR TB in Kinshasa: A Collateral Damage of False Fluoroquinolone Resistant Detection by GenoType MTBDR sl. J Clin Microbiol. 2014; 52(8):2876-2880.
5. Balabanova Y, Nikolayevskyy V, Ignatyeva O, Kontsevaya I, Rutterford CM, et al. Survival of civilian and prisoner drug-sensitive, multi- and extensive drug- resistant tuberculosis cohorts prospectively followed in Russia. PLoS ONE.2011; 6(6):e20531.
6. O'Grady J, Hoelscher M, Atun R et al. Tuberculosis in prisons in sub-Saharan Africa – the need for improved health services, surveillance, and control. Tuberculosis (Edinburgh) 2011; 91:173–178.
7. Biadglegne F, Rodloff A C and Sack U. Review of the prevalence and drug resistance of tuberculosis in prisons: a hidden epidemic. Epidemiol. Infect. 2015, 143, 887–900.
8. Nyangulu DS, Harries AD, Kang'ombe C, Yadidi AE, Chokani K, et al. Tuberculosis in a prison population in Malawi. Lancet.1997; 350(9087):1284–7.
9. Center for Disease Control and Prevention Rapid assessment of Tuberculosis in Large prison of Botswana. MMWR Morb Mortal Wkly Rep.2003; 52(12):250–252.

10. Koffi N, Ngom AK, ka-Danguy E, Seka A, Akoto A, et al. (1997) Smear positive pulmonary tuberculosis in a prison setting: experience in the penal camp of Bouake, Ivory Coast. Int J Tuberc Lung Dis.1997; (3):250–3.

11. Ferrari MJ. Eleven million adults co-infected with AIDS, TB. Can Med Assoc J. 2004; 171:437.

12. Dara M, D Acosta C, Vinkeles Melchers N V S, et al. Tuberculosis control in prisons: current situation and research gaps. Int J Inf Dis .2015; 32 : 111-117.

13. Al-Darraji HAA, Razak HA, Ng KP, Altice FL, Kamarulzaman A. The Diagnostic Performance of a Single GeneXpert® MTB/RIF Assay in an Intensified Tuberculosis Case Finding Survey among HIV-Infected Prisoners in Malaysia. PLoS ONE. 2013 8(9): e73717. doi:10.1371/journal.pone.0073717.

14. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. N Engl J Med .2010; 363: 1005–1015. doi:10.1056/NEJMoa0907847.

15. Steingart K, Sohn H, Schiller I, Kloda L, Boehme C et al. Xpert® MTB / RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Cochrane Database of Systematic Reviews; 2013: Art. No: CD009593

16. Chang K, Lu W, Wang J, Zhang K, Jia S et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert® MTB/RIF assay: a meta-analysis. J Infect.2012. 64: 580–588. doi:10.1016/ j.jinf.2012.02.012.

17. Stop TB Partnership. Global Plan to Stop TB 2011–2015. Geneva, World Health Organization, 2010.

18. Helb D, Jones M, Story E, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010; 48: 229–37.

19. Petroff SA. 1915. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J. Exp. Med. 21: 38–42.

20. Hain Lifescience GmbH. 2009. GenoType MTBDRsl Ver1.0: instruction manual. Hain Lifescience, GmbH, Nehren, Germany.

21. Von Groll A, Martin A, Jureen P, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis and mutations in gyrA and gyrB. Antimicrob. Agents Chemother 2009; 53:4498-4500.

22. Bailey KV, Ferro-Luzzi A. Use of body mass index of adults in assessing individual and community nutritional status. Bulletin of the World Health Organization 1995. 73 (5): 673-880.

23. TB Reach. 2010. http://www.stoptb.org/global/awards/tbreach/

24. Harries AD, Nyirenda TE, Yadidi AE, Gondwe MK, Kwanjana JH, Salaniponi FM. Tuberculosis control in Malawian prisons: from research to policy and practice. Int J Tuberc Lung Dis 2004; 8: 614–17.

25. O'Grady J, Mwaba P, Bates M, Kapata N, Zumla A. Tuberculosis in prisons in sub-Saharan Africa–a potential time bomb. S Afr Med J 2011; 101:107–8.

26. Human Rights Watch. Unjust and unhealthy: HIV, TB and abuse in Zambian prisons. New York 2010.

27. Jurgens R, Nowak M, Day M. HIV and incarceration: prisons and detention. J Int AIDS Soc 2011; 14:26.

28. Chaisson RE, Martinson NA. Tuberculosis in Africa–combating an HIV-driven crisis. N Engl J Med 2008; 358:1089–92.

29. Ali S, Haileamlak A, Wieser A, et al. Prevalence of pulmonary tuberculosis among prison inmates in Ethiopia, a cross-sectional study. PLoS One 2015; 10: e0144040.

30. Telisinghe L, Fielding KL, Malden JL, et al. High tuberculosis prevalence in a South African prison: the need for routine tuberculosis screening. PLoS One 2014; 9: e87262.

31. Moges B, Amare B, Asfaw F, et al. Prevalence of smear positive pulmonary tuberculosis among prisoners in North Gondar Zone Prison, northwest Ethiopia. BMC Infect Dis 2012; 12: 352.

32. Reid SE, Topp SM, Turnbull ER, et al. Tuberculosis and HIV control in sub-Saharan African prisons: "thinking outside the prison cell". J Infect Dis 2012; 205 (suppl 2): S265–73.

33. Noeske J, Ndi NF, Amougou Elo G, Mbondi Mfondih S. Tuberculosis incidence in Cameroonian prisons: a 1-year prospective study. S Afr Med J 2014; 104: 209–11.

34. Ensor L. Overcrowding in South African prisons falls. 2013 [cited 2006 Jul 11]. http://www. bdlive.co.za/national/2013/08/26/overcrowding-in-south-africanprisons-falls

35. Chocano-Bedoya P, Ronnenberg AG. Vitamin D and tuberculosis. Nutrition Reviews 2009;67:289-93.

36. Gibney KB, MacGregor L, Leder K, Torresi J, Marshall C, Ebeling PR et al. Vitamin D defisciency is associated with tuberculosis and latent tuberculosis infection in immigrants from sub-Saharan Africa. Clin Infect Dis 2008;37;113-9.

37. Lonnroth K,Williams BG,Cegielski P, Dye C. A consistent log-linear relationship between Tuberculosis incidence and body mass index. Int J Epidemiol 2010;39:149-55.

Tables

Table I. Frequency of HIV among inmates at the Central Prison Mbuji-Mayi in March 2015.

		n	%
a.	Number of inmates housed in the prison	918	
b.	Number of people who received pretest counseling (b/a)	879	95.7
c.	Number of prisoners who accepted testing (c/b)	753	85.6
d.	Number of prisoners tested (d/c)	539	71.6
e.	Number of HIV-positive prisoners (e/d)	8	1.5
f.	Number of prisoners co-infected with TB and HIV (f/d)	5	0.9

Table II. Main social, demographic and clinical characteristics of TB-RR patients in the Mbuji-Mayi prison on February 2015 (n=14).

Area	Age (years)	Gender	History of treatment	Detection date	Treatment started	HIV status	Xpert® MTB/RIF	Duration of incarceration (months)
1	31	male	NC	10/02/2015	11/02/2015	NEG	RR	21
2	18	male	NC	21/11/2014	11/12/2014	NEG	RR	18
2	20	male	RET	21/11/2014	11/12/2014	NEG	RR	28
2	25	male	NC	14/01/2015	16/01/2015	NEG	RR	12
2	25	male	NC	14/01/2015	16/01/2015	NEG	RR	1
2	55	male	NC	14/01/2015	20/01/2015	NEG	RR	24
2	25	male	NC	5/02/2015	7/02/2015	NEG	RR	19
2	32	male	NC	14/01/2015	20/01/2015	NEG	RR	7
2	35	male	NC	14/01/2015	20/01/2015	NEG	RR	20
2	27	male	NC	16/02/2015	19/02/2015	NEG	RR	36
2	30	male	NC	20/11/2014	11/12/2014	NEG	RR	3
2	35	male	NC	21/01/2015	23/01/2015	NEG	RR	101
2	25	male	NC	22/01/2015	24/01/2015	NEG	RR	21
2	24	male	NC	22/01/2015	24/02/2015	NEG	RR	30

Legend: NC: New case; RET: retreatment case; NEG:negative, RR:rifampicin resistant

Table III. Nutritional status of Inmates Mbuji-Mayi prison in January 2015 (n=752).

