

Performance Characteristics of GenXpert MTB/rif in Armenia

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Abstract

Global TB control efforts have been severely hampered by the lack of diagnostic tests that are accurate, simple to use and can be applied at the point of clinical care. This has been further compounded by the widespread inability to test for drug resistance. We estimated the performance Characteristics of GenXpert MTB/rif of an active case finding program in Armenia where TB notifications is high. Armenia remains in the top ten out of the 27 high multidrug-resistant (MDR) TB burden countries in the world. According to the latest representative 2007 Drug Resistance Survey (DRS) 9.4% of new cases and 43.2% of previously treated cases were multi-drug resistant. Of these, 4% were extensively-drug-resistant tuberculosis (XDR/TB) cases.

Detection of MDR-TB in Armenia remains at 37%. Despite of the fact that in 2013 all detected MDR-TB cases were enrolled in MDR-TB treatment, treatment success rate among 2011 MDR-TB cohort remains 51%, with 26% of patients defaulted therapy mostly due to labour migration, as the main reason of poor outcomes. Moreover, this can be accomplished using unprocessed sputum samples as well as clinical specimens from extrapulmonary sites. We review the development of this assay, its evaluation within the laboratory, its utility among adult and pediatric TB suspects, its use as a screening tool for HIV-associated TB and studies of its implementation at the central level. The Xpert® MTB/RIF assay is a rapid molecular assay that can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of TB and simultaneous assessment of rifampicin resistance to be completed within 2 h.

Keywords: Drug resistance; Epidemiology; Rifampicin; *Mycobacterium tuberculosis*; HIV

Introduction

With 8.7 million incident cases of tuberculosis (TB) and 1.4 million deaths estimated in 2011, TB is a leading cause of morbidity and mortality worldwide. However, public health services globally reported only 5.8 million (66%) of the estimated TB cases in 2011. Moreover, less than 5% of notified TB cases were tested for drug resistance [1], which is often diagnosed after prolonged diagnostic delays. Of the 310 000 notified new and re-treatment cases with pulmonary TB estimated to have multidrug-resistant (MDR)-TB in 2011, just under 60 000 (19%) were reported to the World Health Organization (WHO).

The main reasons for these gaps are inadequate diagnostic capacity and an over reliance on chest radiography and/or sputum smear microscopy as diagnostic tools. Patients with HIV-associated TB, those with sputum smear-negative and/or extra pulmonary disease, and drug-resistant TB patients are particularly affected by the failure of microscopy as a primary diagnostic tool. The "classical" diagnosis of HIV-associated and drug-resistant TB is complex, expensive, slow and technically demanding, relying on conventional culture and drug susceptibility testing (DST). The long delay (up to several weeks) required to obtain results has devastating consequences for patients who go undiagnosed (and therefore untreated or inappropriately treated), or are diagnosed too late.

Detecting more cases, detecting them early and rapidly identifying drug resistance are essential for improving individual patient health and avoiding transmission in the community. This requires universal access and early detection using contemporary tools and innovative strategies.

The past decade has seen unprecedented growth in the TB diagnostic pipeline and accelerated efforts to establish the necessary laboratory infrastructure. Nevertheless, although recommended by WHO, the latest generation liquid culture diagnostics and molecular line probe assays for rapid detection of MDR-TB have not yet solved the diagnostic dilemma in most resource-limited settings, largely due to the need for expensive laboratory infrastructure, extensive biosafety precautions and specialized staff. A new rapid test that overcomes many of the

current operational difficulties was recommended for use by WHO in December 2010: the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is an automated, real-time nucleic acid amplification technology run on the multi-disease platform GeneXpert (Cepheid). The Xpert MTB/RIF assay represents a paradigm shift in the diagnosis of TB and drug-resistant TB by simultaneously detecting *Mycobacterium tuberculosis* and rifampicin resistance-conferring mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 h, directly from sputum samples.

During the period of 1990-2006 the TB mortality in Armenia increased from 4.4 to 8.7 per 100 000 of population followed by decline from 2008 and afterwards. In 2012 estimated TB mortality was 6.3 per 100 000 population, this is far below the Millennium Development Goal (MDG) 6 target to halt TB mortality rate compared with 1990s (Figure 1).

According to the WHO Global TB report TB prevalence in Armenia peaked in 2005-2006 reaching to up to 118 cases (range 55-195) per 100 000 population (Figure 2). Since then TB prevalence consistently decreased in average by 6.3% per year reaching 79 (range 37-137) per 100 000 in 2012. This is far higher than MDG targeted prevalence of 14 per 100 000. However, indirectly estimated TB prevalence has large uncertainty.

