International Journal of Infectious Diseases 56 (2017) 81–84

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

PERSPECTIVE

Enhanced laboratory capacity development: a boost for effective tuberculosis control in resource-limited settings

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ARTICLE INFO

Article history:
Received 22 October 2016
Received in revised form 14 November 2016
Accepted 15 November 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Gabon
Tuberculosis
Research
CERMEL
Laboratory capacity building
South–North collaboration

SUMMARY

Both routine and research tuberculosis (TB) laboratory capacity urgently need to be expanded in large parts of Sub-Saharan Africa. In 2009, the Centre de Recherches Médicales de Lambaréné (CERMEL) took a strategic decision to expand its activities by building TB laboratory capacity to address research questions and to improve routine diagnostic and treatment capacity. Over the past 7 years, a standard laboratory has been developed that is contributing significantly to TB diagnosis, treatment, and control in Gabon; training has also been provided for TB research staff in Central Africa. CERMEL has a cordial relationship with the Gabon National TB Control Programme (PNLT), which has culminated in a successful Global Fund joint application. This endeavour is considered a model for similar developments needed in areas of high TB prevalence and where TB control remains poor to date.

1. Introduction

Despite the overall global progress in tuberculosis (TB) control over the past years, TB remains one of the deadliest communicable diseases. According to the World Health Organization (WHO),[1] there were an estimated 10.4 million new (incident) TB cases globally in 2015, amongst which 480 000 were new cases of multidrug-resistant TB (MDR-TB) and an additional 100 000 were rifampicin-resistant TB (RR-TB). During the same period, 1.8 million people died of TB, including 0.4 million who were HIV-infected.[1] The African region accounts for about one-fourth of the world's cases, and TB incidence is still increasing in many parts of Sub-Saharan Africa, fuelled in part by the HIV-AIDS pandemic. In addition, the emergence of MDR- and even extensively drug-resistant (XDR)-TB provides new challenges and further threatens the control of TB. Whilst there are regions in the world where the

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http://dx.doi.org/10.1016/j.ijid.2016.11.404

extent of the problem has been assessed, at least over the past couple of years, other areas have remained by-and-large neglected, thus limiting the prospects for comprehensive advances in TB control, as laid out in the WHO End TB strategy,[1] if they remain inadequately addressed.

The laboratory plays a critical role in the diagnosis of TB and in the monitoring of treatment. According to the WHO, the effective diagnosis and treatment of TB saved an estimated 43 million lives between 2000 and 2014.[2] However, with the usual meagre resources available and in view of competing health needs in middle- and low-income countries, the laboratory is often neglected and remains very often the weakest link in TB control efforts. Therefore, there is an urgent need to strengthen laboratory services in such countries to ensure early and accurate diagnosis of TB and encourage early enrolment of patients into treatment programmes. To address this gap in the setting of Gabon, the Centre de Recherches Médicales de Lambaréné (CERMEL) in Lambaréné, instigated the establishment of a TB Laboratory in 2009 with very limited seed money. Based on this experience, it is postulated that investing in TB laboratory capacity development is a good choice with measurable and tangible yields over a short time span, allowing stepwise improvements and capacity building to meet local and regional needs. In this article, we share the challenges faced, progress made, and lessons learned in the belief that this model could be replicated in similar semi-rural settings across Africa.

2. The TB challenge

In Gabon, despite efforts made by the Government through the National TB Control Programme, 'Programme National de Lutte Contre la Tuberculose (PNLT)', TB remains a major public health challenge. The incidence of TB (including HIV + TB) is 465 per 100 000 population,[3] placing Gabon amongst the highest-incidence countries for TB worldwide.[1] Access to the laboratory diagnosis of TB is limited, with microscopy (Ziehl–Neelsen) being the only option available in most hospitals and diagnostic centres across the country. As at 2009, culture and drug susceptibility testing (DST) was available only at the Military Hospital in Libreville (the capital city of Gabon). Hence case detection, treatment, and monitoring were sub-optimal, with this constituting a major impediment to the effectiveness of national TB control efforts.