Nutritional status	TB (n=142)	%	non-TB (n=610)	%	Total (n=752)	%
BMI >18 kg/m²	32	22.5%	350	57.4%	382	50.8%
Global malnutrition	110	77.5%	260	42.6%	370	49.2%
Mild	53	37.3%	95	15.6%	148	19.7%
Moderate	27	19.0%	98	16.1%	125	16.6%
Severe	30	21.1%	67	11.0%	97	12.9%

Legend: Severe malnutrition if BMI< 16.0; Moderate if 16.0-16.9; Mild if 17-18.49

Table IV. Priority actions to reduce the burden of TB in the Mbuji-Mayi Central prison.
1. Ensure the screening for TB signs and symptoms of all detainees at the time of prison entry and exit. Continue active and early detection of presumptive TB patients. Raise awareness amongst the detainees, the prison administration and the community of the city of Mbuji-Mayi: each cougher should be tested. Confirm presumptive diagnosis by using Xpert MTB / RIF. Screen for HIV by rapid tests;
2. Initiate appropriate treatment of confirmed TB patients within 24 hours under strict supervision of health care providers;
3. Ensure the systematic screening by chest X-ray of the other detainees , the care providers and the personnel of the prison administration;
4. Feed all inmates nourishing meals, especially the TB / HIV patients under treatment;
5. Ventilate cells
6. TB RR patients may be isolated from other inmates at DIPUMBA Hospital where the mission team was able to obtain a ward for their accommodation. However, the prison administration should find ways to ensure security;
7. Establish compulsory wearing of masks by all TB patients;
8. Establish the compulsory wearing of respirators by all staff entering the prison;
9. Ensure the decongestion of the prison by speeding up the judicial proceedings; and increase space- enforce maximal occupancy of the prison.

Figures

Figure 1. Number of new tuberculosis cases* registered per trimester in the Central Prison of Mbuji-Mayi from 2008 to 2015. (Source: NTP, DRC)

Legend: T1 08: Quarter 1 2008

*TB cases include bacteriologically-positive and clinically-diagnosed tuberculosis patients. Clinical diagnosis was based on at least one tuberculosis-related sign or symptom and/or an X-ray abnormality consistent with tuberculosis. From 2008 to 2014, bacteriological confirmation was based on microscopy on samples collected from passive case finding. GeneXpert was introduced during the last quarter of 2014,.

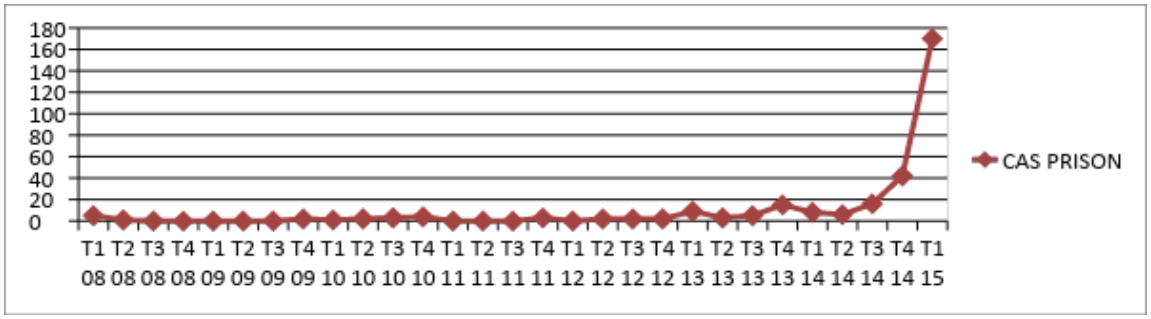


Figure 2. Location of inmates in cells 4 and 7 in Mbuji-Mayi Central prison on February 2015.

Legend:

Illustration of cell 4 (L, 37 m² with 1 door and 2 windows) and 7 (R, 37 m² with 1 door and 1 window). Both cells are "Area 2" and are extremely overcrowded with at least 163 detainees in each. Each individual was assigned a space of 0.22 m². The majority of TB patients were living in the rear of the cell characterized by poor ventilation and lack of sunshine. This illustration represents the real picture of localization of inmates during their life time inside of the cell. A little more space is available at the entrance of the cell, and occupied by the "chief" of the cell.

TB patient

This represents a door

This represents a window

Chapter 4. General discussion

4.1 THE VALUE OF A RAPID DIAGNOSTIC TOOL FOR MDR-TB IN DRC

Antimicrobial resistance represents a major threat to global health and security. In 2014, the World Health Assembly called on all nations and the international community to take every necessary measure to control it, including surveillance of its emergence and spread (1). The development of drug resistance in *Mycobacterium tuberculosis* was first documented in the late 1940s, soon after antibiotic therapy was introduced for tuberculosis treatment (2). It quickly became obvious that combination chemotherapy could prevent the emergence of drug resistance (3) and that patients infected with drug-resistant strains were less likely to be cured (4).

Nevertheless, it was only in the early 1990s that drug resistant tuberculosis began to receive global attention as a public health threat. This coincided with the detection of outbreaks of multi drug resistant (MDR) tuberculosis (defined as resistance to at least rifampicin and isoniazid) that were associated with high mortality among patients co infected with the human immunodeficiency virus (HIV)(5-8). In DRC, the challenge posed by MDR-TB was not clearly defined as a result of the scarce laboratory infrastructure and some logistical limitations. While the WHO estimates classify the country as a high burden country for MDR-TB, less than 2% of this estimated number were detected and put on specific treatment 10 years ago. Kinshasa, the capital city of DRC which accounted for 20% of all TB cases nationwide, was notifying more than 80% of all MDR suspects, and not all of these were laboratory-confirmed (DRC National TB Program data; unpublished).

Our study of the cluster of cases in Mosango demonstrated that MDR-TB was present in DRC even in remote rural areas. To our knowledge this was the first published account of confirmed MDR-TB from a rural area in DRC. Our findings suggest that not only MDR-TB was present in Mosango Health district since at least 2008 but highlighted also the delay in diagnosis and appropriate management of the cases that led to a high case fatality rate. This high case fatality was most likely due to the inadequate retreatment regimen used during several months, as the risk of death with standard short-course chemotherapy is highest when there is resistance to both INH and RMP (9, 10).

By using sequencing data, we showed a set of 4 different drug resistant patterns in the 4 cases suggesting no evidence of a link between these MDR-TB cases. Most likely, the MDR-TB was due to acquired resistance in patients previously treated. Three MDR patients had a strain with a single *rpoB* mutation (526Tyr, 531Trp and 526Leu) and one patient had a strain with a double mutation 531Leu and 633Leu. This double mutation observed in a patient who had been

previously treated 3 times with First-Line Drug treatment, is probably due to acquired drug resistance (11). The mutation 531Leu has been described recently as the most frequent in Kinshasa among relapse and treatment failure TB cases. In a prospective cohort study of TB patients starting retreatment, Van Deun found that this mutation accounts for 63% of all *rpoB* mutations from first recurrence sputum specimens from Kinshasa (12).

Contrastingly, our series consisted of multiple retreatment cases. The MDR-TB patient D had a strain with 526Leu, a mutation that is considered rare by some authors (13). However the *rpoB* 526Leu, which is part of the group called "disputed" mutations described previously by Van Deun, is rather common in DRC and elsewhere (14, 15). Of note, conventional DST at Kinshasa NRL failed to detect it (16, 17). DNA sequencing performed in our study showed the *rpoB* 633Leu in a double mutation, and to the best of our knowledge, this was never described before. Current molecular DST targeting only the RRDR (codons 507-533) is missing it. We doubt its clinical significance, as it has been found only once and accompanying other, more common mutations. If more frequently seen and also in isolation it might be assumed to have significance.

At that time culture growth methods was the only technique used to detect MDR-TB. Culture of sputum on a solid agar medium could provide more reliable DST results but is extremely lengthy and significantly delays the initiation of adequate therapy (18). Liquid-growth based methods and Nucleic Acid Amplifications techniques have demonstrated superior performance in many settings (19, 20, 21). However under field conditions in high TB settings and low income countries, such as DRC, these methods have high technical and logistical requirements that are not easy to meet in a sustainable and accessible way.

