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Review

Burden of pulmonary tuberculosis and challenges related to tuberculosis detection in Ethiopia

Letebrhan Weldemhret[1]

[1] Tigray Health Research Institute, Mekelle, Tigray, Ethiopia

Abstract

Early and rapid diagnosis of *Mycobacterium tuberculosis* in clinical specimen is important for the treatment of patients and control of disease transmission to the community. The disease is largely preventable and curable, but without rapid, and correct diagnostic tools for tuberculosis (TB) infection and drug resistance, it is unlikely that we can meet the national TB elimination program in Ethiopia by 2035. Moreover, drug resistant TB is becoming more common and is a great challenge for the successful control and eradication of TB. The need for rapid, accurate and affordable methods for TB management should be considered by policy makers to improve TB detection rate and reduction of TB related deaths in line with the stop TB strategy by 2030 in Ethiopia.

Key words: tuberculosis, pulmonary, infection burden, detection, Ethiopia.

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Introduction

Tuberculosis (TB) is the most common cause of death from a single infectious pathogen [1,2]. Approximately 4000 people die from TB and nearly 30,000 people are infected with this disease daily [1-3]. There are an estimated 10 million cases of TB globally. There were an estimated 1.2 million TB deaths among human immunodeficiency virus (HIV)-negative people and an additional 208,000 deaths among HIV-positive people in 2019 [2,4]. South-east Asia and the western Pacific regions collectively accounted for 58% of the world's TB cases and the African Region accounted for 28% of the cases. Although, the African region has reported lower number of TB cases compared to the other regions, it accounted 281 cases per 100,000 population, which is double the global average of 133 [2]. Even though, the annual TB incidence and deaths are declining globally, this decline is not fast enough to reach the first milestone of the 'End TB' strategy which has a target reduction of 35% between 2021 and 2025 [2,5].

If left untreated, 70% patients with smear-positive sputa will die within 10 years [7]. Thus, the mortality rate from TB is high. Moreover, it is estimated that 5 to 10% of the 1.7 billion people infected with *M. tuberculosis* would go on to develop TB disease during their life time [1]. It has also been estimated that each individual with untreated smear-positive TB would, on average, infect 10-15 people every year [6].

Drug-resistant TB is becoming a great challenge in the management of TB and this is particularly evident in resource-limited settings [2,7-9]. Globally the mortality and prevalence of TB was 47% and 42% respectively [2]. Mortality with multidrug resistance (MDR) and extensive drug resistance (XDR) is increasing due to lack of diagnostic methods to rapidly and effectively recognize and treat patients [2,4]. An estimated 61% of bacteriologically confirmed TB cases were tested for rifampicin resistance [2,4]. Of these a total of 206,030 people with MDR/RR (Rifampicin resistant)-TB were detected and notified and they included 3.3% of new cases and 18% of previously treated cases of MDR/RR-TB in 2020 [2,4].

Ethiopia is one of the 30 high burden countries for MDR-TB. The overall case detection rate is less than 60% in low-income countries and about 66% globally [10]. The case detection rate in Ethiopia was 71% which was lower than the expected target set for 2020 (85%) [2,11]. Moreover, the nationwide prevalence of bacteriologically confirmed TB was 277 per 100,000 populations in Ethiopia [6]. Of these, 41% were bacteriologically confirmed which is also relatively lower than the expected target (70%) in 2020 [2,9]. Some studies have reported this rate to be as low as 20% in children and HIV infected individuals [7,4].

Direct smear microscopy

Direct sputum smear microscopy is the most widely used method for diagnosing pulmonary TB in developing countries because it is available in most primary health care laboratories [7,12,13]. Sputum smear microscopy has been used for more than a century and it is also the method by which the first bacteriological evidence for the presence of mycobacteria in a clinical specimen was identified [6,14]. It is a rapid and easy procedure to perform, and provides a clue to the physician with a preliminary confirmation of the diagnosis [7,15]. It also provides a quantitative estimation for the number of bacilli being expectorated for assessing the patient's infectiousness [14].

