ORIGINAL ARTICLE

Mycobacterium Tuberculosis Detection in Pulmonary Specimens from Prospective Immigrants; Laboratory Data from Pakistan

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Abstract

Introduction
Pakistan is a high tuberculosis (TB) burden country. As such, pre-immigration TB screening for Pakistani travelers is required by several countries. Positivity rate amongst this group has not been reported previously.

Objectives
To assess the culture positivity rate of *Mycobacterium tuberculosis* (MTB) in pulmonary specimens received from prospective immigrants in Pakistan.

Method
Data for MTB culture requested during 2008-2016 from prospective immigrants were retrieved from laboratory database. TB culture request was based on physician's discretion. Identification and cultivation of MTB was performed at AKU laboratory using standard methods.

Results
A total of 172 cases, for which TB smear and culture was requested were included. MTB culture was positive in 13/172 (7.5%) cases of which 5 were smear positive and 8 smear negative. One case was smear positive and culture negative. Three additional cases were positive for non tuberculous mycobacteria (NTM).

Conclusion
Our data shows a high culture positivity rate in prospective immigrants screened for TB. Use of rapid diagnostic tests such as Xpert MTB/Rif in immigrants' pre and post migration from high TB incidence areas may lead to an early diagnosis and treatment.

Introduction
Tuberculosis (TB) remains one of the world's "deadliest" communicable diseases.[1] In 2015, globally, an estimated 10.4 million people developed TB, 60% of which belonged to India, Indonesia, China, Pakistan, Nigeria and South Africa.[1] Although TB exists mainly in high-burden developing countries (HBCs), an increase in cases in low incidence countries has also been observed.[2] Factors responsible for this increase are HIV co-infection, immigration from TB HBCs, multidrug resistant TB (MDR-TB) and overcrowding within poor communities of large cities.[3] Of these, immigrants from TB HBCs, especially in the first few years have a significant contribution.[4] In the United States and Canada, as well as in various European countries, over 50% of notified TB patients have been reported to be foreign-born,[5] with reactivation of latent TB.[6] Similar reports from European countries such as Denmark, Holland, Sweden, the United Kingdom and Switzerland suggest that foreign-born cases tend to have higher TB incidence; 100/ 100,000 as compared to native population; 15/100,000.[3] These higher rates of imported TB are making TB control more difficult in developed settings.[7]

The findings of a recent meta analysis by Aldridge RW *et al* regarding the pre-entry screening programmes showed that the prevalence ranged from 19.7 cases to 335.9 cases per 100000 cases.[8] However the prevalence was high in immigrants from HBCs. Another meta-analysis by Chanon HY *et al* on the high risk TB population after immigration showed that the cumulative incidence of TB was 2794 per 100000 persons.[9] The rate was higher in the population found to be at higher risk of TB at pre-entrant screening. These meta-analyses conclude that the prevalence and incidence of TB is high in both pre and post immigration population from HBCs and emphasizes on the importance of screening at both levels. In addition to the increasing incidence and prevalence of imported TB, the number of MDR and XDR cases is also increasing notably from Asia and Africa.[10] Immigrant screening for TB is a major component of immigration policies of many countries. The World Health Organization (WHO) recommends symptoms questionnaire or sputum smear for screening and chest X-ray for diagnosis in smear negative cases.[11] Centers of Disease Control and Prevention (CDC), USA recommends PPD skin test or Gamma interferon release assay (IGRA) followed by chest X-ray and TB culture and susceptibility testing.[12]

TB is a major health problem in Pakistan with an estimated

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incidence of 510,000 and a prevalence of 270 per 100,000 population with a 4.2% new of MDR-TB cases.[1,13,14,15] It is estimated that 51% of cases are concentrated in the province of Punjab, followed by 23% in the province of Sindh, 15% in the Khyber Pakhtoonkhwa, and 3.5% in Baluchistan, with the remainder being distributed within the tribal and northern areas and in Azad Kashmir. Therefore, every immigrant travelling from Pakistan has to go through multiple TB screening tests at the local immigration clinics. The proportion of immigrants in which TB is diagnosed during screening is not known.

In this study, we determined the frequency of isolation of *Mycobacterium tuberculosis* (MTB) in pulmonary specimens submitted to our laboratory from immigrants undergoing TB screening.