The latest genotypic techniques might be definitely much easier to set up and run (22, 23). From a public health perspective, rapid and timely detection of TB cases and strengthened capacity to diagnose cases of drug-resistant TB remain thus global priorities for TB care and control.

Results observed during this investigation highlighted the need for a Point-of-Care (POC) diagnostic tool that can be more readily deployed at the district level. This would greatly facilitate the timely detection of drug-resistant TB and facilitate surveillance. Such tests should ideally combine short time to results, high accuracy and ease of use.

4.2 VALIDATION AND FEASIBILITY STUDY

Molecular drug susceptibility testing (DST) methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB. Currently, they are offering speed of diagnosis, standardized testing, potential for high burden settings, and lower level for laboratory biosafety compared to conventional DST (24).

In 2008, the World Health Organization (WHO) has endorsed molecular line probe assay (LPA) technology for rapid detection of MDR-TB that brings results within 2 days –even from clinical specimens (25). The GenoType® MTBDR*plus* assay (MTBDR*plus*, Hain LifeScience GmbH, Nehren, Germany) was one of the first commercially available LPAs. Because of its accuracy and rapidity, genotypic detection of Rifampicin (RMP) and Isoniazid resistance the MTBDR*plus* has emerged as an essential tool for the diagnosis of MDR-TB (26, 27). It has also been suggested as an alternative approach for conducting Drug Resistance Surveys (DRS) in settings with limited capacity to perform phenotypic DST (28).

In 2009, the company Hain Lifescience introduced a new format of the LPA, the GenoType® MTBDR*sl* test (MTBDR*sl*), for the rapid determination of genetic mutations associated with resistance to FQs, second line injectable drugs and ethambutol. The main mechanism of resistance to FQs in *Mycobacterium tuberculosis* is caused by mutations affecting DNA gyrase, which consists of the GyrA and GyrB subunits, encoded by the *gyrA* and *gyrB* genes, respectively (29). Most mutations conferring bacterial resistance to FQs occur in a short segment termed the quinolone resistance determining region (QRDR) in the *gyrA* gene (30, 31). Analysis of the QRDR alone by genotypic tests has been suggested as sufficient for rapid identification of the vast majority of FQ-resistant *M. tuberculosis* strains with an estimated sensitivity around 85% for FQ resistance (32, 33). The identification of resistance to FQs by the MTBDR*sl* is based on this principle. The format of the MTBDR*sl* is similar to that of the MTBDR*plus* and it also has a turn-around-time of 48 hours. MTBDR *sl* (version 1.0) was the first commercial second-line line probe assay (SL-LPA) for detection of resistance to second-line TB drugs. In 2015, the manufacturer developed and made commercially available version 2.0 of the MTBDR sl assay. Version 2.0 detects the mutations associated with FQ and second line injectable drug (SLID) resistance detected by version 1.0, as well as additional mutations. Once a diagnosis of RR-TB or MDR-TB has been established, SL-LPA can be used to detect additional resistance to second-line drugs, in, *gyrB* and the *eis* promoter for version 2 (33,34).

In the beginning of our study in 2011, we did use version 1, later on we worked with version 2, when it became available on the market. Our study reported that also the specificity of MTBDR*sl* LPA was inadequate for FQ-resistance during a validation study of the assay in Kinshasa. Our results warrant caution in the interpretation of the MTBDR*sl* when the only sign of resistance is the absence of WT2 band hybridization, without the presence of confirmatory mutation bands. Such instances may lead to the false interpretation as FQ resistance in settings with high prevalence of the 80Ala and 90Gly polymorphisms that do not confer resistance. Performance of the MTBDR*sl* LPA could thus be improved by omitting all *gyrA* WT probes and adding the few missing mutant probes well documented to confer FQ resistance, so that all clinically relevant mutations are confirmed by a mutant band. According to the MTBDR*sl* results, the proportion of FQ resistance among MDR-TB (i.e. RMP-resistant) samples was 15%. Only through sequencing, the proportion of FQ resistance dropped to 5%. This is the first assessment of the performance

of LPA technology under routine diagnostic conditions in the capital city of DRC, one of the 22 TB high burden countries.

In low-income countries with high TB burden as DRC, resistance to FQ is not routinely tested because of the very limited laboratory infrastructure. New molecular techniques that do not have the same biosafety requirements as conventional techniques have the potential to overcome this problem and are an alternative for periodic or continuous surveillance of resistance against this important class of drugs. During this study, a local validation of a novel molecular assay has been assed; its accuracy was compared to a reference, composed of standard pheno- and genotypic techniques.

Our results are important for clinical practice. For MDR-TB management, knowledge on FQ susceptibility is crucial as FQs are the core drugs in all the second-line drug regimens. Failure to detect mutations conferring resistance (i.e. poor sensitivity) or over-detection of false resistance (i.e. poor specificity) results in poor programmatic management of MDR-TB cases. FQ-resistance in *M. tuberculosis* has a major impact on MDR-TB patient outcome, and removal of FQ seriously jeopardizes the strength of the second-line regimen, so false positive results should be avoided. Molecular differentiation of the *gyrA* mutations 80Ala and 90Gly has important clinical consequences, since these mutations are not associated with FQ resistance. In Kinshasa, managing MDR-TB cases based on results of the MTBDR*sl* LPA only would thus have been detrimental for patient prognosis.

Our conclusion is that the Genotype MTBDR *pl/sl* could improve the programmatic management of MDR-TB in Kinshasa as the test results are available within 48 hours and give guidance on appropriate treatment regimens.

4.3 The burden of MDR-TB and Surveillance of drug resistance in Kinshasa, DRC

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 Surveillance 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 (35).

Obviously, the true burden of TB drug resistance cannot be established without an appropriate and accurate laboratory test. Prior to the implementation of new molecular tools in DRC, evidence on the magnitude, distribution, and trends of MDR-TB in DRC was scanty, since data on drug resistance were only generated at specific sites at the subnational level or in the framework

of research projects. In 1998, during the Kinshasa survey performed by the Antwerp Supra-National TB Reference Laboratory (SRL), the proportion of MDR-TB was 2.2% among new cases of TB and 22.1% among retreatment cases. FQ resistance was not tested in those days [Van Deun A; unpublished data].

Surveillance of FQ resistance is essential to track the emergence of XDR-TB, especially in areas such as the DRC where they are widely used for treatment of other bacterial infections. WHO suggested that in settings currently lacking the capacity for exhaustive surveillance based on routine DST of all previously treated TB cases, sentinel surveillance based on a sample of previously treated cases, i.e. all first-line failure and relapse cases, should be used as an integral part of programmatic monitoring of MDR-TB.

Laboratory-based surveillance should evaluate the susceptibility of *M. tuberculosis* to INH and RMP; and if resistance to RMP is present, then susceptibility to the FQs and second-line injectable agents most often used in the setting should also be tested (36). After validation, in the DRC context, of the molecular line probe assay (LPA) that was endorsed by the WHO in 2008 and subsequently the implementation of GeneXpert, it became possible to determine the magnitude of drug resistance particularly RMP, MDR-TB and FQ resistance, in the patients with recurrence (failure or relapse/reinfection) after primary treatment of tuberculosis in Kinshasa, the capital of DRC with about 10 million inhabitants. In parallel, to evaluate the feasibility of a simplified and more efficient strategy for monitoring of drug resistance in DRC. Our prime objective was not to unravel the SLID resistance problem.

This study provides the first report on monitoring of MDR-TB and FQ resistance in Kinshasa, DRC. From 2005 to 2013, among failure and relapse cases with valid DST results, 346 (19.5%) showed resistance to RMP and INH. This proportion of MDR-TB amongst recurrent cases is similar to the one observed in the survey of retreatment cases done in 1998 [Van Deun A; unpublished data] but is 1.5 fold higher than the current DRC estimates (13%, 95% CI 0.2-28) (37). This proportion is also higher than the MDR-TB prevalence reported in the remaining 8 African TB high burden countries in 2013 (37). Our data suggest that the overall frequency of MDR-TB remained at the same level in Kinshasa between 2005 to 2009. An apparent increase of MDR-TB occurred in our series after 2010 but this finding is biased by drastic changes in population sampled and DST methods

With the implementation of LPA in Kinshasa, the proportion of valid DST has significantly been raised (82.0 % versus 68.0 % with Conventional DST). In a vast country such as DRC, faced with numerous logistical problems, the implementation of molecular DST methods with fewer requirements for laboratory biosafety, could improve significantly the scaling up of programmatic management and surveillance of TB drug resistance.