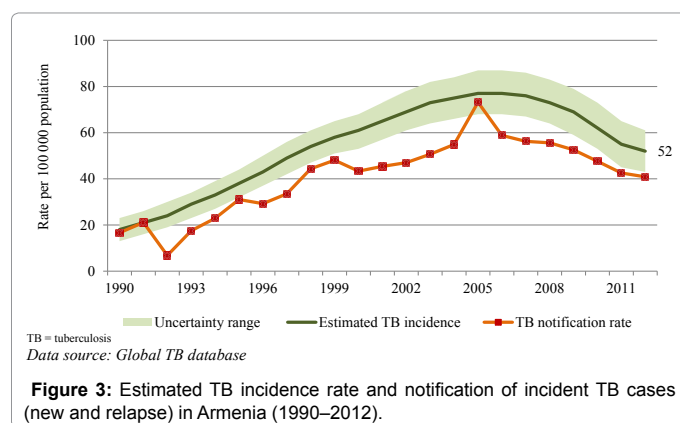
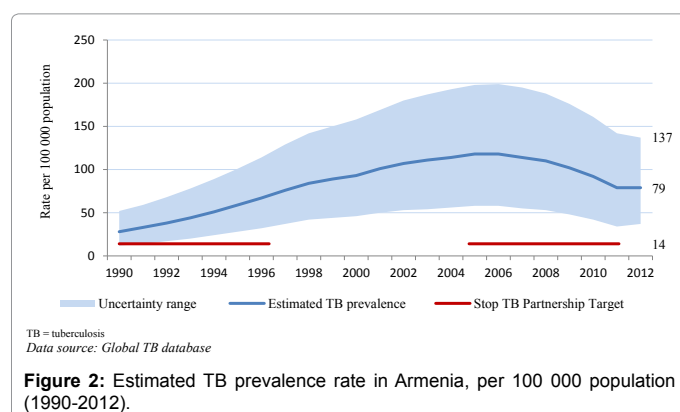
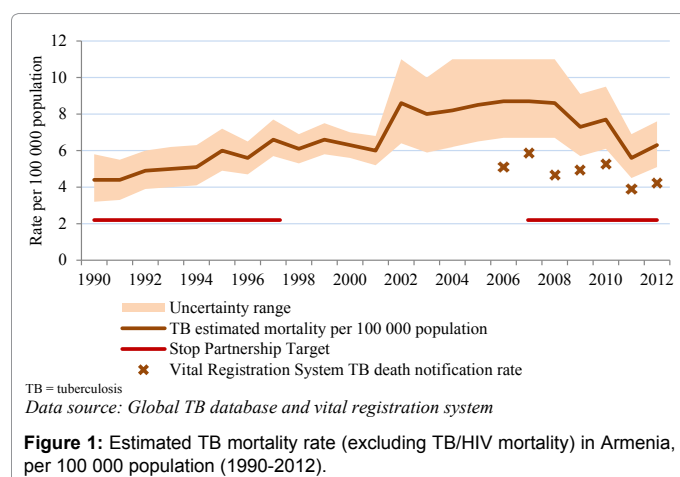
TB incidence in Armenia has sharply increased reaching to its peak in 2005-2006 to level of 77 cases per 100 000 (Figure 3). From 2008 the trend reversed. The gap between notification and estimated incidence across the last years notably reduced. This indicates improvement in

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case detection. Thus, in 2012 the estimated TB incidence is 52 (range: 43-61) and notification of incident TB cases is 41 per 100 000.

At the national level, the number of notified TB cases (all forms) increased from 1 570 cases (equivalent to 51.7 per 100 000) in 2003 to a peak of over 2 300 (77.0 per 100 000) cases in 2005 (**Figure 4**). After 2005, the number of notified TB cases steadily declined. In 2013, in total 1 457 TB cases (48.9 per 100 000) were notified (**Figure 5**). This is the lowest number and level of TB cases recorded over last decade.

The relative numbers of new smear positive TB cases among all new TB cases notably varied over the time (**Figure 5**). Over the last ten years the proportion of smear positive TB cases among all new decreased from 40% in 2003 to 27% in 2013 with some sharp year-to-year fluctuations suggesting about some weakness in surveillance.

The percent of new cases that are extra pulmonary increased gradually from 18% in 2003 to 24% in 2013 again with sharp fluctuation in between 2009-2010 (**Figure 6**).

Figure 7 provides trends in age specific notification rates of new TB cases from 2008 -2012. As it is shown the age specific notification rate consistently decreasing in all age groups except these aged over 65 years. Decrease is notable especially in the young age groups.

Across the last eight years proportion of male TB patients is more or less stable ranging from 71% to 77% among new TB cases (2013 data include relapse cases too). Proportion of males was highest in 2006-2007 then decreased up to 71% in 2010 and again gradually increased in recent three years (**Figure 8**).

Proportion of retreated TB cases among the notified all TB cases in recent ten years notably increased. Overall proportion of retreated TB cases varied from 9% to 41% at national level. There are very sharp year-to-year changes in proportions of notified retreated TB cases between 2004-2005 and 2005-2006 and 2008-2009 indicating weakness of surveillance. Within the recent five years, the notification of retreated cases during the recent five years is free of such sharp variations, suggesting an improvement of surveillance system (**Figure 9**).

Drug resistant tuberculosis

Currently, all TB patients detected in Armenia according to the current national protocol undergo culture testing and drug susceptibility testing (DST) at the NRL. According to the NRL database, in 2013 of 456 patients with positive culture/Xpert MTB/Rif results 438 (96%) had documented DST results. In total 104 (21%) patients among these with DST results were identified as rifampicin resistant/multi-drug resistant (RR/MDR) TB cases (**Figure 10**).

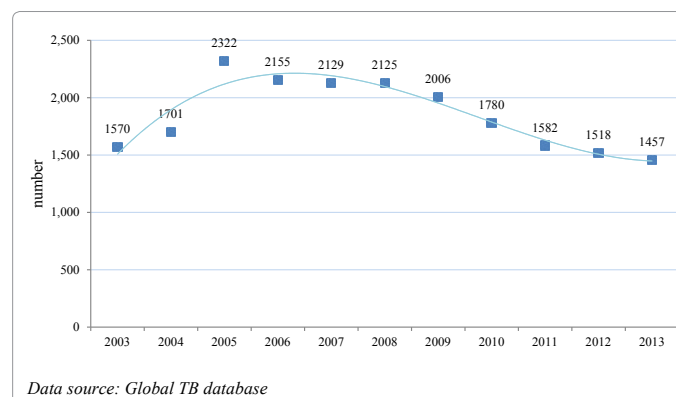


Figure 4: Notification of TB (all forms) number; fitted with polynomial trendline, Armenia, 2003-2013.