CERMEL, formerly known as the Medical Research Unit (MRU) of the Albert Schweitzer Hospital, Lambaréné, Gabon, was established over 30 years ago in order to study major causes of disease burden in the local population.[4,5] Over the years, it has grown to become a leading African Research Centre, specializing in the pathophysiology and treatment of infectious diseases prevalent in Central Africa. Malaria research has been the main focus of CERMEL since inception, and a series of studies on the epidemiology of malaria and the clinical development of novel antimalarial combination therapies and vaccine candidates has been performed; the results of these studies have been published in high impact scientific journals and they have led to changes in medical practice.[6–8] CERMEL also has a track record of conducting highly successful clinical trials, with the most recently completed trials with a high clinical impact being (1) the RTS,S malaria vaccine trial (MAL 55),[9] (2) the GMZ2 candidate malaria vaccine trial,[10] and (3) the Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) study.[11] In recent years, CERMEL has also diversified its research portfolio to include other infectious diseases including TB and HIV and has maintained a strong partnership with the Institute of Tropical Medicine, University of Tübingen, in Germany.[12]

3. Strategies for overcoming the TB challenge

3.1. Laboratory infrastructure development

In view of limited resources precluding the construction of a new TB laboratory building, a large building space previously used as an archive was refurbished; this refurbishment took place between July 2009 and January 2010 (Figure 1a). The refurbished space was partitioned into four separate rooms, serving as the laboratory office, sample reception room, slide preparation room, and culture/DST room (Figure 1b). An additional external room adjacent to the laboratory was also acquired for use as a dedicated molecular mycobacteriology laboratory. Starting with basic microscopy, the capabilities of the laboratory were expanded to cover TB culture, DST, and molecular diagnostics. Timelines for the major deliverables in the laboratory development process are shown in Table 1. Right from the outset, the Government of Gabon, through the Ministry of Health (MoH), and the PNLT were engaged in the process. Regular joint meetings were held, culminating in the signing of an official partnership and collaboration agreement in 2014 between CERMEL and the MoH. With this partnership, it was possible to obtain necessary government approvals with minimal delay.

3.2. Staff training and competency

A standardized and systematic format of laboratory staff training both locally (on site) and internationally (abroad) was established. Local training consists of documented training in each of the analytical sample processes in the laboratory, namely TB microscopy, culture, DST, and the use of rapid molecular tests (Xpert MTB/RIF and line probe assays). Training is done in

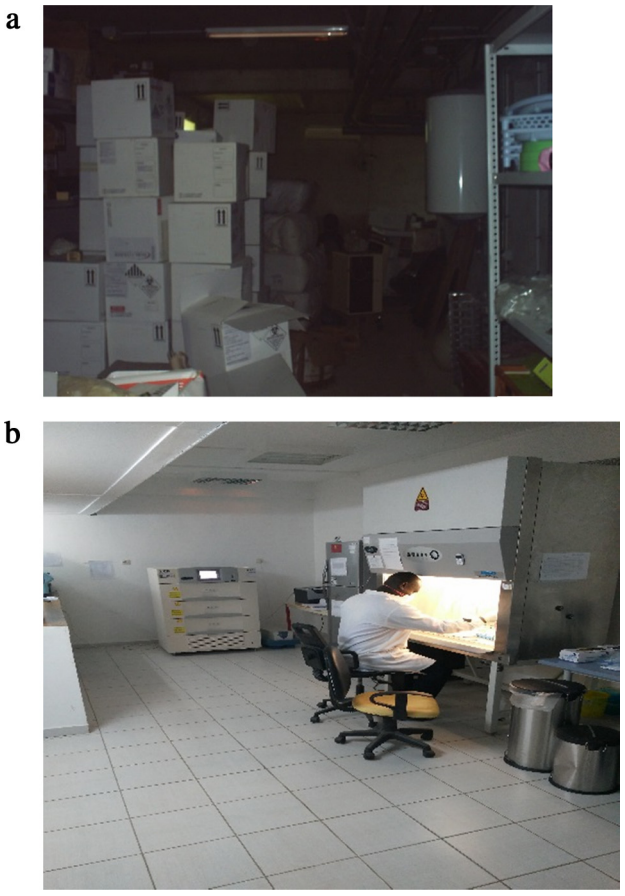

Figure 1. (a) The laboratory as an archive before being refurbished. (b) New look of the TB culture room after the refurbishment had been completed.