Our findings provide the DRC NTP with evidence that the new molecular techniques or approaches could, in the specific context of DRC, improve the programmatic management of MDR-TB. Genotype MTBDR (version 2) and Xpert® MTB/RIF both substantially reduce the turnaround time to get results compared to culture and DST. As shown on **Figure 1,** since its installation in 2012, the implementation of the Xpert network has made it possible to increase the number of diagnoses of confirmed drug-resistant cases. In DRC, at the end of 2016, more than 70% of the estimated number of MDR TB among retreatment cases have been detected and put on specific treatment. All of these are laboratory-confirmed.

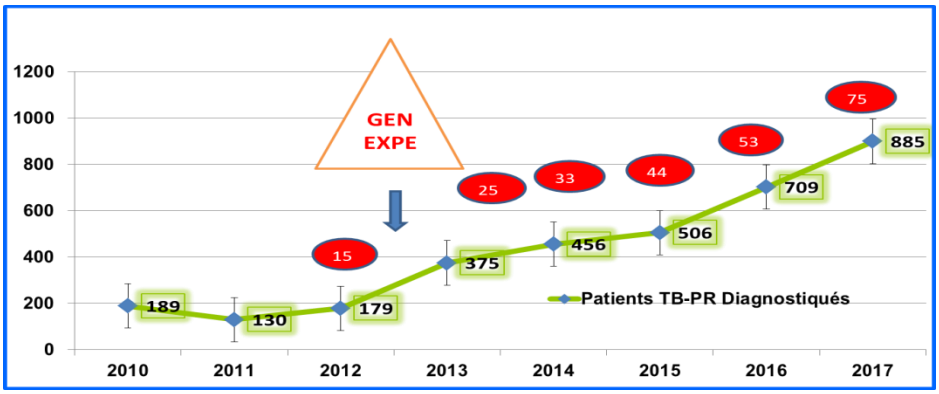


Figure 1: The implementation of Xpert® MTB/RIF and the increase of TB RR cases in DRC.

4.4 Emergence of TB/MDR-TB in the Mbuji-Mayi prison in the Democratic Republic of Congo, an outbreak investigation

The introduction of the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) assay is considered an important breakthrough in the fight against TB and MDR-TB (38-40). WHO recommends that people suspected of having pulmonary TB and considered to be at risk of harboring MDR-TB bacilli should receive the Xpert® MTB/RIF tests as a primary diagnostic test (41).

In low income countries, such as the DRC, due to the financial and logistical issues, the Xpert® MTB/RIF machines are located in national or provincial reference laboratories and mostly used for the detection of RMP resistance among MDR suspects, and not for routine TB detection. At the end of 2013, the NTP of DRC introduced the Xpert® MTB/RIF technology in each province, mainly motivated by the lack of data on the MDR-TB burden and the extremely long turn-around times with conventional methods.

In the perspective of End TB, WHO strongly recommended to intensify active case findings strategies specially among vulnerable populations. In parallel of our study, we evaluated the feasibility of a simplified and more efficient strategy for monitoring of drug resistance in DRC. As, in the meantime, GeneXpert, a new molecular tool, became available in the market, we used it to document the emergence of TB/MDR-TB cases in Mbuji-Mayi central prison settings in order to guide appropriate treatment and infection control measures.

Our findings demonstrate the high burden of TB and drug-resistant TB in a large prison in DRC. The prevalence of TB in this prison was 39 times higher than the estimate of 549 cases per 100 000 in the general Congolese population (42) and is 3.5 times greater than the prevalence reported from Zambian prisons (43).

A number of studies in prison populations in Sub Saharan Africa (SSA) have reported higher rates of TB (5,6, 12) and HIV prevalence (43,44) than in Mbuji-Mayi central prison, where the overall HIV prevalence was rather low (1.4%) though somewhat higher (2.3%) among inmates with TB. Not all consenting prisoners were tested though due to lack of HIV-tests, so these figures have to be interpreted with caution.

Several risk factors likely contribute to the high prevalence of TB in prisons in general: inmates often originate from groups with low socio-economic status in which TB is more prevalent, most prisons do not screen for TB at imprisonment, and the characteristics of the prisons, with overcrowding and poor ventilation, further enhance the risk of spread of TB (45- 50).

In Mbuji-Mayi central prison, our findings suggest that overcrowding, nutritional status and length of incarceration seem to be the three major risk factors that contributed to the spread of TB disease in prison. These risk factors are well documented as the major risk factors for high TB prevalence in prisons (45, 51-56). Especially the overcrowded conditions deserve attention. The Central Prison of Mbuji-Mayi was built in 1950 for a capacity of 150 inmates, i.e. for at most 16 persons per cell. During our investigation we observed 918 persons were incarcerated in the same premises, and cells contained more than 102 persons, six times more than their capacity. Overcrowding was exacerbated by the other factors: very poor ventilation in the cells (1 door and 1 window per cell usually covered by clothes and other possessions) and lack of exposure to sunshine. This could explain why the majority of TB cases were retrieved in the back of the cell where ventilation and sunshine are most lacking. The length of incarceration was associated with TB. We noted in this prison that only 202 (22%) detainees had actually been sentenced. Lack of judges and delay in prosecution seem to be the major causes of this high length of incarceration. The judicial proceedings were accelerated after our investigation, and three months later the total number of inmates was rapidly decreased.

The Mbuji-Mayi region is classified among the “5 hot-spot” regions for TB and TB-RR (DRC First Drug Resistance Survey 2017, unpublished data). Delay in diagnosis of TB in Mbuji-Mayi prison was recognized as a major limitation for TB control efforts in this correctional setting. Prison-based TB control measures in DRC are limited, as so far only passive case detection based on microscopy is deployed in prisons nationwide in DRC. Overall, active case finding using the more sensitive Xpert® MTB/RIF technology increased TB case detection by 19 fold over passive case detection. In prisons, passive and active case-finding should be carried out simultaneously and systematically. A combination of these two approaches will substantially increase case detection and likely contribute to TB control in this highly vulnerable population.

The GeneXpert® MTB/RIF offers rapid and accurate diagnostic results from biological specimens with minimal staff training requirements. Our findings highlight that the lack of well-equipped laboratory facilities with well-trained personnel were key barriers to TB control in this prison.

The introduction of the GeneXpert® MTB/RIF assay is considered an important breakthrough in the fight against TB and multidrug-resistant (MDR)-TB. For the first time, a molecular test is simple and robust enough to be introduced outside a reference laboratory setting, detecting TB and rifampicin-resistant TB as a proxy for MDR-TB. The GeneXpert should be considered as a promising new diagnostic system which is fully automated closed system that performs both sputum sample processing and real-time PCR, producing results within 2 hours. The huge advantage of Xpert® MTB/RIF is that is capable of detecting the MTB complex and simultaneously detecting RMP resistance.

During our accuracy evaluation, sensitivity and specificity was 100% according the genetic sequencing using the RMP resistant and sensitive MTB complex isolates. We conclude that Xpert® MTB/RIF is an accurate, rapid and appropriate tool to be installed for use at point-of-care in prison clinics.

Although testing with GeneXpert® MTB/RIF does not require additional laboratory equipment, the sophisticated nature of the device requires care in handling, i.e., a stable and uninterrupted electrical or battery supply to avoid interruption of the procedure and subsequent loss of results, adequate storage space for the cartridges, and dedicated staff to perform testing.

4.5 ON THE DIAGNOSIS OF DRUG RESISTANT TB IN DRC

4.5.1. Genotypic methods for drug resistant detection

When we initiated our thesis, no genotypic test of drug susceptibility was performed in DRC. Molecular-based testing now allows to obtain rapid test results regarding drug susceptibility. Our research provided the country with the first genotypic results in the diagnosis of drug-resistant TB. Table I indicates that out of 961 MDR cases tested for resistance to second-line drugs, 69 (7%) were found pre-XDR-TB and 13 (1.4%) XDR-TB.