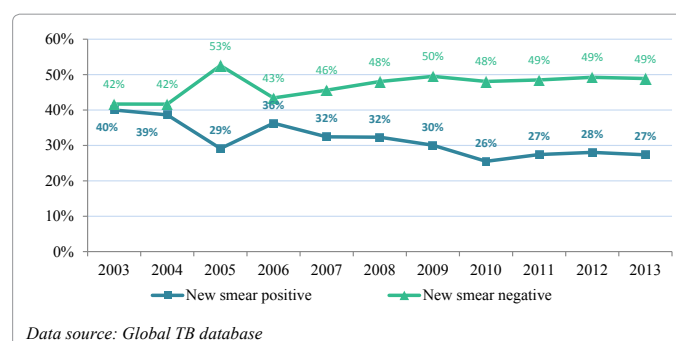
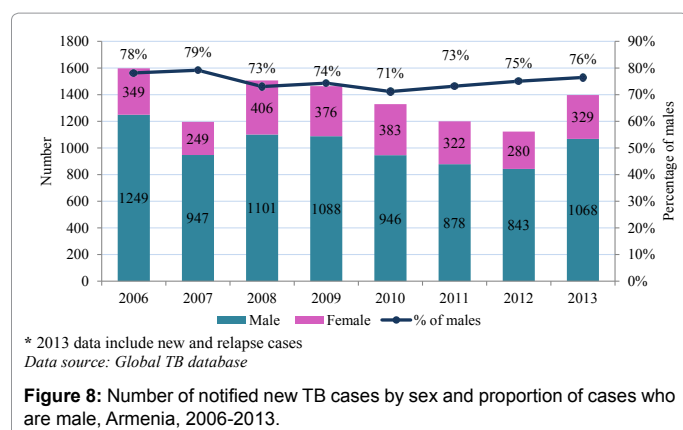
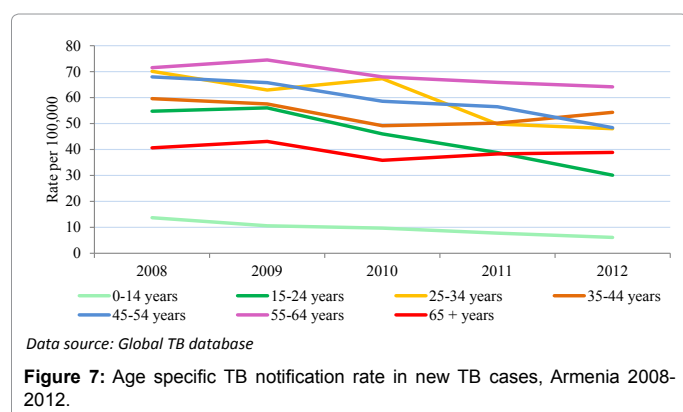
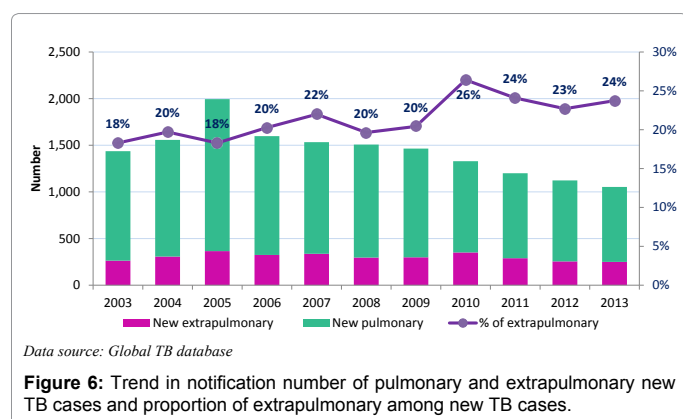


Figure 5: Proportion of new smear positive and smear-negative pulmonary TB cases among all new TB cases.



According to routine drug resistance surveillance results in 2013 the percentage of MDR-TB among new registered cases was 11.6% (43/371) and 34.3% (23/67) among the previously treated cases (Figure 11). The proportion of MDR-TB among new and previously treated cases remains more or less stable. It is important that the total number of MDR-TB patients detected in recent three consecutive years remains around 100. Wide variation of percentage of MDR-TB patients among new and previously treated cases between some consecutive years (2009-2010, 2010-2011) and implausible results in 2011 (MDR-TB among new and retreated are equal) most probably is related to weakness in accuracy of classification of treatment history of patients and most probably double counting. But even accounting this fact it could be concluded that absolute number of MDR-TB patients in recent five years reduced annually in average by 22%. Universal access to MDR-TB treatment since 2010 (even with moderate treatment

success rate), access to early drug resistance testing and administration of drug resistant (DR) TB treatment to prevent further amplification most probably are the main contributing factors for the stabilization of MDR-TB prevalence among TB patients and reduction of absolute number of MDR-TB observed in Armenia in recent years.

According to National TB C

TB/HIV Coinfection: In recent years the number of recorded TB/HIV coinfecting cases in Armenia increased sharply, however, this might be an artefact associated with the increased coverage of HIV testing among TB patients. In general, there has been major progress in implementing TB/HIV interventions, such as testing TB patients for HIV and providing co-trimoxazole preventive therapy (CPT) and antiretroviral therapy (ART) to HIV-positive TB patients. Coverage of HIV testing is high: in recent three year over 95% of TB patients have documented HIV results. Proportion of HIV positive people among notified TB patients in 2011, 2012 and 2013 (when coverage was reasonably high) was correspondingly 3.3%, 5.2% and 4.7% (Figure 12).

Of 67 HIV-positive TB patients notified in 2013 49 (71.6%) patients were enrolled on CPT and the same number of patients started on ART. However, before 2010 both ART and CPT coverage were quite limited. (Figures 13 and 14).