Ziehl-Neelsen (ZN) stained smears prepared directly from sputum specimens is the most widely used technique for diagnosing TB in resource-limited areas [15,16]. ZN microscopy is very specific but it has low and variable sensitivity that ranges from 20 to 80%, often depending on the diligence with which specimens are collected, smears are made, and stained smears are examined [17,18]. The sensitivity can be as low as 20% in children and HIV-infected people [15]. Due to its low sensitivity, the World Health Organization (WHO) has recommended that the traditional ZN microscopy be replaced by fluorescent light-emitting diode (LED) microscopy in settings in both high- and low-volume laboratories as it saves time and improves sensitivity [17,19]. In addition, it has qualitative, operational, and cost advantages over ZN microscopy [16]. Fluorescent microscopy (FM) enhances the microscopic field of the smear to be seen resulting in more effortless examination of the specimen and making it easier to count bacilli [8]. Moreover, FM has 10% more sensitivity and comparable specificity with ZN microscopy [19]. Since fluorochrome-stained smears can be examined at lower magnifications, it takes less time to examine these smears than to examine smears stained with ZN stain and still results in higher sensitivity and similar specificity [17]. Although smear microscopy is specific, it has low sensitivity and cannot differentiate *Mycobacterium tuberculosis* (MTB) from non-tuberculous mycobacteria (NTM) or atypical mycobacteria and drug resistant TB [13,18].

Mycobacterium TB Culture

Culturing of *Mycobacterium tuberculosis* is a sensitive and accurate technique for detecting and differentiating *Mycobacterium* species [8,12,14,20]. Culturing mycobacteria is mainly done on solid and or liquid media [14]. This method is much more sensitive than smear microscopy, allowing for the detection of as few as 10 bacteria per mL and also provides grown isolates for species identification, drug susceptibility testing and genotyping [14,21]. There are two types of TB culture methods: manual and automated culture.

Solid TB culture media

Solid media is most commonly an egg-based media used for MTB growth and drug susceptibility test (DST) Among the egg-based media, LJ media such as Middle brook 7H10, Middle brook 7H11 and Dubose oleic-albumin agar are recommended [22]. Solid culture methods are less expensive compared to liquid culture systems, but the results are often delayed due to the slow growth of MTB [20]. TB culture and DST using solid media can take 6 weeks or more due to slow growing characteristics of the organism [8]. The length of time required for the growth of *Mycobacterium tuberculosis* complex (MTBC), and to interpret the result is 4 to 8 weeks [8,23]. During this time patients may be inappropriately treated and drug-resistant strains may continue. In addition, LJ media varies from batch to batch in its ability to support the growth of TB depending on the quality of the eggs used and achieving consistent drug concentrations when performing susceptibility testing. Thus, it is recommended that all mycobacterial isolates should be identified to the species level at least to the extent that the MTBC isolate is differentiated from nontuberculous mycobacteria (NTM) [17]. Despite these limitations, there are strains of the MTBC that will grow better on solid media and thus, this is considered the gold standard for the detection of TB and the practice is to inoculate at least one tube each of solid and liquid media [22].

Liquid culture media

Mycobacterial growth indicator tube (MGIT) is the most commonly used automated TB culture media. Liquid media are preferable for rapid initial identification of mycobacteria, but majority of the resource-poor countries depend on LJ egg-based medium for the growth of MTBC isolates [3,22]. The mycobacterial growth indicator tube (MGIT) 960 (Becton Dickinson, BIORAD, Hercules, USA) uses middlebrook 7H9 broth in a 7 mL plastic tube and a fluorescence quechin based oxygen sensor embedded at the bottom of the tube. The BACTEC MGIT 960 system contains the Middlebrook 7H9 medium, oleic albumin dextrose catalase (OADC), polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azolocillin (PANTA), and an oxygen-quenched fluorochrome embedded in silicone at the bottom of the

