

Research Article

CULTURING, IDENTIFICATION AND DRUG RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* IN SPUTUM SPECIMEN

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Abstract: The development of low-cost, fast, and reliable methods for detecting *Mycobacterium tuberculosis* (MTB) infection and drug susceptibility is critical for tuberculosis control. The new microscopic examination of liquid drug susceptibility assay (MODS) examines early MTB colonies in a liquid medium, which permits for more convenient diagnosis and testing of drug susceptibility. The sensitivity of MODS (91%) was superior as compared to the sensitivity of different culture methods (92%). The MGIT and MODS were used to monitor tuberculosis-positive sputum samples for isoniazid and rifampin susceptibility. Concordance between MODS and MGIT was found in 89 percent of cases. MODS are the fastest method used for diagnostic and susceptibility testing (median, 10.0 or 9 days). MODS is a fast, low-cost, responsive, and particular method for detecting and testing MTB susceptibility; it is specifically well-suited utilize in growing countries with high infection rates and a growing number of multidrug-resistant cases.

Introduction

Tuberculosis (TB) is a major contributor to the worldwide burden of disease and has gain considerable attention in recent years, particularly in low and middle-income countries (Pio *et al*, 1999). The genus *Mycobacterium* belongs to the family of *Mycobacteriaceae*. The most familiar of the species are *Mycobacterium tuberculosis*, the causative agents of tuberculosis (TB). Tuberculosis (TB) is an infectious airborne disease. The name *Mycobacterium*, meaning Fungus like bacterium is obtained from the mold-like appearance of *Mycobacterium tuberculosis*, when expand in liquid media. They are non-motile, aerobic, non-copulating and non-sporing. It seems to like to grow in a well-ventilated area of the upper layers of the lungs. Tuberculosis generally impact the lungs in humans known as Pulmonary tuberculosis but it can also harm intestine, meninges, bones, and joints, lymph glands, skin and other tissues of the body mutually called extra pulmonary tuberculosis. It occurs more in people with a weakened immune system and

adults. When this disease affects animals like cow; this is known as bovine tuberculosis (Gleissberg *et al*, 2001).

Mycobacterium tuberculosis has a double duration of 18 hours and clinical practice can take about 6 to 8 weeks to develop. Resistance to dehydration and can survive the expected cough. The cell wall contains a complex lipid chain such as mycolic acid, simple chain fatty acids to form acid-fast components, wax D and phosphatides (Levinson W, 2010). In some microorganisms the presence of fats and waxy substance typically interfere with the staining. These microorganisms cannot be stained by Grams stains unless some special treatments square measure done. It is estimated that from two thousand to two thousand ten at least 9 cases were reported over the year. One and half million people die due to this disease in every year. While, the 95% deaths have been found in moderate income countries. TB is mostly found in children in the poorest countries where more than a hundred thousand children die every year. In United States about ten to twelve million people are infected by the *Mycobacterium*

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tuberculosis. In contrast to the decline of infectious disease within the African nations has dramatically accumulated. TB is next main reason of death among young people from infectious disease in these countries having nearly half percent of HIV infected population. Isolation, cultivation, identification and drug resistant mycobacteria in sputum specimen are very important to upgrade the identification as part of the worldwide TB control efforts. Over 60 other species of mycobacteria have been recognized, some of which are low-grade pathogens in man (Ali *et al*, 2010). The development of antimicrobial resistance is one of the many challenges. Tuberculosis has the potential to improve genetic mutations that make it more resistant to many effective antibiotics (WHO, 2010).

After the introduction of effective antibiotics within the nineteen fifties, TB incidence declined steadily. The rate of nearly 2000 deaths annually, have continued to decrease. It is currently demonstrated that one third of the world's population is sick with this disease. The occurrence of tuberculosis in United States has declined over the years and now it is almost deceased. Much in 1907, the incontestable presence of Gram-positive granules is cold symptoms plus wherever there is no proof of acid-fast bacilli however that might could manufacture infectious disease once injected into susceptible animals. Much suggested that these granules are nonacid fast quick kind of tubercle bacilli. The mode of direction is on face to face intake of dustier bacilli contain in bead nuclei of spit up sputum tubercle bacilli square measure no heritable from persons with active sickness who are excreting viable bacilli by means of coughing, sneezing or talking.

Mycobacteria square measure quickly killed by ultraviolet light even through glass, and by heat at 60°C for 15 to 20 min. Culture may be killed by exposure to daylight for two hours but bacillus in sputum may remain alive for 20 to 30 hours. Sputum smear microscopy is used as an important or pressing priority to spot or diagnose infectious disease within the patients in specific analysis space. The most usual specimen for diagnosis of pulmonary tuberculosis is sputum which consists of pus and mucus secretions coughed up the sputum into in a very clean wide-mouthed instrumentation. For decades, microscopy research has been used because initial take a look at to sight active tuberculosis (TB). Despite it is ubiquity, microscopy is suboptimal as result of its low sensitivity, high inter-operator variability, is largely unhelpful in extra pulmonary and childhood TB, and does not sight drug resistance. Over-came

some shortcomings of sputum smear microscopy, supported its increased sensitivity and coincidental detection of resistance to rifampicin (Steingart *et al*, 2013).