Treatment outcomes

According to the NTP records the treatment success rate among the new smear positive pulmonary TB patients during the last decade decreased from 79% in 2002 to 63% in 2011. Some decrease in treatment success rate is most probably linked to the increased MDR-TB burden

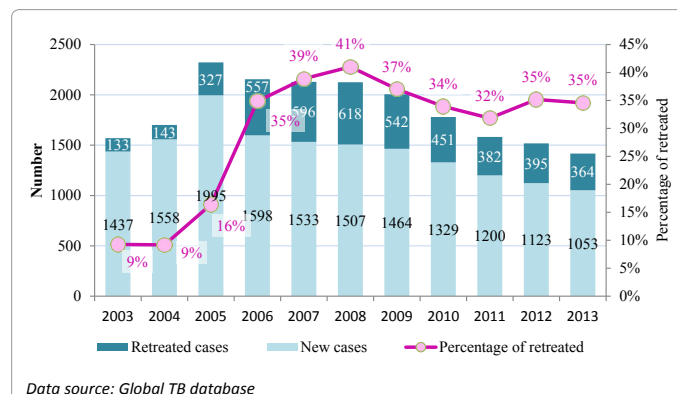


Figure 9: Number of notified new and retreated TB cases and proportion of previously treated TB cases, Armenia 2003-2013.

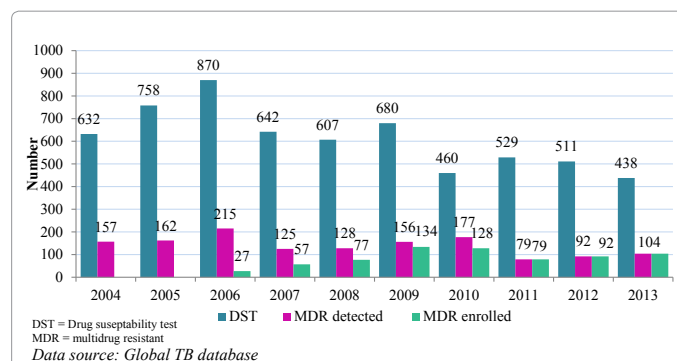
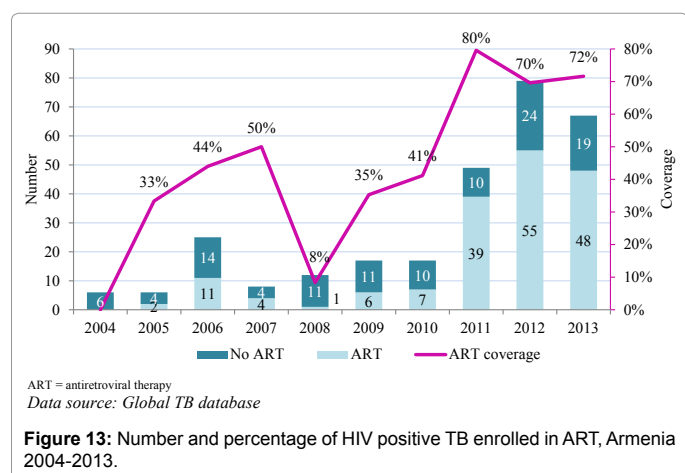
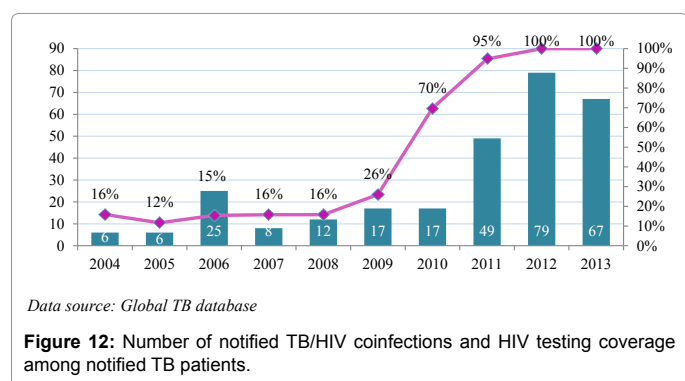
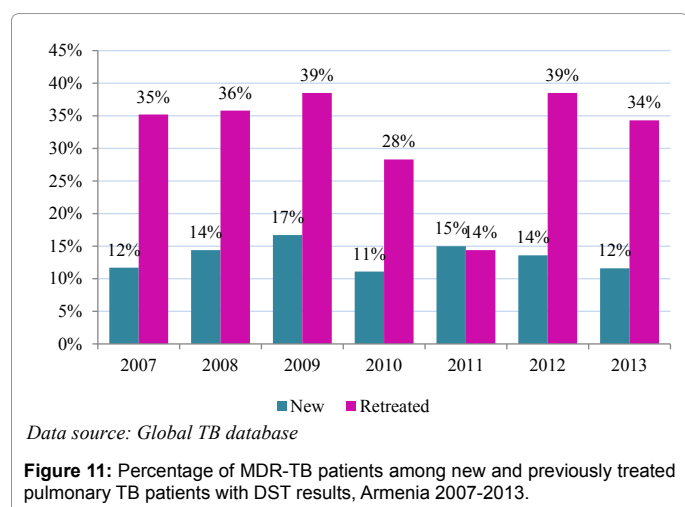


Figure 10: Number of DTS, MDR-TB detection, and treatment in Armenia, 2004-2013.