tube. MGIT tubes may be read manually under an UV light or placed in an MGIT 960 instrument, where they are incubated and monitored for increasing fluorescence every 60 min. Growth can also be visually observed by the presence of small granules or flakes [11]. Moreover, MGIT contains a nutrient-rich medium where contaminating bacteria are inhibited by the addition of a cocktail of antibiotics. The growth of bacteria, including mycobacteria, is indicated by fluorescence, which increases as oxygen decreases in the tube. The instrument detects this fluorescence in the medium using UV light and complex computer algorithms [8,22].

The liquid culture technique increases the detection rate of MTB by 10% over solid media, and the rapid growth in liquid culture results in reduced delays for DSTs compared with conventional solid media [8,23]. However, it is more prone to contamination, and there is need to control large volumes of infectious material by implementing adequate biosafety measures [8,12]. Moreover, WHO has recommended the use of liquid culture and rapid species identification [18]. Thus, a combination of antimicrobial agents, such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azolocillin) is added to the liquid medium to suppress the growth of contaminants.

Drug susceptibility test

Drug susceptibility test (DST) for MTB uses both phenotyping and genotyping techniques [7,8]. Phenotypic methods involve culturing of *Mycobacterium tuberculosis* in the presence of anti-TB drugs to detect growth or inhibition of growth. Genotyping methods target specific molecular mutations associated with resistance against individual drugs. Phenotypic DST methods are performed as direct or indirect tests on solid or liquid media. In direct testing a set of drug-containing and drug-free media are inoculated directly with concentrated specimen. Indirect testing involves inoculation of drug-containing media with a pure culture grown from the original specimen [24].

Nucleic acid amplification tests

Due to the limitation of conventional microbiological diagnosis in diagnostic delay, advanced molecular techniques are introduced to identify and confirm the MTBC more accurately and rapidly in clinical specimens [6,8]. The nucleic acid amplification assay (NAAT) is used for both detection and identification of the MTBC [25]. The assay uses either polymerase chain reaction (PCR) or loop-mediated isothermal amplification for the detection of MTBC isolates [17]. The application of this technology has become routine in many high-income countries with low TB prevalence. The LAMP assay (Eiken Chemical Co. Ltd., Tokyo, Japan) is a novel, isothermal nucleic acid amplification method which is simple, rapid, and with high specificity and that might be adaptable to resource-limited settings [26].

These techniques are commonly used for confirmation of smear positive results or for primary diagnosis [14]. The NAAT technique detects nucleic acids from dead as well as live *M. tuberculosis* and, therefore, it can remain positive for long periods in patients who have completed tuberculosis therapy. Its application is also limited due to the complexities of DNA extraction, amplification and detection, and biosafety concerns [21]. Hence, it is limited to resource rich settings and to some research areas due to the cost and complexity.

Line probe assays

Line probe assay (LPA) is a new molecular technology identifying the presence or absence of specific mutations associated with rifampicin and isoniazid resistance in the genome of the MTBC [15]. It is a rapid and accurate test to identify cases with MDR-TB. LPA is directly performed on clinical samples and offers an enormous advantage to the patients. The LPA protocol involves DNA extraction from clinical specimens or cultured material, multiplex amplification with biotinylated primers and reverse hybridization [21]. The turnaround time of LPA ranges from 24 to 48 h which is short compared to conventional DST methods, and hence, it is highly advantageous for high-burden settings [27]. Data from systematic reviews and meta-analyses indicate that line probe assays have a sensitivity of ≥ 97% and specificity of ≥ 99% for the detection of rifampicin resistance alone and a sensitivity ≥ 90% and specificity of ≥ 99% for combination with isoniazid on isolates of *M. tuberculosis* and on smear-positive sputum specimens [8].