Table 1
Timeline of major deliverables in the development of the TB laboratory

Deliverable	Timeline (for acquisition)
Infrastructure	
• Refurbishing of laboratory space	July 2009
• Microbiology safety cabinet (MSC) class II	June 2012
Establishment of basic and culture methods	
• Light microscopy	From January 2010 to May 2012
• Fluorescence microscopy	
• MGIT 960 (BD, Belgium)	
Establishment of molecular methods	
• COBAS TaqMan 48 (Roche, Germany)	From June 2014 to January 2015
• GenoType MTBDRplus (Hain, Germany)	
• FluoroType MTB (Hain, Germany)	
• GenoType MTBDR sl (Hain, Germany)	
• GeneXpert MTB/RIF (Cepheid, USA)	
External training/collaboration	
• National Reference Centre for Mycobacteria, Forschungszentrum Borstel, Borstel, Germany	July 2011
• Molecular and Experimental Mycobacteriology, Research Centre Borstel, Borstel, Germany	February 2015
Collaboration with Gabon National TB Control Programme (PNLT)	
• Formal collaborative agreement signed with the PNLT	November 2014
• Support for a successful PNLT application to The Global Fund for TB treatment in Gabon, for which we are a sub-beneficiary	October 2015

a step-wise manner. A new staff member will usually start with TB microscopy, which takes between 1 and 2 months. At the completion of training in each technique, the trainee must pass a competency assessment (CA) test before being allowed to perform a given test routinely in the laboratory and before proceeding to train on the next technique. Through international collaboration, some members of the staff have been further trained in specialized techniques at the National Reference Centre for Mycobacteria and in the Molecular and Experimental Mycobacteriology department of the Research Centre Borstel, both in Borstel, Germany. This external training has included performing DST with different concentrations of anti-TB drugs, Sanger sequencing, real-time PCR, and whole-genome sequencing analysis.

3.3. Quality assurance

International ATCC (American Type Culture Collection) reference strains of *Mycobacterium tuberculosis* complex (MTBC) are used to check the quality of the procedures and processes. The laboratory is also enrolled in TB Microscopy, Culture and DST of the Microbiology Proficiency Testing Scheme of the National Health Laboratory Service (NHLS), Sandringham, South Africa. Microscopy external quality assessment (EQA) samples are sent to us three times a year, while culture and DST are sent twice a year. Furthermore, an internal audit of the laboratory is carried out once a year.

3.4. Regional workshop on the laboratory diagnosis of TB

To address the acute shortage of expertise and enhance the capacities of technicians/scientists in this sub-region, a workshop on current methods for the diagnosis of TB was organized and took place in Lambaréné from January 12 to 16, 2015. Fourteen technicians and scientists from Cameroon, the Republic of the Congo, and Gabon participated in the workshop within the framework of the Central Africa Networks on Tuberculosis, AIDS and Malaria – CANTAM (http://www.cantam.org). The Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung, BMBF), Germany, sponsored the workshop, which combined lectures and practical sessions (including participant hands-on activities) on diverse topics including state-of-the-art culture techniques and microscopic and molecular diagnostic methods.

3.5. Understanding the regional clinical TB epidemiology

The (co)prevalence of TB and HIV in the Central African region was reviewed[13] and an epidemiological study of TB was conducted in Lambaréné (the PanEpi study) between 2012 and 2013,[14] during which 201 adult and paediatric TB patients were enrolled with active follow-up; 66% had bacteriologically confirmed TB and 95% had pulmonary TB. The HIV co-infection rate was 42% in adults and 16% in children. *Mycobacterium tuberculosis* and *Mycobacterium africanum* were identified in 82% and 16% of 108 culture-confirmed TB cases, respectively. Isoniazid and streptomycin yielded the highest resistance rates (13% and 12%, respectively). The MDR-TB rate was 4% (4/91) in new TB cases and 31% (4/13) in retreatment TB cases. Treatment success was achieved in 53% of patients. In TB/HIV co-infected patients, the mortality rate was 25%. It was aimed to better understand local perceptions and health-seeking behaviour with regard to TB in the region,[15] and along the line, obstacles in the care for paediatric TB cases[16] and problems with first- and second-line drug supplies were identified and discussed.[17]