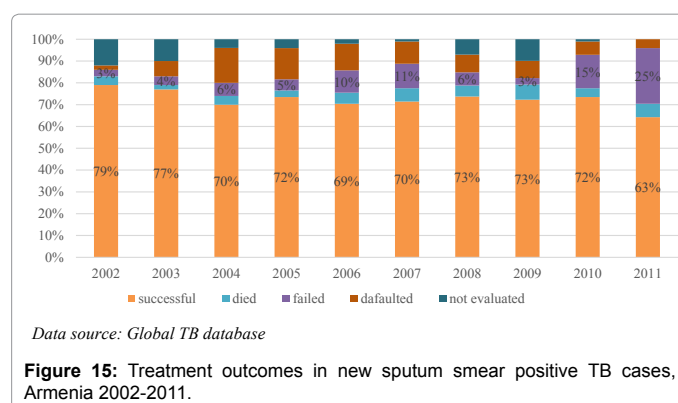
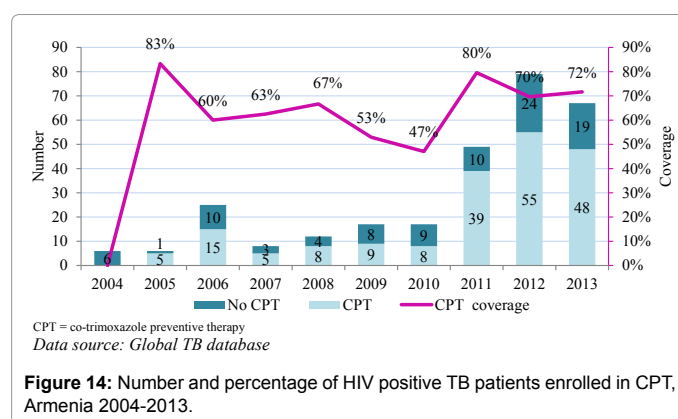
among TB cases as well as increase in percentage of patients with TB/HIV coinfection. However, based on NTP data it is difficult to conclude about real trends in treatment outcomes. Discussion with NTP staff and review of records indicated common practice of nonadherence to recording and reporting guidelines, specifically the patients detected as MDR-TB were not assigned as “treatment failure”. This practice partially was improved in 2010 and 2011 (following the previous mission review), which explains sharp increase in treatment failure in 2010 and especially in 2011 cohorts. At the same time it is noteworthy that proportion of defaulters, for instance, reduced notably (Figure 15).

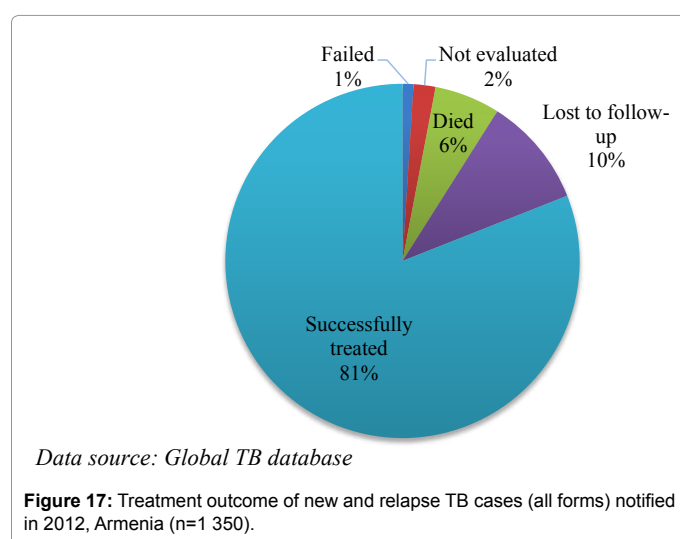
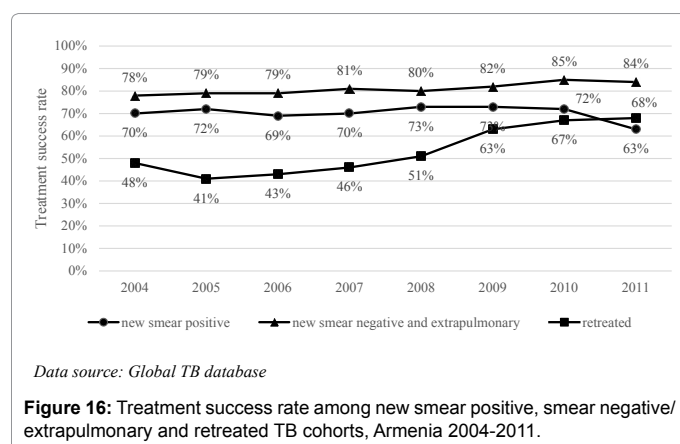
While treatment outcomes of smear negative/extra pulmonary TB cases and retreated cases in 2004 - 2011 improved from 78% to 84%

in smear negative/extra pulmonary TB patients and from 48% to 68% in retreated TB cases (Figure 16). Increase in favourable outcomes of retreated cases is most probably associated with the improved access to MDR-TB diagnosis and DR-TB treatment.

Figure 17 shows the treatment outcomes of all new and relapse cases (bacteriologically confirmed or clinically diagnosed, pulmonary or extra pulmonary) of TB patients notified in 2012 according to the WHO revised recording and reporting framework and case definitions. According to new case definitions treatment, success rate in new regular TB cases is 81% and failure rate is 1% only.

According to latest available data submitted to the WHO (2012), main TB indices showed decline in compare with previous years. Thus, TB incidence (including HIV+TB) was reported 52 (43-61) per 100,000 people with the absolute number of around 1,500 cases registered (1,300-1,800), TB prevalence (including HIV+TB) of 79 (37-137) per 100,000 and TB mortality (excluding HIV+TB) of 6.3 per 100,000, which places Armenia in the list of high-burden countries for TB in the WHO European Region. Case detection (all forms) increased from 74% (63-90) in 2011 to 79% (67-95) in 2012. Similarly as in previous year, TB mostly affected people of economically active age group (15-24 years) and mostly male (4.2 male/female ratio among new cases smear positive). Treatment outcomes of drug-susceptible TB are showing good results and consistently improved in compare with previous year from 62% (cured + treatment completed) to 80.1% (cured + treatment completed) in 2012 among new SS+ cases. Similar for retreatment cases percentage of patients successfully treated (cured + treatment completed) increased from 68.2% in 2011 to 76.2% in 2012. Default to treatment is mostly related to issues of labour migration to Russia. However, despite the successes in managing drug-susceptible TB, drug-resistant tuberculosis is a major challenge to the effectiveness of





National Tuberculosis Program in Armenia, placing the country in the list of 18 high-burden countries for MDR-TB in the WHO European Region. According to the 2007 drug resistance survey, MDR-TB among never treated patients was 9.4% and 43.2% among previously treated cases with 4% of XDR cases. Estimated burden of MDR-TB in 2012 was 9.4 (7-12) and 43 (38-49) among retreatment TB cases. A total 102 MDR-TB cases notified in 2013, 4% were notified as XDR-TB cases.