However, LPA is currently limited to culture isolates and direct testing of smear-positive sputum specimens. Thus, it does not replace conventional culture and drug susceptibility testing in which culture is still required for smear-negative specimens, and conventional drug susceptibility testing to confirm resistance to drugs other than isoniazid and rifampicin [8,25]. Rapid and more sensitive molecular techniques are needed due to increased incidence of TB drug resistance [12,13].

GeneXpert MTB/RIF assay

GeneXpert MTB/RIF assay is a rapid fully automated molecular diagnostic test that detects simultaneously MTBC and mutations associated with rifampicin resistance with minimal sample handling. The test can be carried out within 2 hours and is conveniently placed at the point of patient care [21]. The WHO endorsed the Xpert MTB/RIF rapid molecular assay in December 2010 for simultaneous MTBC and rifampicin detection in areas with high risk of MDR-TB and HIV-associated TB infections [8,21]. Furthermore, the global TB community agreed to roll out and scale up the Xpert MTB/RIF assay in high TB burden countries by developing polices, guidelines and frameworks to support and incorporate into their national TB control programs [28]. The Xpert MTB/RIF assay is a single use sample-processing cartridge system with integrated multicolor real-time PCR assay [20]. Moreover, this method is the only fully integrated instrument that can detect MDR-TB with minimum user input unlike other diagnostic technologies [10,29]

The use of GeneXpert MTB/RIF assay as a replacement or add-on test to smear microscopy has increased the number of TB cases identified by approximately 30% [21]. Many researchers have validated the GeneXpert MTB/RIF assay using controlled trials and in field conditions. Systematic reviews have concluded that it provides efficient performance for detecting both MTB and RIF resistance [21,28,30]. The assay can analytically detect 5 copies of purified DNA, and 131 cfu/mL of MTB spiked sputum [21]. Moreover, none of the resistant strains were detected as susceptible nor were the susceptible strains reported as resistant for rifampicin detection by Xpert MTB/RIF assay [4]. The sensitivity of GeneXpert MTB/RIF assay among smear-negative and smear-positive suspected TB were 86.3% and 90.6% respectively [30]. Its sensitivity ranges from 59.5% to 86.3% in smear negative cases. However, when GeneXpert MTB/RIF assay was used as an initial test replacing smear microscopy, it showed a pooled sensitivity of 88% and specificity of 98% [14]. The GeneXpert MTB/RIF assay has a sensitivity of 79% and specificity of 98% among HIV co-infected individuals [30]. Another study indicated that the GeneXpert MTB/RIF assay has an additional yield of 23% over smear microscopy among smear positive contacts [9].

Despite its speed and general applicability, the GeneXpert MTB/RIF assay does not differentiate between dead and live TB bacilli which is necessary for observing patient response to chemo-therapeutic intervention. In addition, this method is unable to differentiate MTB from NTM.

Conclusions

The tuberculosis disease is a global challenge due to the number of active TB cases and deaths worldwide, mainly in resource-poor settings. Tuberculosis is a national challenge in Ethiopia due to the higher prevalence of active TB diseases and deaths than the average global TB incidence. Despite the advantages of molecular tests, conventional microscopy and culture remain necessary for patients' follow-up treatment regimens. Moreover, culture-based DST methods are currently the only methods available for accurate testing of susceptibility to second-line drugs. The GeneXpert MTB/RIF assay is a molecular technique which was recently introduced for simultaneous detection of MTB complex and rifampicin resistance. Limited access to appropriate diagnostic tools remains a barrier to diagnosis and treatment and many people live with active TB. There is no fully self-integrated method available which is rapid and meets the expected sensitivity needed for diagnosis. There is urgent need for rapid and correct diagnostic methods to overcome the challenges in resource-poor countries.

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Corresponding author

LetebrhanWeldemhret, MSc in Medical Microbiology.
Tigray Health Research Institute, Hawelti, Mekelle, 1547,
Ethiopia, East Africa, Africa
Tel: +251914269399
Fax: +251-034-241-446
Email: letishw23@gmail.com

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