3.6. Understanding the regional molecular epidemiology of drug-resistant TB

Furthermore, this laboratory is contributing MTBC strains to a multi-centre study in collaboration with the Molecular and Experimental Mycobacteriology Research Centre Borstel, Borstel, Germany to gain an insight into the distribution and spread of MTBC lineages across the African continent, as well as the emergence and transmission of MDR strains in the participating countries. Of interest, MTBC samples were collected from 2011 onwards and sent to the Research Centre Borstel, Germany for DST and classical molecular genotyping (24-locus MIRU-VNTR (mycobacterial interspersed repetitive unit–variable number tandem repeats) and spacer oligonucleotide typing). In addition, all strains were analyzed by whole-genome sequencing (Illumina MiSeq and NextSeq 500). The preliminary results indicate that of 346 isolates, 9.5% were already MDR and 72.7% of the MDR strains are grouped into one cluster of strains (Beckert et al., unpublished). The consortium is currently developing single nucleotide polymorphism (SNP)-based PCR assays with the ultimate goal of enabling the monitoring of MDR to prevent the undetected spread of MDR-TB at its partner sites in Africa.

3.7. Improved patient management including drug-resistant TB

Clinical research is an essential tool to build patient care capacity and to foster staff development. To that end, since the laboratory became operational, clinical studies have been conducted on the prevention of early mortality by presumptive TB treatment in HIV-infected patients initiating antiretroviral therapy (the PROMPT trial),[18] and on the immune reconstitution inflammatory syndrome among HIV-infected patients starting antiretroviral therapy in Gabon (the IRIS study).[19]

Following the identification of MDR-TB cases in the Lambaréné area, this medical emergency was responded to by building drug-resistant TB treatment capacity at Georges Rawiri Hospital in Lambaréné, in close collaboration and with the support of the MoH and WHO. All patients are included in a prospective cohort study, with the 'Bangladesh regimen' forming the blueprint of the

treatment strategy. Following culture conversion, the first cohort of four MDR-TB patients has recently been discharged from the hospital, with more patients now receiving treatment (Ateba-Ngoa, unpublished).

4. Lessons learned

Close collaboration with the relevant stakeholders within a country is essential for forging a successful alliance.A South–North partnership can be an excellent platform to launch, and sustain, a high-level TB laboratory.An appropriately equipped and adequately staffed laboratory forms the nucleus of improved understanding of the local epidemiology and provides the basis for good patient care, extending from drug-sensitive to drug-resistant TB.

5. Conclusions

Sub-Saharan Africa continues to bear a disproportionately high burden of TB disease, and the lack of adequate infrastructure is hindering TB control efforts. Gabon, for instance, continues to rank amongst the countries with the highest TB incidence per population rates in the world, and at the same time has weak laboratory infrastructure and manpower to support TB epidemiological surveillance, diagnosis, and treatment. Over the past few years, we have established a TB diagnostic facility from scratch at the Medical Research Centre (CERMEL) in Lambaréné, the centre of Moyen-Ogooué Province in Gabon, despite constrained resources, and this has enabled progress in understanding the regional epidemiology of TB and has allowed improved and expanded treatment. The country-wide role of the laboratory in TB diagnosis, treatment (including monitoring), and control cannot be overemphasized, and this may serve as a model for other smaller countries with a high TB incidence, which must be taken into account when seriously gearing up towards implementing the End TB strategy on a global scale.

Funding: CERMEL is a not-for-profit private institution, depending entirely on obtaining competitive funding. We succeeded in securing competitive research grants from the following institutions/funding agencies: The University of Tübingen, Germany; The European and Developing Countries Clinical Trial Partnership (EDCTP) through PanACEA (Pan African Consortium for the Evaluation of Antituberculosis Antibiotics) (grant number IP.2007.32011.012); CANTAM (grant number CB.2007.41700.006); The Federal Ministry of Education and Research (BMBF) (grant number 01DG14012); The German Centre for Infection Research (DZIF) (grant numbers TTU 02.805 and TTU 02.805_01); and the UBS Optimus Foundation. In 2015, we supported The Gabon PNLT in a successful application to The Global Fund to treat MDR-TB in Gabon, for which we are a sub-beneficiary (http://www.theglobalfund.org/en/portfolio/country/grant/?k=b6ca5f6e-3381-48da-a278-03fc09da1a15&grant=GAB-T-MSPS). The funders had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit this manuscript for publication.

Conflict of interest: None.

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