TDR-SGS 16 -29

Prevalence and Antimicrobial Resistance of Mycobacterium tuberculosis among Citizens, non-national Resident and Refugees in Lebanon: a nationwide study (PAR-MTB)

Report

Pr. Monzer HAMZE

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Introduction

Tuberculosis remains one of the most fatal diseases in the world. According to the latest data of the World Health Organization (WHO), there were 10.4 million new cases of tuberculosis (TB) and around 1.7 million people died from the disease in 2016 worldwide. The WHO major goal of ending the TB epidemics by 2030 is threatened by the increasing numbers of multi drug resistant (MDR) estimated at nearly 490,000 in 2016 (WHO, 2017a).

Lebanon hosts nearly 1.1 million UNHCR registered Syrian refugees and 500,000 Palestinian refugees, in addition to another 500,000 of non-registered Syrian refugees (MoPH, 2017) .

Lebanon migrant workers also contribute to the incidence of TB among the Foreigner non Syrian refugees (FNSR), linked to probable reactivation of remotely-acquired latent tuberculosis infection (LTBI) following migration from low/intermediate-income high TB burden settings (Pareek *et al.*, 2016)

Lebanon is an upper-middle income with low burden TB country with in estimated incidence of 740 cases (12/100 000) and mortality rate of 1/100 000 in 2016 according to WHO (WHO, 2017b).

According to the National TB program (NTP), the treatment success rate is much higher in the Lebanese population (around 90%) than among the non-Lebanese patients of which 50% leave the country before the completion of their treatment. Cases of Syrian patients with treatment interruption due to insecurity in Syria were identified. These conditions favor the risk of emergence of drug resistance (MoPH, 2017).

The above uncertainties around the estimated incidence and notified cases reflect existing diagnostic gaps.

Study objectives

The aim of this project is to gain a first more comprehensive overview of the epidemiological situation of TB in Lebanon. More specifically, the main objectives are to establish more solid diagnostics of TB cases and measure the actual incidence of drug resistance in the country by using phenotypic and molecular testing of samples collected nationwide.

Materials and Methods

Ethical Statement

This national survey was approved by the ethical committee of the Doctoral school of sciences and technology - Lebanese University, the members of the Lebanese NTP and the Ministry of Public Health (MoPH). Informed consent was obtained from patients included in the study.

Study design and study population

From June, 1st, 2016, collection of the samples from all Lebanon except the Northern region (samples from this region are directly collected by Laboratoire Microbiologie Santé et Environement –LMSE) is centralized in the TB reference center in Beirut and then transferred to LMSE with the help of the International organization for migration (IOM), as explained below. Before the samples are transferred to LMSE, the reference center ensures a significant proportion of TB laboratory services including microscopic examination and a molecular test for *Mycobacterium tuberculosis* (*M. tuberculosis)* identification and detection of common mutations in *rpoB* gene, associated to rifampicin resistance (GeneXpert). Regardless of the molecular results, thrice a week, the samples are transported to LMSE.

Microscopic, phenotypic and molecular testing in Lebanon

Microscopic examination was done using Ziehl-Neelsen coloration with a fast cold staining kit (Kit Quick-TB, RAL diagnostics) for the detection of Mycobacteria. Samples received from the TB reference center in Beirut were subjected to reexamination for confirmation.

All microscopy positive and negative samples were decontaminated using NaOH together with N-acetyl-cysteine and cultured using solid (Lowenstein-Jensen LJ) and liquid (BBL MGIT Mycobacteria Growth Indicator Tube) cultures.

Positive primary cultures were detected after 1-4 weeks on liquid media and 6-12 weeks on solid media. To confirm identification of *M. tuberculosis* complex (MTBC), Anyplex MTB/NTM Real-time detection Kit (Seegene) was used.

Upon confirmation of MTBC, phenotypic drug susceptibility testing (DST) was performed by diluting the primary isolate and then inoculating it into drug containing and drug free MGIT media. DST was performed with the BD BACTECTM MicroMGIT Reader by following the manufacturer's recommendations, which included using the BACTEC MGIT SIRE kit (Becton Dickson, Sparks, MD, USA) for isoniazid (INH), rifampin (RMP), streptomycin (SM), and ethambutol (EMB) with a critical concentration of 0.1 mg/L, 1.0 mg/L, 1.0 mg/L and 5 mg/L respectively. In cases of resistance to INH and RMP, testing was performed for the following second-line line drugs: Amikacin (AK), Kanamycin (KM) and Levofloxacin (LVX) with a critical concentration of 1 mg/L, 2.5 mg/L and 1.5 mg/L respectively.

Patient data collection

A standardized form was used to collect patient data, comprising patient age, gender, nationality, residency, status of the disease, family history (Annex). Data were anonymized for subsequent analysis.

Results

Samples, cultures and study population

In order to have a general image on the burden of TB in Lebanon, a nationwide collection was started from June 2016 on. As of November 2017, 1239 samples from 1069 different patients were collected, of which 370 were bacteriologically and molecularly confirmed as MTBC and 36 as NTM (table 1).

Table 1: Number of notified TB cases from January 2016 to November 2017

Collection period	**June 2016- November 2017**
Bacteriologically confirmed MTB	370
Bacteriologically confirmed NTM	49
Negative cultures	496
Culture in progress	152
Samples excluded from the study	2
Total	1069

After exclusion of second samples received from same patients, the study population of confirmed MTBC cases from June 2016 to November 2017 thus includes 370 patients, of which 52.16 % % were female.