Armenia has achieved considerable gains in tuberculosis control in recent years and the Millennium Development Goal of halving the global prevalence has already been reached. There was concern, however, about the slowing rate of reduction, partly because of hard-to-reach populations maintaining reservoirs of infection. This has led to a growing interest in active case finding (ACF) program that aim to identify persons with tuberculosis (TB) who might otherwise continue to infect others for a long infectious period before seeking care, if they do so at all. The program can also offer better diagnostic capacities than those in routine services, particularly for asymptomatic and sputum smear-negative patients. Multidrug-resistant tuberculosis (MDR-TB) has emerged as a global health threat. Its situation is worsened by the presence of multidrug resistant (MDR) strains of *Mycobacterium tuberculosis*, the causative agent of the disease. In recent years, even more serious forms of drug resistance have been reported. Given poor treatment outcomes of multidrug-resistant tuberculosis, there is a pressing need for rapid drug susceptibility testing of multidrug-resistant *Mycobacterium tuberculosis* against rifampicin. This review aims at evaluating these rapid assays.

National tuberculosis (TB) programs are often faced with the difficulty of choosing an appropriate rapid assay. Besides field factors such as costs, sustainability and accessibility, test performance in terms of predictive values and turnaround time fundamentally influence the choice of rapid assays. Rapid genotypic tests can yield results within 1 day in either culture isolates or clinical specimens. Although the turnaround time of a rapid phenotypic assay is generally longer than that of a genotypic counterpart by 1-2 weeks in comparable samples, it is probably important to consider test reliability before turnaround time.

Aims and Objectives

This review article has three primary objectives. The first is to describe the dynamic process followed by WHO in policy development for TB diagnostics, using the example of Xpert MTB/RIF assay as a pathfinder. The second is to summarize subsequent evidence on the use of Xpert MTB/RIF, clarify common misconceptions about the technology, and provide perspectives on the role of the assay in improved case detection and care delivery. The third is to summarize the relevance of the technology for TB prevalence surveys and drug resistance surveillance, its impact on case and treatment outcome definitions, and discuss issues around affordability, sustainability, ethics and research priorities.

Case Finding

There is no active TB case finding in Armenia: patients are referred individually to primary healthcare system (general physicians) based on complains of cough or other respiratory symptoms. General practitioners usually start antibacterial treatment with broad spectrum of antibiotics for two-three weeks. If the symptoms continue the patients are referred to TB doctors, who further refer TB suspects for laboratory tests (microscopy, Xpert MTB/Rif/LPA, culture). Usually they prescribe anti-tuberculosis treatment without receiving microbiological confirmation. At the same time, not all physicians are well familiar with TB case definition and main symptoms for suspecting TB, which leads to a number of patients being referred to TB laboratory diagnostics without TB-related symptoms.

Methods

Study design

We conducted the assessment of performance characteristic of Xpert MTB/RIF TB for active case finding (ACF) among presumed TB and HIV adults at the republican TB hospital in Armenia.

Sputum specimen collection and processing

Enrolled subjects were asked to provide two sputum specimens (>2 mL each). Sputum samples were not induced, but collection was directly-observed by nurses. One sputum specimen from each patient was analyzed using GeneXpert MTB/RIF according to manufacturer's instructions [2,3]. From the remainder of the first specimen and the second-day sputum specimen used for mycobacteriological liquid culture. Upon arrival at the laboratory, samples were stored at 4°C for next-day processing, which included decontamination using N-acetyl-L-cysteine and sodium hydroxide and centrifugation before staining a sample from the deposit using. Regardless of smear microscopy results, pellets were inoculated into the liquid medium mycobacteria growth indicator tube (MGIT) 960 culture system (BD Diagnostics, USA). The reference laboratory utilizes BACTEC MGIT 960 liquid culture system to determine drug susceptibility testing (DST) for isoniazid, rifampicin, streptomycin and ethambutol with the confirmation of MDR-TB

(resistance to isoniazid and rifampicin) using Genotype MTBDRplus assay (Hain Lifescience, Germany).

Statistical analysis

Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of the Xpert and their respective 95% confidence intervals (95% CI) were calculated compared to the results of the gold-standard MGIT 960 liquid culture using med calculation statistical software [4].

The following criteria are used for Xpert MTB/RIF testing in Armenia:

- 1) All TB suspects that resulted sputum smear negative irrespectively if the suspect has had a TB diagnosis in the past or not. For each patient 1 sample should be tested by the Xpert MTB/RIF. Only in exceptional cases, a 2nd sample may be tested when the first Xpert MTB/RIF test result was negative. Such exceptional cases include, if there is a clinical worsening situation and if there is an insufficient within Chest X-ray examination and Xpert MTB/RIF (false positive results of Xpert MTB/RIF), (requested by the TB specialist). In this case the Xpert MTB/RIF assay is considered as the second test after sputum microscopy for TB diagnosis and rifampicin resistance.
- 2) All TB suspects living with HIV or presenting strong clinical evidence of HIV infection in HIV prevalent settings. The Xpert MTB/RIF is considered for such suspects as the first test for TB diagnosis (and rifampicin resistance).