Table I. Genotype MTBDR plus and sl test results in the NTP-DRC from 2012 to 2017

Years	Number of specimens tested	Number of MDR	Number of pre-XDR	Number of XDR
2012	245	84	8	1
2013	193	60	4	0
2014	42	5	4	0
2015	62	59	9	3
2016	191	191	16	7
2017	562	562	28	2
Total	1295	961	69	13

Our findings are important for clinical practice. For MDR-TB management, knowledge on FQ susceptibility remains crucial as FQs are the core drugs in all the second-line drug regimens. FQ-resistance in *M. tuberculosis* has a major impact on MDR-TB patient outcome, and removal of FQ seriously jeopardizes the strength of the second-line regimen. Failure to detect mutations conferring resistance results in poor programmatic management of MDR-TB cases.

Futhermore, our results highlight that genotypic methods have an important role in surveillance, especially given the limitations of conventional phenotypic methods, and accelerate the

availability of results. There is therefore an urgent need for rapid and easy-to-perform molecular tests of drug susceptibility for a wide range of antituberculosis drugs.

4.5.2. Genotypic methods to enhance the MDR-TB surveillance system

In DRC with a high burden of TB, there was inadequate capacity for routine testing of all previously treated TB patients for resistance to drugs. Less than 2% of presumptive MDR-TB estimated number were detected and put on specific treatment before 2009. Extremely long turn-around times for laboratory results to reach the treating clinician increase the risk of the spread of resistant strains. We provided the NTP of the DRC with evidence that the novel molecular techniques could, in the specific context of DRC, improve the programmatic management of MDR-TB. Table 2 shows that the rollout of GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), is starting to increase access to susceptibility testing for RR-TB, from 2012 to 2017 a total of 91 616 Xpert tests were performed. Among the valid results, 11.19% (3038/21553) RR-TB cases were detected. The proportion of invalid results is around 10%.

Table 2. Xpert MTB/RIF results in DRC from 2012 to 2017.

YEARS	XPERT MTB/RIF RESULTS										
	(MTB-)	(MTB+ RIF S)	(MTB+ RIF R)	(MTB+ RIF IND.)	ERROR	INVALID	NO RESULT	TOTAL TEST XPERT	% RR+ out of total XPERT	% TB+ out of total XPERT	% non valid (I) out of total XPERT
2012	7828	915	66	48	964	5	3	9829	0.67%	10.47%	9.89%
2013	9917	1975	376	116	1035	16	24	13459	2.79%	18.33%	7.99%
2014	11478	2570	467	137	1606	47	57	16362	2.85%	19.40%	10.45%
2015	5051	2561	517	77	672	334	205	9417	5.49%	33.50%	12.86%
2016	5915	2949	729	166	699	235	229	13275	5.49%	**28.96%**	8.76%
2017	17545	6863	883	138	1696	1064	231	29274	3.02%	**26.93%**	10.21%
TOTAL	**57734**	**17833**	**3038**	**682**	**6672**	**1701**	**749**	**91616**	**3.31%**	**23.52%**	**9.95%**
							% RR within MTB+		**11,19%**		

However, much greater efforts are needed to meet the WHO target of universal drug susceptibility testing.

In DRC, 70% of the estimated number of MDR TB among retreatment cases have been detected and put on specific treatment during 2017. All of these are laboratory-confirmed and **50.7%**(449/885) of MDR-TB have their FQ or SLID DST results using LPA as shown on Table 2. At the same tine DRC is working to remove all barriers for a successful implementation of the PMDT plan, it should improve the quality and the extend of services provided by the TB laboratories network with a regard to FQ and SLID DST testing in particular.

We wish to continue the scaling up of the use of molecular tests within the country in a programmatic way. In the perspective of End TB, our findings led to a large innovative and future-oriented project called: **TB Fast Track program**. This project aims for "rapid" screening for TB and drug-resistant TB and for setting up " a fast" treatment that is "adequate", ie compatible, with the type of *Mycobacterium tuberculosis* carried by the patient within a period not exceeding 48 hours. The TB Fast Track program includes 3 critical phases. The first is the use of Xpert MTB/RIF for the rapid screening of TB and RR-TB among TB suspects in a vision that is both operational and programmatic. The second phase will be the evaluation of the instantaneous electronic transmission of strategic information and confirmation of the initiation of appropriate treatment by the clinician in the field. The last phase will be the scaling up of the concept in the largest medical centers within the country. (See table 3)

Table 3. Overview of the DRC NTP results with PMDT.

2009	**27%** of DST coverage among retreatment cases;
	Less than **5%** of MDR-TB as FQ or SLID DST results using phenotypic methods;
2017	**70%** DST coverage among retreatment cases by using Xpert MTB/RIF;
	50,7%(449/885) of MDR-TB as FQ or SLID DST results using LPA.
Achievements	
From 130 MDR-TB cases detected in 2011, 885 RR-TB cases detected in 2017,which represents an increase of 6.8 fold;	
85% of the 2013 cohort with the long regimen of treatment success versus 95% for the 2015 cohort with the short course regiment;	
Bedaquiline compassionate use for 45 XDR patients since 2015.	
PMDT Plan for 2018-2020	
% of new and relapse TB patients tested with a WHO- recommended rapid diagnostic(WRD) at the time of diagnosis in the 5 "hot-spot"provinces of DRC:	70%
% of DST coverage among retreatment cases:	100%
% of RR-TB cases with FQ and SLID DST results:	100%
% RR-TB and XDR-TB on "adequate" treatment:	100%
Is the LNRM/PNLT/DRC accredited according the ISO 15189-2012 standard?	YES

Chapter 5. Conclusion and recommendations

5.1 Conclusion

TB remains a major global health problem. It causes ill-health for approximately 10 million people each year and is one of the top ten causes of death worldwide. For the past 5 years, it has been the leading cause of death from a single infectious agent, ranking above HIV/AIDS. This is despite the fact that, with correct case management, i.e. a timely diagnosis and correct treatment, most people who develop TB disease can be cured.

The End TB Strategy was endorsed by WHO's 194 Member States during the 2014 World Health Assembly, and covers the period 2016–2035. The SDGs were adopted by United Nations (UN) Member States in September 2015, and cover the period 2016–2030. The SDGs and the End TB Strategy share a common aim: to end the global TB epidemic. Targets set in the End TB Strategy include a 90% reduction in TB deaths and an 80% reduction in TB incidence by 2030, compared with 2015. The 10% per year fall in incidence that is needed by 2025 has been previously achieved only by countries within the wider context of Universal Health Coverage (UHC) and broader social and economic development.

UHC means providing all people with access to needed health care services of sufficient quality to be effective, without their use imposing financial hardship. To lower the number of new TB cases to less than 10 per 100,000 population by 2035 ("end the global TB epidemic") and achieve a 95% reduction in TB deaths by 2035, the world needs a technological breakthrough by 2025 that will allow an unprecedented acceleration in the rate at which TB incidence falls between 2025 and 2035. This will only happen with substantial investment in R&D in the years up to 2025, so that new tools such as a post-exposure vaccine or a short, efficacious and safe treatment for latent infection that could substantially lower the risk of developing TB among the approximately 2 billion people that are already infected, are developed.

The only rapid test combining diagnosis and DST of TB, currently recommended by WHO, is the Xpert® MTB/RIF assay (Cepheid, USA). It can provide results within 2 hours, and was initially recommended (in 2010) for diagnosis of pulmonary TB in adults. Since 2013, it is also recommended for use in children and to diagnose specific forms of extra pulmonary TB. In our hands it proved an effective tool for documenting rapidly the extent of an outbreak in a prison population and curtailing it.

Laboratory services, although crucial for national disease control programmes, are often the weakest link in the health system, receiving low priority and inadequate resources. For TB control, quality controlled bacteriological examination is essential for the diagnosis and

management of TB patients. Laboratory strengthening is a priority for the Stop TB Strategy, including improved access to and use of existing diagnostics as well as the development and implementation of appropriate new technologies. TB laboratory services should be integrated within the national system of the laboratory services. At the central level, the national reference laboratory for TB can either be located within the NTP or it may be part of the general laboratory system with close links to the NTP. At the regional and peripheral levels, TB laboratories should be fully integrated within the reference hospitals, district hospitals and health centres. The laboratories at the regional and peripheral levels are multi-purpose, with technicians performing tests for the diagnosis of a wide variety of diseases. Involvement of accredited non-state sector laboratories into the NTP laboratory network should be considered.