Material and methods

Search strategy and study selection

Few sample size which was evaluated using the Buderer's formula that obtain 95 percent sensitivity or specificity with much lesser than five percent accuracy at such a ninety five percent CI was 60 percent TB among of suspects. We performed sputum from the at least 322 of consecutive affected cases of TB with help of both average LJ media or fluid on the micro plates. So the growth that was present in LJ media which are solid through pictorial colonial testing. Meanwhile colony which was found in usually broth and micro plate sources with very little visibility. We compared sensitivity, specificity, pollution levels, effect time, and cost of these two tests (Kidenya *et al*, 2013). Mycobacterium species *smegmatis* mc2155 (Carriere *et al*, 1997; Jacobs, Jr. *et al*, 1993; Riska & Jacobs, Jr. 1998) and mc24502 used to distribute existing phages grown in Middlebrooks 7H9 media containing 0.2% glycerol, 0.5% serum albumin (BSA), fraction V, 0.2% dextrose and 0.09% NaCl (selected SADC) with 0.05% Tween-80 and 150 g / ml hygromycin (for mc24502). (Svetoslav Bardarov *et al*, 2002).

Laboratory methods

Sample collection

Patients with tuberculosis suspected of having high-risk or tuberculosis-resistant. MTB samples among victims in HIV- contaminated hospitals, samples each patient collected only one study, 20 of which came from abdominal washing of 12 patients those were unable to give sufficient sputum specimen (Moore *et al*, 2006). The most usual specimen for identification of pulmonary tuberculosis is liquid body substance that consists of pus and mucus secretions coughed up from the respiratory organ. Patient instructed to cough up the sputum into a clean wide mouthed container. If the sputum is scanty, a 24 hours sample also tested. Sputum samples on three days raised probability of detection (Kunkel *et al*, 2016).

Growth detecting using (LJ) medium

It is a particular media known for the cultivation and isolation of *Mycobacterium tuberculosis* commonly called LJ medium (Elbir *et al*, 2008). It adds Congo red and malachite green to inhibit the growth of unwanted/contaminated bacteria. The contaminated specimen (100 ml) was placed in two test tubes of solid LJ media which was being made by following usual procedure (Central TB Division, 2009). For

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preparation of LJ media, 1st a table salt solution consisting of 2.3 g of monopotassium phosphate (KH₂PO₄), 0.23 g of MgSO₄, 0.6 g of magnesium 2-hydroxypropane -1,2,3-tricarboxylate, 3.5 g asparagine (amino acid), 11 ml glycerol, 20 ml glycerol (grade reagent), was first developed. - Malachite green, 3.0% solution (originate with 3.0 g malachite green dye and 100 ml of ddH₂O). The components were mixed in a series of ddH₂O, then lowered to 25°C and deep-freeze. Then 600 ml of table salt solution is mingled with 1000 ml of hatched eggs. Whole eggplant was spread on aseptic vessels, sealed in 6-7 ml volumes then placed in incubator in sealed container for 55 minutes at 85uC. LJ center is later refrigerated for up to 4 weeks because replicating time of *M. tuberculosis* was slow (15–20 hours) as compared with to bacteria (Kidenya *et al*, 2013). When *M. tuberculosis* is allowed to grow on LJ medium they appear as brown, granular colonies. Each classification was tested for morphology and pigmentation (Cattamanchi *et al*, 2009). The church of the emergence of the colonies was noted. In the event of contamination, sputum specimen was again contaminated and re-designed for patient guidance and excluded from the study (Davis *et al*, 2011). In addition, whether no growth was showing for eight weeks even contamination was there, the cultures were thrown away or lab forms were properly filled. Additionally, to the cultural characteristics, usual Ziehl-Neelsen slides are create with feature colonies that appear earlier week 8 to ensure the appearance example acid-fast of bacilli (Kiraz *et al*, 2007).

DST using BACTEC MGIT

DST was accomplished by using BACTEC Mycobacteria Growth Indicator (MGIT) Tube 960 SIRE Kits, Sparks, USA, Becton Dickinson, the AST Set Carrier and the BACTEC MGIT machine with fundamental isolates acquired from Lowenstein Jensen (LJ) media. The manufacturer's DST laboratory protocol is available there. The BACTEC-MGIT system delivered an automatic result after strict adherence to the procedure. As a quality check, each test batch included the reference strain H37Rv MTB (ATCC 27294). The final vital concentrations for Isoniazid and rifampin were 0.1 and 0.4 g/ml for Isoniazid and 1 g/ml for rifampin (Ejigu *et al*, 2008).

