COMPARISON BETWEEN NEWER DIAGNOSTIC APPROACHES AGAINST CURRENT DIAGNOSTIC APPROACH IN DIAGNOSING PULMONARY TUBERCULOSIS IN HOSPITAL AL SULTAN ABDULLAH UiTM

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ABSTRACT

Introduction

A wide spectrum of laboratory techniques has been developed to diagnose tuberculosis (TB). This study compares the performances of light microscopy (Kinyoun stain), fluorescence microscopy (Auramine stain), and molecular (Xpert MTB/RIF Ultra), against the gold standard method of culture (Mycobacteria Growth Indicator Tube, MGIT) in diagnosing pulmonary TB.

Materials and methods

This study is a single-centre, prospective, cross-sectional study conducted at Hospital Al-Sultan Abdullah (HASA) UiTM in Malaysia, from June 2021 to May 2022. A total of 109 respiratory samples from patients clinically suspected of pulmonary TB which fulfilled inclusion and exclusion criteria were tested with microscopic, molecular and culture methods.

Results

MGIT culture and sensitivity result were used as reference standard. Sensitivity and specificity for AFB detection by Xpert MTB/RIF Ultra, light microscopy and fluorescent microscopy were 93.8% and 92.5%, 56.3% and 98.9% as well as 37.5% and 98.9%, respectively. Sensitivity and specificity of MTB detection by Xpert MTB/RIF Ultra were 93.8% and 92.5%, whereas those for rifampicin-resistant detection by Xpert MTB/RIF Ultra were 100% and 100%.

Conclusion

For AFB detection, Xpert MTB/RIF Ultra (94%) sensitivity was far superior than both light (56%) and fluorescent (38%) microscopic methods. However, the specificity for all three methods were fairly comparable, at 99% for both microscopic methods but at slightly lower 93% for molecular method. Sensitivity and specificity of Xpert MTB/RIF Ultra for MTB detection and rifampicin-resistant detection, were above 90% and 100%, respectively.

INTRODUCTION

Tuberculosis (TB) is one of the top 10 causes of death and the leading cause of a single infectious agent worldwide. TB is caused by *Mycobacterium tuberculosis* (MTB) which typically affects the lungs (pulmonary TB). In 2020, an estimated 10 million people were infected with TB worldwide. Due to the COVID-19 pandemic, the underreported number of newly diagnosed TB fell from 7.1 million to 5.8 million in 2020. As a result, TB death increased from 1.2 million in 2019 to 1.3 million in 2020 due to reduced access to TB diagnosis and treatment. A total of 206030 people with multidrug or rifampicin-resistant TB (MDR/RR-TB) were detected in 2019, a 10% increase from 186883 in 2018 but reduced by 15% to 177 100 in 2020[1]. MDR-TB is a public health crisis and a health security threat. In Malaysia, 25173 cases of TB were recorded nationally in 2018, and an incidence rate of 92 cases per 100000 population[2].

MTB is an aerobic, non–spore-forming, non-motile bacillus with a high cell wall content of high-molecular-weight lipids. Growth is slow, the generation time is 15 to 20 hours, and visible growth takes 3 to 8 weeks on solid media[3]. A wide spectrum of laboratory techniques has been developed to diagnose active TB. The diagnostic tests included microscopy acid-fast bacilli (AFB) smear either using a conventional light microscope (Ziehl-Neelsen / Kinyoun) or fluorescence microscope (Auramine Rhodamine), culture either on the solid or liquid media, nucleic acid amplification (NAA) for example polymerase chain reaction (PCR) and nucleic acid amplification with Xpert MTB/RIF or Xpert MTB/RIF Ultra. AFB smear microscopy is an inexpensive but less sensitive test with a lower detection threshold of 10,000 organisms per millilitre of sputum for smear positivity. The sensitivity of sputum AFB smear compared with culture is approximately 60%, and unable to distinguish between MTB and other mycobacteria[3,4,5,6]. However, most laboratories now use a fluorochrome stain

(phenolic auramine/ auramine-rhodamine) which is more sensitive and allows more rapid slide reading than conventional microscopy[4, 5].

Culture remains the gold standard for diagnosis, but it is time-consuming. Culture on solid media takes 3 to 8 weeks for visible growth, and culture on liquid media turns positive between 10 (smear positive) to 20 (smear negative) days[3, 5, 7]. As for NAA tests, eg., PCR, it requires advanced laboratory techniques. To address these issues, Xpert MTB/RIF has been proposed to facilitate TB diagnosis and rifampicin detection. Due to the easy format, it is used both in laboratories and at the bedside as a point-of-care test (POCT). However, its sensitivity is imperfect, particularly in smear-negative and HIV-associated TB, and some limitations remain in determining rifampicin resistance[8].

As a result, the Xpert MTB/RIF Ultra assay was developed by Cepheid as a next-generation assay to overcome these limitations. Ultra assay incorporates two different multi-copy amplification targets (IS6110 and IS1081) and a larger DNA reaction chamber than Xpert MTB/RIF. Rifampicin resistance detection has also been improved in Ultra by relying on interpreting the melting curves in the active site of rpoB[8]. With no decrease in sensitivity compared to Xpert MTB/RIF, Xpert MTB/RIF Ultra can identify with increased specificity rifampicin resistance-associated mutation.

With the availability of rapid NAA testing, Xpert MTB/RIF Ultra assay, test results can be reported weeks earlier than culture, leading to improved patient management and outcomes. However, it is strongly recommended by CDC that NAA tests be performed and interpreted in the context of a comprehensive testing algorithm that includes AFB smear and culture as well as drug susceptibility testing (DST) to maximize its benefit to patient management.

Current methods used at microbiology unit in Department of Clinical Diagnostic Laboratory Hospital Al-Sultan Abdullah, UiTM for diagnosing TB are conventional light microscopy using Kinyoun stain (on-site) and culture (outsourced to referral laboratory). As