Efforts to improve the laboratory performance should be well coordinated to avoid fragmentation and creation of separate disease-specific services. In DRC context, HIV testing should be offered to all TB suspects along with sputum examination. Based on national policies, HIV tests may be carried out in TB laboratories and/or by health-care providers. The strategic orientations for laboratory strengthening have to focus on:

- improving smear microscopy;
- strengthening and expanding capacity for molecular testing;
- adapting and transferring existing technologies to resource-limited settings;
- contributing to development and testing of new diagnostic tools under field conditions.

WHO strongly recommends that programmes generate population-representative data of drug resistance surveillance (DRS) for new patients, for the different categories of re-treatment patients (failure after Category I, failure after re-treatment, lost to follow-up and relapse) and for other high-risk groups.

Designing an effective case-finding strategy depends on this information. Availability of DRS data for the different groups also enables calculation of the number of patients who should enter the programme; this in turn greatly facilitates programme planning and drug procurement.

The DRC NTP programme may not have sufficient laboratory capacity to provide drug susceptibility testing (DST) of all patients. Where targeted DST surveys identify a risk group or groups of patients with a high proportion of MDR-TB (which may exceed 80%), the use of empiric MDR-TB short or conventional regimens in all patients in that sub-group may be justified.

The three risk groups commonly considered for direct enrolment for a MDR-TB regimen are:

- Category II failures (chronic TB cases);
- TB patients who are close contacts of MDR-TB cases;
- Category I failures who received a full course of treatment.

The proportion of MDR-TB in these three groups may vary considerably. It is therefore important to confirm MDR-TB through the use of DST (to, at least rifampicin) for all patients who start a MDR-TB regimen. With the expanded use of Xpert® MTB/RIF this should be feasible.

5.2 RECOMMENDATIONS

From the research work presented in this thesis, I would like to derive the following specific recommendations directly resulting from the empirical work for policy:

1. To establish a routine and comprehensive surveillance system for all previously treated cases in DRC, which is the first step towards routine drug susceptibility testing for all TB patients, requires not only the implementation of new diagnostic technology (Xpert® MTB/RIF in periphery combined with LPA at central level) but also to address a number of programmatic issues related to DST coverage and expansion of access to short course drug regimens for MDR-TB cases.

2. All barriers limiting access to DST should be removed urgently. NTPs should grasp the opportunities created by the roll-out of the new generation molecular tools to improve and to support the surveillance of drug resistance.

3. Given the high risk of TB in prison settings, there is an urgent need for policy makers, programme managers, and scientific communities to implement and support effective control programmes to protect not only the health of prison inmates but also the health of the wider community. As overcrowding and poor nutrition seem to be major drivers of the transmisison, we recommend that living conditions in prisons should be improved and inmates should have access to better diet. Dedicated health programs should be implemented on a continuous basis. Overcrowding in prisons is a huge risk factor and should be avoided.

Beyond this work, I would like to suggest also some general recommendations to the country:

4. More attention and funding should be dedicated to strengthening the entire DOTS-plus program to achieve global TB control and avoid treatment stock-outs for all patients, including the increasing number of patients recognized to have resistant tuberculosis.

5. Besides early detection and adequate treatment of drug-resistant TB, there are other interventions that are fundamental to limit the emergence and spread of DR-TB: early detection and adequate treatment of drug-susceptible tuberculosis, effective infection control measures. health system strengthening, addressing underlying risk factors and social determinants.

As a final word, I would like to point out that in this thesis I have tried to analyze the weaknesses, constraints and challenges related to improving laboratory services for the diagnosis of TB and MR TB within my country, DRC. The process of writing this thesis was long and not easy. During my thesis project, international technological breakthroughs forced me to adapt the diagnostic policy in the NTP in my capacity as TB manager, and hence to revise my thesis objectives and calendar accordingly. However, the result of this process of "in-depth" analysis, review of scientific evidence and our own findings are now the bedrock of the first strategic plan for the development of the TB laboratories network in DRC, which is a sub-sectoral plan, of the DRC's Sanitary Development Plan for the period 2016-2020. Indeed, my thesis work contributed to policy formulation and allowed the NTP to more clearly define strategy taking into account the realities of our context as well as the crucial parameters for improving the diagnosis of TB and MDR-TB in the DRC.

References to Chapters 4 and 5

1. Resolution WHA67/25: antimicrobial resistance. Presented at the 67th World Health Assembly, Geneva, May 24, 2014 (Resolutions and Decisions; Annexes [WHASS1/2014WHA67/2014/ REC/25]). Geneva: World Health Organization, 2014.
2. Crofton J, Mitchison DA. Streptomycin resistance in pulmonary tuberculosis. Br Med J 1948; 2: 1009-15.
3. Cohn ML, Middlebrook G, Russell WF Jr. Combined drug treatment of tuberculosis. I. Prevention of emergence of mutant populations of tubercle bacilli resistant to both streptomycin and isoniazid in vitro. J Clin Invest 1959; 38: 1349-55.
4. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. Am Rev Respir Dis 1986; 133: 423-30.
5. Nosocomial transmission of multidrug-resistant tuberculosis among HIV-infected persons — Florida and New York, 1988– 1991. MMWR Morb Mortal Wkly Rep 1991; 40: 585-91.
6. Frieden TR, Sterling T, Pablos-Mendez A, Kilburn JO, Cauthen GM, Dooley SW. The emergence of drug-resistant tuberculosis in New York City. N Engl J Med 1993; 328: 521-6.
7. Monno L, Angarano G, Carbonara S, et al. Emergence of drug-resistant Mycobacterium tuberculosis in HIV-infected patients. Lancet 1991; 337: 852.
8. Moro ML, Gori A, Errante I, et al. An outbreak of multidrug resistant tuberculosis involving HIV-infected patients of two hospitals in Milan, Italy. AIDS 1998; 12: 1095-102.
9. Schreiber YS, Herrera AF, Wilson D, Wallengren K, Draper R, et al. (2009) Tuberculosis retreatment category predicts resistance in hospitalized retreatment patients in a high HIV prevalence area. Int J Tuberc Lung Dis 13(10):1274-80.
10. Lan NTN, Lademarco MF, Binkin NJ, Tung LB, Quy HT, et al. (2001) A case series: initial outcome of persons with multidrug-resistant tuberculosis after treatment with the WHO standard retreatment regimen in Ho Chi Minh City, Vietnam. Int J Tuberc Lung Dis 5(6):575-8.
11. Lipsitch M, Levin BR (1998) Population dynamics of tuberculosis treatment: mathematical models of the roles of non-compliance and bacterial heterogeneity in the evolution of drug resistance. Int J Tuberc Lung Dis 2:187–199.
12. Van Deun A, Maug AK, Bola V, Lebeke R, Hossain MA, et al. (2013) Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol 51:2633–2640.

13. Yip CW, Leung KL, Wong D, Cheung DTL, Chu MY, et al. (2006) Denaturing HPLC for high-throughput screening of rifampicin-resistant Mycobacterium tuberculosis isolates. Int J Tuberc Lung Dis 10:625–630.

14. Ohno H, Koga H, Kohno S, Tashiro T, Hara K (1996) Relationship between rifampin MICs and rpoB mutations of Mycobacterium tuberculosis strains isolated in Japan. Antimicrob Agents Chemother 40:1053–1056.

15. Van Deun A, Barrera L, Bastian I, Fattorini L, Hoffmann H, et al. (2009) Mycobacterium tuberculosis strains with highly discordant rifampin susceptibility test results. J Clin Microbiol 47:3501–3506.

16. Quezada C M, Kamanzi E, Mukamutara J, De Rijk P, Rigouts L, et al. (2007) Implementation validation performed in Rwanda to determine whether the INNO-LiPA Rif.TB Line Probe Assay can be used for detection of Multidrug-Resistant Mycobacterium tuberculosis in low-resource countries. J Clin Microbiol 45:3111–3114.

17. Pfyffer GE, Welsher HM, Kissling P, Cielslak, Casal M, et al. (1997) Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid media for recovery of acid-fast bacilli. J Clin Microbiol 35:364-368.

18. Somoskovi A, Kodmon C, Lantos A, Bartfal Z, Tamasi L, et al. (2000) Comparison or Recoveries of Mycobacterium tuberculosis Using the Automated Bactec MGIT 960 System, the BACTEC 460 TB System and Lowenstein-Jensen Medium. J Clin Microbiol 38: 2395-2397.

19. Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, et al (2005) Meta-analysis of BACTEC MGIT 960 with or without solid media ,for detection on mycobacteria. J Clin Microbiol 42(5):2321-2325.