In terms of patient residence among the eight Lebanese governorates (Akkar, North Lebanon, Mount Lebanon, Beirut, South governorate, Baalbak-El Hermel, Beqaa and Nabatiyeh), Mount Lebanon was the most represented, enclosing 32.7% of TB patients

and this due to high number of population in that area specially the presence of migrant workers. To note, 2.4% of TB patients resides in jail.

Lebanese nationals represented 29,2% of the total TB cases. Among the foreign-born patients, 26.8% were Syrians, 2.7% Palestinians while 37.8% were classified as Foreigner non-Syrian refugees (FNSR). Finally, 3.5% of patients whom the data was not provided.

Drug resistance

Drug susceptibility test was performed on all bacteriologically confirmed *M.tuberculosis.* Out of the 330 confirmed MTBC cases, 232 DST results are already available and the rest is in progress. Among the cases with available results, 80.2% of total TB patients were susceptible to all 4 first line drugs, 3.9% have mono-resistance to isoniazid, 0,4% with mono-resistance to rifampicin, and 9.5% mono-resistance to streptomycin. Seven cases were resistant to isoniazid and streptomycin.

One case of MDR was resistant to rifampicin and isoniazid only, while an additional 2 were resistant to all 4 first-line drugs. The latter patients were of Syrian, Lebanese and Ethiopian nationalities, respectively.

In addition, three cases of extensively drug resistant (XDR) were detected by phenotypic drug susceptibility testing. The latter patients were of Syrian, Sudanese and Ukrainian nationalities.

Discussion

While the TB incidence decreased in Lebanon until 2011, the NTP reported a 27-percent increase in notified cases starting from 2012 (Cousins, 2014) . According to WHO and MoPH, compared to the preceding year, the number of cases among the non-Lebanese raised from 200 to 300, which was attributed to the arrival of Syrian refugees after the war started in Syria in 2011 (WHO and MoPH, 2016). In addition, the number of cases notified among Lebanese citizens also increased from 298 in 2011 to 330 in 2012. This

trend was subsequently confirmed, with a dramatic increase in notified TB cases in Lebanon in 2014, 2015 and 2016, peaking at 681, 664 and 679 respectively. An expansion was especially seen among the Syrian refugees is marked with 109, 139 and 147 notified cases respectively (MoPH, 2017).

In order to have a better overview on the real TB situation in Lebanon, the study was done on a national level. All samples received from the 9 TB centers in Lebanon were tested (microscopic examination, molecular testing and culture). From the total samples received to LMSE, only 370 were bacteriologically confirmed as *M. tuberculosis.* Looking to patient's nationalities, the highest proportion was among the FNSR (37.8%) and this is due to the influx of migrant workers to the country. 99 TB cases were among Syrian refugees and non-refugees.

Most of the cases came from patients that resides in Mount Lebanon (32.7%) as well as in a previous study by Hamze et al (Hamze *et al.*, 2010).

Moving to drug resistance profiling, out of the 3 MDR cases, 1 was diagnosed as a new TB case and the remaining 2 were previous TB patients. Same for the XDR patients.

Only 1 cases of mono resistance to rifampicin was phenotypically confirmed.

According to other studies conducted in Lebanon, the overall MDR-TB prevalence was variable: with 25% in 2012-2013 (Araj *et al.*, 2016). An addition, an earlier study with 6 MDR cases were phenotypically confirmed between July 2003 and October 2005 (Rahmo *et al.*, 2012).

There is a lack of precision on the real incidence of TB cases and the anti-TB drug resistance that might be due to an incomplete diagnostic coverage in Lebanon over the past years.

Conclusion and recommendations

According to the current results, this first nationwide study lead in Lebanon indicates that the incidence of drug resistance remains relatively low for the moment. Sample and culture collection will be continued in order to see if this trend is maintained or tends to increase over time.

As elsewhere, for obtaining a complete diagnosis including drug resistance profiling, 4-6 weeks are needed on average in our setting using classical liquid based identification and DST. Albeit faster, the available molecular tests (GeneXpert MTB/RIF and AnyPlex) provide limited information for predicting sensitivity and resistance to first and second anti-TB drugs. In addition, these tests are not able to differentiate members of MTBC and identify NTM species.

Therefore, the evaluation of a new diagnostic assay need to be performed as a potential future alternative to at least part of this current, slow and costly diagnostic algorithm.

References

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Annex

UNIVERSITE LIBANAISE

Ecole Doctorale des Science et Technologie

Plateforme Azm pour la Recherche en Biotechnologie et ses Applications

Laboratoire de Microbiologie Santé et environnement

Information concernant un Tuberculeux

Code CMUL:.....**Référence:**.....

1) Informations concernant le patient:

Nom:…… P rénom: …….

Age:…… A dresse: ……..

Sexe: …… Médecin traitant : ……

Nationalité*: ……

* Si Syrien : Refugié ☐ Oui (date d'entrée : …../…./….)

☐ Non

Date d'apparition des symptôme :……..... A mbulant / Hospitalisé

Nature de prélèvement : …….

Contact avec une personne atteinte de tuberculose

☐ Oui → Famille / Hors famille

→ Avant / Simultané

☐ Non

Statut de la maladie

☐ Tuberculose récente

☐ Rechute

☐ Suspicion

☐ Contrôle sous traitement

☐ Contrôle après arrêt du traitement

Type de tuberculose

☐ Pulmonaire

☐ Extra – pulmonaire (Préciser : ……..)

2) Analyses effectués au laboratoire:

- **Test à la tuberculine :** IDR : Induration : ……..
- **Examen microscopique :** Résultat du Ziehl : Positif / Négative (Grade :…….)