All adult patients (>18 years) presenting to the Republican TB dispensary within the 3- month period (2013 and 2014 and categorized as listed in Table 1) with suspected *M. tuberculosis* and who underwent sputum production as part of screening were eligible included in this analysis. Respectively, in total 277 and 343 specimens from 318 and 534 suspected patients routinely sent to the NRL Armenia being acid fast bacilli negative performing GenXpert MTB/RIF. In that period in the NRL only one GeneXpert MTB/RIF and at the end of 2014 two machines were installed. For all 277 and 343 specimens GenXpert MTB/RIF has been performed and simultaneously culture on liquid media (BD MGIT 960) were inoculated. Out of samples in 2013 and in 2014 1.44% and 0.87%. Cerebrospinal fluid (CSF), 4.3% and 7.58% pleural, and 0.36% and 0.6% bronchial lavage have been included. The remaining samples comprised pus (0.36%), bone 0.36% and 0.47%, 2.16% and 2.04% urine, genital tract 0.7% and 2.04%.

Patients in the “not MTB group were diagnosed respectively with pneumonia (26) and (2), pneumothorax spontaneity (4) and (2), acute bronchitis (4) and (1), chronic bronchitis (1) and (2), malignant tumor of lung (6) and (4), tumor of lung and bronchi (4) and (6), carcinoma (1), prolonged fever of unknown origin (1), residual changes after TB (27) and (49), other defeats excluding TB (26) and (14), bronchopneumonia (11) and (15), pleuropneumonia (14), hydrothorax (4) and (2), clinical recovery of TB (1) and (3), emphysema (1) and (3), ovary cancer (1), pleurite (3), abscess of lung (2) and (5), other disease of urogenital tract (2) and (1), BCG (0) and (5).

Of those classified as having definite, probable, or possible MTB were (139/50.18%/year 2013), (232/67, 64 %/ year 2014). In total during the 2014 HIV/TB cases were 179/1540/12%.

Performance of Xpert MTB/RIF compared to MGIT culture. Table 1 describes the accuracy of Xpert MTB/RIF using mycobacterial culture as the reference standard for the various sample types and among the 277 and 343 specimens *M. tuberculosis* positivity determined by Xpert MTB/RIF respectively were 14.4% (40/277) and 28.3% (97/343). The sensitivity and specificity of Xpert MTB/RIF versus MGIT culture on 277 and 343 specimens were 88.89 % (95% CI: 75.93 % to 96.25 %) and 100% (95% CI: 97.96 % to 100.00 %), PPV and NPV 100.00

Diagnosed cases	Sets of data	Resistant Strains (GenXpert)	Susceptible Strains (GenXpert)	Negative (GenXpert)
Infiltrative tuberculosis	47	3	11	33
Disseminated miliary tuberculosis	14	1	6	7
Focal tuberculosis	21	2	2	17
Tuberculosis of Kidney	6	-	-	6
Exudative Plevritis	12	-	1	11
Tuberculosis meningitis	4	-	-	4
Knee-joint tuberculosis	1	-	1	-
Bronchadenitis	1	-	1	-
Suspected having HIV	5	-	-	5
Polyserositis tuberculosis	3	-	1	2
Mesenteric adenitis tuberculosis	1	-	-	1
Peripheral lymph node	1	-	1	-
Peripheral lymph node	1	-	-	1
Vertebral tuberculosis (sample: sputum)	3	-	-	1
Primary tuberculosis complex	3	-	3	-
Primary tuberculosis complex	1	-	-	1
Tuberculosis in liver cirrhosis	1	-	-	1
TB not confirmed	139	-	-	139

Table 1: Study period 2013 QU 4th.

% (95% CI: 91.11 % to 100.00 %), 97.31 % (95% CI: 93.83 % to 99.11 %) respectively, 85.45 % (95% CI: 73.33 % to 93.49 %), 100.00 % (95% CI: 98.49 % to 100.00 %), PPV and NPV 100.00 % (95% CI: 92.38 % to 100.00 %), 96.84 % (95% CI: 93.86 % to 98.62 %) respectively.

The prevalence of TB respectively was just under 14.4% and 11.2% (14% SS+cases, period 2014) using both methods (liquid culture as the gold standard and GenXpert MTB/rif) for the SS-cases. Xpert increased the case detection by 16.04% (47/293, using one sample, period 2014) compared to smear microscopy negative with a specificity of 100%. The overall sensitivities of Xpert were 88.89% and 85.45%. High cases of smear-negative disease were related to an absence of cough with presence of sputum and low volume of sputum.

The sensitivity and specificity of rifampicin resistance versus MGIT DST respectively for SS- cases were 88.89 % (95% CI: 51.74 % to 98.16 %), 100.00 % (95% CI: 98.19 % to 100.00 %) and PPV 100.00 % (95% CI: 62.91 % to 100.00 %) and NPV 96.30 % (95% CI: 80.97 % to 99.38 %), 71.43 % (95% CI: 47.83 % to 88.65 %), 100.00 % (95% CI: 87.94 % to 100.00 %), PPV 100.00 % (95% CI: 78.03 % to 100.00 %) and NPV 82.86 % (95% CI: 66.34 % to 93.39 %), respectively. However, there were also one and six confirmed false negative results vs DST (period 2013 and 2014).

Screening for HIV-associated TB year 2014

Total HIV-infected (267 cases) and suspected HIV prisoners (1540 cases) at a single site provided two early-morning sputum specimens to be examined using ZN microscopy, BACTEC MGIT 960 liquid culture and a single Xpert. The sensitivity, specificity, NPV and PPV of Xpert were calculated relative to gold-standard results using MGIT 960 liquid culture and has evaluated the utility of Xpert MTB/RIF for screening for HIV-associated TB among patients with advanced immunodeficiency prior to starting antiretroviral therapy (ART). Out of 267 88 were HIV/ TB SS- cases and 6 were HIV/TB SS+.

TB diagnosis is also extremely challenging since symptom screening, chest radiology and smear microscopy which often takes

one day except culture-based diagnosis which takes many weeks. In a prospective study during the 2014, a cohort of 432 diagnosed TB patients suspected having HIV based of the presence of symptoms by obtaining one or two sputum samples.