Material & Methods

Setting

Our study was conducted at a Clinical Microbiology Laboratory, Karachi, Pakistan, that receives approximately 20 specimens/year from immigrants for MTB culture. These requests are generated by the prescribing physicians working for or referred from immigration screening programs on the basis of positive TST or significant findings on chest radiographs.

Study design

Cross-sectional study

Specimen selection

All the pulmonary samples (sputum or bronchial washings) from immigrants undergoing TB screening identified from laboratory database were included. Data was retrieved from 2008-2016 and included age, gender, year of isolation, specimen type, positivity and susceptibility pattern against isoniazid, rifampin, streptomycin, ethambutol, ethionamide, ofloxacin, capreomycin, amikacin and kanamycin. Duplicate specimen from the same patient was excluded.

Microbiological methods

Isolation of Mycobacterium tuberculosis (MTB)

Acid Fast Bacilli (AFB) smear: AFB smears were performed using Kinyoun's and Auramine O stains. The following quantitation was used: 1-19/40 fields AFB – Rare, 20-199/40 fields AFB seen – (1+), 5-50/field AFB – (2+), >50/field AFB (3+).

AFB culture

TB culture was performed using Lowenstein-Jensen (LJ) (Remel™, Thermo fisher scientific™, Kansas, USA), Mycobacterium Growth Indicator Tube (MGIT), (BD, Thermofisher scientific, Dublin, Ireland) and Middlebrook 7H10 agar (BD, Thermofisher scientific) for all of the specimens. MTB was isolated from clinical specimens using standard microbiological procedures, and it was identified by typical colony morphology and PNB (para-nitrobenzoic acid) sensitivity. In addition to these standard identification procedures pigmentation and rate of growth were also observed.

Antimicrobial susceptibility test

Agar proportion method was used to determine susceptibility against isoniazid (1 µg), rifampin (5 µg), streptomycin (1 µg), ethambutol (25 µg), ethionamide (25 µg) (BD, Thermofisher scientific™, Dublin, Ireland), ofloxacin (10 µg) (Oxoid™, USA), capreomycin (4 µg/ml), amikacin (5 µg/ml), kanamycin (4 µg/ml) (Sigma Aldrich®). Middlebrook 7H10 agar supplemented with 10% oleic acid albumin dextrose catalase (OADC) was used for susceptibility testing.[16] MTB H37Rv was used as a control.

Ethical approval

This study has been exempted from ethical approval by the institutional ethical review committee (ERC number: 4067-Pat-ERC-16). Due to social and financial impact of a positive TB result on prospective immigrants' future, patients were not contacted and no clinical information was collected. This study is based on laboratory data only. The results were reported by the laboratory to the prescribing physicians.

Results

A total of 172 immigrants were recruited during the study period. These included 87 male (50.8%). Mean age of study participants was 53 years (SD±20.5). Pulmonary specimens included were mostly sputum 168 (97.6%) and bronchoalveolar lavage 4 (2.3%). In all those patients, who had a positive TST or radiological findings consistent with TB and could not produce sputum, bronchalveolar lavage was done. Of these 172 immigrants, 13 were positive for MTB. These included 5 smear positive culture positive cases and 8 smear negative culture cases. Additionally, one sputum specimen was smear positive and culture negative however, the patient had a prior history of TB treatment. A total of 13 cultures were positive for *Mycobacterium tuberculosis* (Table 1). Three cases grew NTM. On susceptibility testing 10 (77%) cases were both isoniazid and rifampicin susceptible, 1 (7.6%) case was isoniazid monoresistant, 1 (7.6%) MDR and 1 (7.6%) XDR. Xpert MTB/Rif data was available for four culture positive cases and of these three were Xpert MTB/Rif positive and one was negative.

Discussion

A rising trend of imported TB is being observed worldwide. In US alone it has been observed that the rate is 13.4 times higher in foreign born as compared to the general population.[17] TB culture plays an important part in immigrant screening policies, especially in high risk and vulnerable population.