20. Bogard M, Vincelette J, Antinozzi R, Alonso R, Fenner T, et al. (2001) Multicenter study of a commercial, automated polymerase chain reaction system for the rapid detection of Mycobacterium tuberculosis in respiratory specimens in routine clinical practice. Eur J Clin Microbiol Infect Dis 20:724–731.

21. Piersimoni C, Scarparo C, Piccoli P, Rigon A, Ruggiero G, et al. (2002) Performance assessment of two commercial amplification assay for direct detection of Mycobacterium tuberculosis complex from respiratory and extra pulmonary specimens. J Clin Microbiol 40:4138–4142.

22. Hillemann D, Rüsch-Gerdes S, Richter E (2007) Evaluation of the Geno-Type MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol 45:2635–2640.

23. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, et al. (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert® MTB/RIF test

for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377(9776):1495–505.

24. World Health Organization. 2013. The use of molecular line probe assay for the detection of resistance to second-line anti tuberculosis drugs: expert group meeting report. (WHO/HTM/TB/2013.01). World Health Organization, Geneva, Switzerland.

25. World Health Organization.2008. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement. World Health Organization, Geneva, Switzerland.

26. Barnard M, Albert H, Coetzee G, O'Brien R, Bosman ME. 2008. Rapid molecular screening for multidrug resistant tuberculosis in a high volume public health laboratory in South Africa. Am. J. Respir. Crit. Care Med. 177:787–792.

27. Hillemann D, Rüsch-Gerdes S, Richter E. 2007. Evaluation of the GenoType MTBDR*plus* assay for rifampin and isoniazid susceptibility testing of *Mycobacterium tuberculosis* strains and clinical specimens. J. Clin. Microbiol. 45:2635–2640.

28. Rigouts L, Hoza A S, De Rijk P, Torrea G, Chonde T M, Basra D, Zignol M, van Leth F, Egwaga S M, Van Deun A. 2011. Evaluation of the Genotype® MTBDR*plus* assay as a tool for drug resistance surveys. Int. J. Tuberc. Lung Dis. 15:959–965.

29. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG.1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393: 537–44.

30. Cheng AFB, Yew WW, Chan EWC, Chin ML, Hui MM, Chan RCY. 2004. Multiplex PCR amplimer conformation analysis for rapid detection of *gyrA* mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob. Agents Chemother. 48:596–601.

31. Matrat S, Veziris N, Mayer C, Jarlier V, Truffot-Pernot C, Camuset J, Bouvet E, Cambau E, Aubry A. 2006. Functional analysis of DNA gyrase mutant enzymes carrying mutations at position 88 in the A subunit found in clinical strains of *Mycobacterium tuberculosis* resistant to fluoroquinolones. Antimicrob. Agents Chemother. 50:4170–4173.

32. Chang KC, Yew WW, Chan RCY. 2010. Rapid assays for fluoroquinolone resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. J. Antimicrob. Chemother. 65:1551-1561.

33. World Health Organization. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs. Policy guidance. World Health Organization, Geneva, Switzerland. WHO/HTM/TB/2016.07 http://apps.who.int/iris/bitstream/handle/10665/246131/9789241510561-eng.pdf?sequence=1

34. WBarnard M, Warren R, Gey Van Pittius N, van Helden P, Bosman M, Streicher E, Coetzee G, O'Brien R. 2012. Genotype MTBDR *sl* line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory. Am. J. Respir. Crit. Care Med. 186:1298-305.

35. Antimicrobial resistance: global report on surveillance 2014. Geneva: World Health Organization, 2014.

36. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 4th ed. WHO/HTM/TB/2009.422. 2009. http://apps.who.int/iris/bitstream/10665/44206/1/9789241598675_eng.pdf. Accessed 6 Oct 2015.

37. World Health Organization. Global tuberculosis report 2014. Annex 2 Country Profiles: Democratic Republic of the Congo - p.130. 2014. http://www.who.int/tb/publications/global_report/gtbr14_annex2_country_profiles.pdf?ua=1. Accessed 6 Oct 2015.

38. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. N Engl J Med .2010; 363: 1005–1015. doi:10.1056/NEJMoa0907847. PubMed: 20825313.

39. Steingart K, Sohn H, Schiller I, Kloda L, Boehme C et al. Xpert® MTB / RIF assay for pulmonary tuberculosis and rifampicin resistance in adults (Review). Cochrane Database of Systematic Reviews; 2013: Art. No: CD009593

40. Chang K, Lu W, Wang J, Zhang K, Jia S et al. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert® MTB/RIF assay: a meta-analysis. J Infect.2012. 64: 580–588. doi:10.1016/ j.jinf.2012.02.012. PubMed: 22381459.

41. Stop TB Partnership. Global Plan to Stop TB 2011–2015. Geneva, World Health Organization, 2010.

42. Kaswa MK, Aloni M, Nkuku L, Bakoko B, Lebeke R, Nzita A, and al. A pseudo-outbreak of pre-XDR TB in Kinshasa: A Collateral Damage of False Fluoroquinolone Resistant Detection by GenoType MTBDR sl. J Clin Microbiol. 2014; 52(8):2876-2880.

43. TB Reach. 2010; http://www.stoptb.org/global/awards/tbreach/. Accessed 15 February 2016.

44. Al-Darraji HAA, Razak HA, Ng KP, Altice FL, Kamarulzaman A. The Diagnostic Performance of a Single GeneXpert® MTB/RIF Assay in an Intensified Tuberculosis Case Finding Survey among HIV-Infected Prisoners in Malaysia. PLoS ONE. 2013 8(9): e73717. doi:10.1371/journal.pone.0073717.

45. O'Grady J, Hoelscher M, Atun R et al. Tuberculosis in prisons in sub-Saharan Africa – the need for improved health services, surveillance and control. Tuberculosis (Edinburgh) 2011; 91:173–178.

46. Harries AD, Nyirenda TE, Yadidi AE, Gondwe MK, Kwanjana JH, Salaniponi FM. Tuberculosis control in Malawian prisons: from research to policy and practice. Int J Tuberc Lung Dis 2004; 8: 614–17.

47. O'Grady J, Mwaba P, Bates M, Kapata N, Zumla A. Tuberculosis in prisons in sub-Saharan Africa–a potential time bomb. S Afr Med J 2011; 101:107–8.

48. Human Rights Watch. Unjust and unhealthy: HIV, TB and abuse in Zambian prisons. New York 2010.

49. Jurgens R, Nowak M, Day M. HIV and incarceration: prisons and detention. J Int AIDS Soc 2011; 14:26.

50. Chaisson RE, Martinson NA. Tuberculosis in Africa–combating an HIV-driven crisis. N Engl J Med 2008; 358:1089–92.

51. Ali S, Haileamlak A, Wieser A, et al. Prevalence of pulmonary tuberculosis among prison inmates in Ethiopia, a cross-sectional study. PLoS One 2015; 10: e0144040.

52. Telisinghe L, Fielding KL, Malden JL, et al. High tuberculosis prevalence in a South African prison: the need for routine tuberculosis screening. PLoS One 2014; 9: e87262.

53. Moges B, Amare B, Asfaw F, et al. Prevalence of smear-positive pulmonary tuberculosis among prisoners in North Gondar Zone Prison, northwest Ethiopia. BMC Infect Dis 2012; 12: 352.

54. Reid SE, Topp SM, Turnbull ER, et al. Tuberculosis and HIV control in sub-Saharan African prisons: "thinking outside the prison cell". J Infect Dis 2012; 205 (suppl 2): S265–73.

55. Noeske J, Ndi NF, Amougou Elo G, Mbondi Mfondih S. Tuberculosis incidence in Cameroonian prisons: a 1-year prospective study. S Afr Med J 2014; 104: 209–11.

56. Ensor L. Overcrowding in South African prisons falls. https://www.businesslive.co.za/bd/national/2013-08-26-overcrowding-in-south-african-prisons-falls/ (accessed Jan 1, 2016).

Remerciements

A l'issue de ce travail, je réalise pleinement que cette thèse de doctorat est le fruit d'une coopération et collaboration enrichissantes entre la Belgique et la RD Congo. Je tiens à remercier sincèrement toutes les personnes qui ont contribué à notre formation et à la réalisation de ce vaste programme de coopération, notamment le Professeur Docteur Bruno GRYSEELS.