Accuracy retained in specimens from HIV-infected patients, showing pooled values of 81.25 % (95% CI: 54.34 % to 95.73 %) sensitivity and 100.00 % specificity (95% CI: 95.28 % to 100.00 %) and PPV 100% (95% CI: 75.12 % to 100.00 %), NPV 96.25% (95% CI: 89.42 % to 99.18 %). Accuracy estimates for rifampicin resistance detection were with pooled sensitivity of 85.71 % (95% CI: 42.23 % to 97.63 %) and pooled specificity of 100.00 % (95% CI: 95.15 % to 100.00 %), PPV 100.00 % (95% CI: 54.05 % to 100.00 %), NPV 98.68 % (95% CI: 92.86 % to 99.78 %).

The assay also correctly identified 26 cases of MDR-TB and 6 cases of MDR-TB/HIV, reducing median time to diagnosis by over 5 weeks and showing the great potential of the assay to reduce the risks of nosocomial MDR-TB and MDR-TB/HIV outbreaks in HIV care and treatment settings.

Diagnostics classification

For this study, patients were classified as having MTB if no other diagnosis was made and the attending physician made the decision to treat for MTB based on the clinical algorithm. In addition, patients diagnosed with MTB were classified as having definite, probable, or possible MTB using the standardized case definition. Xpert MTB/RIF results were not included in the case definition, because it was the test under evaluation. Definite MTB was defined as a clinical syndrome consistent with MTB, with *M. tuberculosis* isolated in sputum MGIT culture. All patients who did not meet the criteria or did not receive treatment for MTB and received an alternative discharge diagnosis were classified as not having MTB.

Discussion

Xpert MTB/RIF is a rapid, specific test for the diagnosis of MTB. As with other tests for MTB, a negative result cannot exclude a diagnosis of MTB. Xpert MTB/RIF's introduction for pulmonary specimen testing has required changes in clinical and diagnostic algorithms and changes in the requisition of samples and their processing and the laboratory and clinic workflow, to name a few of the consequences. It has also exposed general weaknesses in the general health care systems for TB, one being ongoing linkage to care post diagnosis. Among these clear specimens, pleural fluids and CFS performed most poorly. This may be due to specimen collection, storage, and preparation techniques due to low numbers of bacteria.

To our knowledge, this is the first time we report the diagnostic performance of a single GeneXpert MTB/RIF assay for sputum smear negative samples in improving pulmonary TB case detection among HIV-infected and TB suspected inmates. There are a number of limitations in this current assessment. First, most of the included cases studies were case-control by design and it was difficult to tell whether cases and controls had been selected from the same or similar cohorts. Estimates based on case-control study are often difficult when cases and controls belong to different cohorts. Nonetheless, sensitivity analysis restricted to studies of possibly higher quality has corroborated the main findings of the current review. Second, the number of samples used for individual cases was relatively small. This could have reduced the statistical power for detecting more positive cases and showing differences between assay subgroups.

In this study specimen routinely sent to the laboratory were investigated with the standard algorithm (smear microscopy and culture) without performing Xpert MTB/RIF testing. Based on the low number of CSF and pleural fluids conclusions the analysis cannot be extrapolated to

the two specimens. This might improve if Xpert MTB/RIF will be used as the first-line diagnostic on the received specimen. Centrifuged compared to non-concentrated fluid and pus specimens resulted in more positive Xpert MTB/RIF results (but not statistically significant).

This report provides local data to support the introduction of TB screening of pulmonary and extra pulmonary specimens with Xpert MTB/RIF technology and similarly follows the pulmonary Xpert MTB/RIF algorithm with confirmation by DST and its use in the context of clinical suspicion. It is acknowledged that the sample size for homogenized tissue biopsy specimens, bone and other extra pulmonary samples in this study is low, but the sensitivity of Xpert MTB/RIF appears comparable to that of MGIT for these specimens.

At the same time in this study, we report for the first time, the diagnostic performance of a single GeneXpert MTB/RIF assay in improving pulmonary TB case detection among HIV-infected (year 2014). Overall, diagnosed active pulmonary TB disease was remarkably high 48% (127/267) among these HIV-infected patients in dedicated HIV units.

With a very high sputum AFB smear-negativity, a single Xpert had sensitivity for the diagnosis of active TB disease respectively for 2013 and 2014 (88.89 % and 85.45 %) but high specificity (100%) and for rifampicin resistance sensitivity 88.89% and 71.43% detection compared to the liquid DST. The new convenient assay markedly increased the number of active TB cases detected in this population, potentially at much earlier TB disease burden, where profoundly symptomatic disease is common at the time of clinical presentation.

Finally, intensified case finding (ICF) programs support identification of earlier TB cases, especially with less advanced TB disease and low bacterial burden, but potentially insufficient to be detected using the Xpert assay.

Conclusion

The Xpert MTB/RIF test on one sputum sample rapidly and correctly identified the majority of adults with culture confirmed pulmonary tuberculosis with high specificity. MTB/ RIF assay is thus a promising rapid diagnostic test for both pulmonary and extra pulmonary. Prospective studies are required to evaluate using two sample of sputum for the Xpert MTB/RIF assay to increase the positivity rate of sputum negative cases. A better knowledge of the mechanisms of drug resistance of *M. tuberculosis* and the relevant molecular mechanisms involved will improve the available techniques for rapid drug resistance detection and will help to explore new targets for drug activity and development.

The new diagnostic tool is markedly superior to sputum smear microscopy, however, missed almost half of Chest-X-ray diagnosed pulmonary TB cases 66.6% (86/129) in the predominantly smear-negative sample. The high TB prevalence reported here affirms the importance of intensified TB case finding in settings and warrants further exploration of multiple diagnostic approaches using the Xpert technology, potentially combined with other diagnostic screening modalities according to National TB program.

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