Our study reports 7.5%culture positivity rate in pulmonary specimens from asymptomatic individuals who were screened for immigration purposes. As observed in the population based National TB prevalence survey conducted in Pakistan from

Table 1: shows the frequency of smear and culture positivity of TB in study population and their susceptibility pattern

Cases	Smear results	Culture results	Susceptibility pattern
Case 1	Positive	Positive	Both rifampicin and isoniazid susceptible
Case 2	Positive	Positive	Both rifampicin and isoniazid susceptible
Case 3	Positive	Positive	Both rifampicin and isoniazid susceptible
Case 4	Positive	Positive	Both rifampicin and isoniazid susceptible
Case 5	Positive	Positive	XDR TB †
Case 6	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 7	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 8	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 9	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 10	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 11	Negative	Positive	Both rifampicin and isoniazid susceptible
Case 12	Negative	Positive	Isoniazid monoresistant*
Case 13	Negative	Positive	MDR TB**

*Mono-resistance: resistance to any one first-line anti-TB drug
**Multidrug resistance: resistance to both isoniazid and rifampicin
†Extensive drug resistance: Multidrug resistance alongwith resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin).[25]

2010 to 2011, total number of smear positive cases were 233/8521(2.7%) and 278/8521 (3.2%) culture positive cases. The survey also showed that 39% of the 207 definite AFB smear positive cases had no cough but abnormal radiological findings. The findings of the prevalence survey are consistent with findings of our study, both indicating high prevalence of TB in an otherwise asymptomatic population of Pakistan.[13]

In the health and demographic survey of Pakistan 2012-2013, 33 percent of males and 10 percent of females migrated to other countries, 29 percent of which was urban population. The most common reason for this immigration were mostly work and educational opportunities. In case of females, marriage was the commonest factor identified.[18] Our study lacks clinical and the demographic data of the immigrants included.

Over the past three decades, the TB notification data from industrialized countries has been variable but the outcome is same: decreased incidence in native population and an increasing incidence in foreign population. However the data from various studies suggest that the diagnosis of active TB was lower at the time of immigration.[19] It was also seen in Taiwan that the average annual TB notification rate in the foreign-born population was greater than that in the Taiwan-born population (94.0/100,000 vs. 72.0/100,000). 73% of these foreign born belonged to mainland China and Vietnam.[20]

In a study conducted in Turin, Italy from 1991 through 2010, a total of 27,358 socially marginalized immigrants attended the screening program out of which a total of 557 (2%) were definite cases of active TB. 75% of this population belonged to high TB burden countries. Similarly, prevalence of TB in 2.7% in immigrants was observed in a study conducted in an otherwise low TB incidence area in Western Europe.[4]

Culture still remains the gold standard for diagnosis of active tuberculosis. The main drawback of culture is that it takes 2-8 weeks that causes delay in diagnosis and immigration process. AFB smear is also rapid and inexpensive but it has low sensitivity of 64% and specificity of 98%.[21] Rapid molecular methods should be evaluated for inclusion in the screening policies.

Conventional PCR assay is known to have 77.3% and 99.9% sensitivity and specificity.[22] Comparatively, sensitivity of the Xpert MTB/Rif in smear and culture-positive pulmonary specimens was found to be 100% and in case of smear-negative pulmonary specimens, the sensitivity and specificity of the test is 74.2% and 98.3% respectively.[23] Xpert MTB/Rif will not only aid in rapid diagnosis but also in early start of therapy preventing transmission and emergence of MDR TB. As suggested by Samaraweera S *et al* addition of Xpert MTB/Rif in the algorithm of immigration screening policy may help in identifying cases early.[24]

Our study does not represent the cumulative TB positivity rate from all the immigrants from Pakistan. We have only included the samples of individuals referred by the physicians to our laboratory after initial screening. The proportion of cases that are referred to other laboratories is not known to us. The rate of positivity reported therefore might be an overestimate.

The high positivity rate highlights an urgent need to estimate the actual burden of active TB in this population. Further multicenter studies are required to determine the cumulative positivity rate in this population. This will help to make a policy to screen these individuals effectively.

In summary, our study finds that there is a high culture positivity rate (7.5%) in asymptomatic individuals who underwent screening for immigration purposes. This was a single center study and we suggest further multicenter population based studies to determine the actual culture positivity rate in this population. There is also a need to evaluate the role of rapid diagnostic methods such as Xpert MTB/Rif as they will lead to early diagnosis and management of these individuals.

Declarations

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Disclaimer

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i. 45th Union World Conference on Lung Health, Barcelona, Spain, 2014. The abstract has been published in the abstract book.
ii. 38th Annual PAP Conference/ 3rd Joint Conference of the Societies of Pathology in Collaboration with the Royal College of Pathologists & British Association of Pakistani Pathologists, Lahore, Pakistan, 2015

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