Mes remerciements les plus sincères vont ensuite aux promoteurs de cette thèse :

- *Professeur Docteur Marleen BOELAERT, qui depuis notre première rencontre en 2004, a cru en moi. Elle m'a ouvert les portes de la famille de l'Unité d'Epidémiologie et de Contrôle de maladies de l'Institut de Médecine Tropicale d'Anvers (IMT). A la fin de mon Master en Santé Publique, option Contrôle des maladies à l'IMT Anvers, elle m'a suggéré de poursuivre mes études en réalisant un PhD. Elle m'a totalement adopté, encadré et permis d'arriver au bout de cette thèse. Je ne cesserai de la remercier pour sa clairvoyance, sa rigueur scientifique tout au long de ma formation doctorale et sa marque de générosité à mon égard ;*
- *Professeur Docteur Jean Jacques MUYEMBE TAMFUM, notre maître et promoteur de ce travail, dont la connaissance sans limites de la microbiologie médicale, et la notoriété n'a cessé de nous séduire tout au long de notre cursus. Cher Professeur, la rigueur scientifique et l'abnégation dans la recherche sont là des valeurs essentielles que vous nous aurez inculquées. Vous êtes et resterez toujours pour nous cette source de savoir intarissable où l'on vient s'abreuver, mais aussi la meilleure référence à suivre. Je me souviendrai toujours de ce jour de janvier 2003 quand je vous informais de ma volonté de parfaire ma formation dans votre service de microbiologie. Votre réponse fut : « Viens, j'ai besoin de travailler avec des gars passionnés dans la recherche ». Je n'ai jamais oublié. Vous m'avez ouvert le monde du savoir.*
- *Professeur Margareta IEVEN, qui en dépit de ses lourdes responsabilités a bien accepté de m'encadrer tout au long de mon parcours de recherche doctorale.*
- *Docteur Armand Van DEUN, expert incontestable dans la lutte contre la tuberculose, qui a guidé mes premiers pas dans la recherche portant sur la thématique laboratoire et contrôle de la tuberculose. Je n'oublierai jamais son*

souci permanent du travail bien accompli. Voilà 12 ans maintenant depuis qu'il m'accueillit dans la famille de l'unité de Mycobactériologie de l'IMT Anvers.

- *Professeur Docteur Bouke DE JONG, pour la bienveillance dont elle a toujours fait preuve à mon égard. Votre leadership et encadrement m'ont permis de finaliser cette recherche doctorale et d'arriver au bout de cette thèse.*

Je remercie vivement les Professeurs Docteurs Lut LYNEN et Bob COLEBUNDERS, membres du comité individuel de revue de ma recherche doctorale et tous les distingués membres du Jury pour avoir accepté sans hésitation d'examiner notre travail.

Je tiens à remercier toute mon équipe de recherche de l'INRB et des Cliniques Universitaires de Kinshasa : Docteurs Muriel Aloni, Nadine MAKUTH et mes techniciens de laboratoire Léontine NKUKU et Servet KIBONZA, sans votre support on n'y serait pas parvenu.

Mes remerciements je les adresse également à l'équipe de la Coordination Provinciale Lèpre et Tuberculose de Kinshasa : aux Docteurs Valentin Bola, Nicolas Nkiere, Brian BAKOKO, Nicole ASHAMBI et aux techniciens de laboratoire Rossin LEBEKE et Albert NZITA.

J'exprime tous mes remerciements à l'ensemble du staff de l'Unité de Mycobactériologie et de l'Unité d'Epidémiologie et Contrôle des maladies tropicales de l'IMT. Merci pour vos encouragements, vos conseils et le climat favorable d'apprentissage lors de mes nombreux séjours à Anvers : un grand merci pour Epco HASKER, Tullia Carla Enrica BATTAGLIOLI, Leen RIGOUTS, Karin JANSSENS, Ciska MAECKELBERGH, Arabella HUYS, Greet VERHULST, Inge VAN CAUWENBERG et Evelien PAESSENS, pour tout ce que vous avez fait pour moi.

Un très grand merci « fleuri » à Anne Marie TROOSKENS (IMT) et Deby KRISTIN (UA). J'ai particulièrement apprécié vos compétences professionnelles et académiques. Vous avez été pour moi des atouts déterminants.

Je tiens à remercier vivement Helga BODGES et tout son staff du service des étudiants à l'IMT,, pour votre serviabilité combien remarquable et votre disponibilité.

Je remercie le Département de la reprographie de l'Université d'Anvers pour le travail de qualité réalisé.

Je souhaite remercier les éminences grises des Cliniques Universitaires de Kinshasa : Alma mater pour tout.

Au Professeur Docteur Donatien KAYEMBE NZONGOLA NKASU, Chef du Département de Biologie Médicale à l'Université de Kinshasa à nos débuts, pour nous avoir admis comme assistant au sein du Département ; mais surtout pour nous avoir sensibilisé sur l'importance et le rôle du Médecin Biologiste dans le Congo renaissant.

Comment oublier de remercier à sa juste valeur :

- *Professeur Docteur Samuel MAMPUNZA qui nous orienta judicieusement à parfaire notre formation en Microbiologie médicale dès la fin 2002 ;*
- *Les Professeurs Docteurs Zacharie KASHONGWE et Jean-Marie KAYEMBE, qui m'ont soutenu et permis de découvrir que la Tuberculose était ma passion scientifique.*

Que les Professeurs Docteurs Raphaël KALENGAYI MBOWA, MBAYO KALUMBU, CHIRIMWANI, KABONGO MPOLESHA, Thadée ODIO WOBIN, Paul MULUMBA MADISHALA, MVUMBI LELO, Pascal LUTUMBA, Dieudonné MUMBA et Octavie LUNGUYA trouvent ici l'expression de notre franche estime pour l'encadrement combien remarquable qu'ils nous ont assuré.

Je dis merci au Dr Mulangu SABWE, mon ami et frère, mon compagnon de lutte, pour toutes ces années de dur labeur sur le terrain et pour ton soutien sans faille.

Merci à tous les Chefs de Travaux, Spécialistes, Assistants de notre Département de Biologie Médicale pour les moments de partage scientifique.

Je tiens à remercier sincèrement le Docteur Max SALFINGER du Colorado State Department of Health ; Prof Antoinette TSEFU de l'Ecole de Santé Publique, Mme Karen HAWKINS, CDC Advisor Kinshasa ; Professeur Robin RYDER et Professeur Docteur Annelies VAN RIE , UNC at Chapel Hill : grâce à qui j'ai eu l'opportunité d'être admis comme Visiting Scientist à Albany, NY, USA par le biais du programme APHL / CDC /

NCID. Cette expérience a grandement contribué à façonner notre carrière dans la thématique « Biologie médicale orientée vers la Santé publique ».

Les mêmes sentiments de gratitude, nous les exprimons envers les Docteurs André NDONGOSIEME, Jean Pierre KABUAYI, Etienne BAHATI, Jean Paul OKIATA et Georges BAKASWA, Directeurs du Programme National de Lutte contre la Tuberculose (PNLT) et à tous les agents, pour avoir favorisé et facilité notre intégration au PNLT de la RDC. Il en est de même pour les Docteurs Pamphile LUBAMBA, Représentant de la Fondation Damien Belgique en RDC, ainsi qu'Henriette WEMBANYAMA, de l'OMS, pour leurs encouragements et sages conseils.

Au Docteur Gratien BOLIE MUBIALA qui, le premier, nous initia à l'art de guérir, nous offrant ainsi l'opportunité de côtoyer le monde professionnel.

A mes amis, de tout temps, avec qui nous avons débuté la carrière professionnelle et avons partagé un même idéal de travail et de réussite : Docteurs Jean-Jacques YANGA, Eric KAYEMBE, Jean-Jacques NGALA, Jean-Paul TSHIANI, Julien SHABANI, Jérôme KABUYA, Jean Michel KAYUMBA et Jean-Jacques MPUTU et Serge DITU KABEMBA, je vous dis merci !

A vous tous, que je n'ai pas pu citer nommément ici, qui avez contribué à la réalisation de ce travail, par votre soutien tant moral que financier et /ou de quelque nature que ce soit, trouvez ici l'expression de toute ma profonde gratitude et reconnaissance.

Dr Michel KASWA-KAYOMO

Anvers, le 06 juillet 2018