Microscopic observation of drug susceptibility (MODS)

The Microscopic observation of drug susceptibility (MODS) assay test was performed in both indirect & direct methods. The tests were performed on 12 well cultured plates. In which each test contained a 2 mL Middlebrook 7H9 broth, 200 microliter (μL)

suspension, and 34 microliter (μL) of significant antimicrobial drugs. The suspension of McFarland, approximately 104 CFU / mL of isolates was used. The test tube (control) was set on the 1st row of the plate. In every plate, two antibiotics combined with a combination of isoniazid and streptomycin (I&S) and ethambutol and rifampicin (E&R) were administered randomly (WHO, 2013). A direct method test was performed with 140 microliter (μL) of a contaminated sputum sample which is prepared from the pump-extracted mixture as indicated. Indirect tests were performed with appropriate MTB colonization equipment. Bad control contained only MODS media. Good control consisted of MODS and MTB media with a combination of 1: 100 and 1:10 PZA. All the plates were placed at 37°C and tested twice a week after daily contamination for up to 42 days. Detectable serpentine clusters also known as pellicle formation shown good culture. Growth seen in both drug free and drug containing sources were resilient, while drug free trials and no growth in drug induced media outlets shown that testing may be separated from experimental drugs (park *et al*, 2002). Broth from Mycobacteria Growth Indicator (MGIT) tubes was poured into MODS sources to determine whether the cord formed in the acidic area could be detected easily. Intermediate broth for DST persistence of streptomycin, isoniazid, rifampicin, and ethambutol (SIRE) was prepared in a laboratory (Owusu and Newman *et al*, 2020).

Results

Culture Detection of *M. tuberculosis* by LJ Media, and MODS Assay

During the study period, 321 suspected cases of pulmonary tuberculosis which were enrolled, and all were included in the research. 51.1 percent of the people tested positive for HIV, while 60.4 percent tested positive for ZN. According to medical criteria, 62.3 percent of the cases were pulmonary tuberculosis. Liquid broth detected 89% pulmonary tuberculosis cases, 95 percent confidence interval, 84.7 percent to 93.3 percent while LJ detected 77.0 percent, or 95 percent of confidence interval, is 71.2 percent to the 82.8 percent and their pvalue = 0.0007. The liquid assay correctly identified 95.9% of the 121 patients without tuberculosis as negative, while the LJ solid assay correctly identified 93.4% as negative. As a result, sensitivity of liquid assays was 89 percent and its specificity which was 95.9 percent.

TAT for MODS and LJ medium

In each sample, turnaround time which was calculated from date of the following inoculation for example sample processing and availability date

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of positive-results concurrent identification or susceptibility results. The median TAT for MODS was nine days, by a range for example five to twenty nine days. Whereas LJ's was 21 days and an IQR which was used for almost fourteen –twenty eight days. The period to positive culture results was evaluated about 138 samples which were positive including both micro of broth culture or L. Hence the amount of time it required to diagnose tuberculosis using micro broth culture was substantially faster than using LJ culture (P-value less than 0.0001).

Comparison of MODS with BACTEC-MGIT

The results which obtained by the MODS or BACTEC-MGIT that were in agreement usually for almost 55 isolates 94.5 percent, 23 susceptible, 32 resistant have 0.894 and 58 isolates at the low concentrations that tested for usually INH susceptibility (0.1 g/ml). Among the discordant samples, one strain which tested vulnerable with MODS while resistant with such as MGIT, or two strains that tested resistant mostly with MODS yet susceptible with the MGIT. For 57 strains tested for RMP susceptibility, there was full agreement between MODS and MGIT results (98.3%); 38 susceptible, 19 resistant. With MODS, the one discordant isolate was susceptible, but with MGIT, it was resistant. For fifty seven strains 98.3 percent, the analyses including its two methods which detecting the MDR-TB were very similar; 19 were MDR or 38 were not MDR is 0.961.

Discussion

Drug-resistant tuberculosis (DRTB) is a major obstacle to global TB control development. HIV including AIDS care or treatment programmes which are also involve in jeopardy as a result previous emergence of the XDR-TB between the patient of HIV-infected in South Africa, as well as more mortality that comes with it (Gandhi *et al.*, 2006). The key diagnostic method involve in DOTS strategy, sputum smear microscopy, is the inadequate which resolve this kind of emerging problem in the TB regulation. Some work has been done in this field recently, or new methods that detecting the TB drug resistance. Traditional solid-media of culture that takes three –four weeks to produce outcomes or have sensitivity from seventy six to eighty four percent; while twenty three and four is much better than the sputum smear of microscopy, but it still misses a significant percentage of following TB cases. Liquid media is the modern recommended culture or DST method of the choice, as it quicker and more sensitive than solid culture (Moore *et al.*, 2006). Therefore total of 25 studies testing the MODS were found in

our study. MODS had 92 percent pooled sensitivity and a 96 percent specificity, while MGIT had an 87 percent pooled sensitivity and a 98 percent specificity. Even in stratified studies, however, there was significant variability in these figures. Both tests had low reagent and material costs. In the same studies 38, the average or normal time from the receipt to analysis MODS took 9.2 days to complete inside the lab or 11.5 days usually for the MGIT, which was quicker than with traditional solid or liquid cultures. Hence proportion of infected specimens for the both assays that was minimal, and they were comparable to traditional cultures.

Conclusion

The study tell that liquid assay example micro of the broth culture including MODS with *Mycobacterium tuberculosis* reported in the literature are early, feasible, or inexpensive tool for evaluating pulmonary tuberculosis and outperforming traditional LJ of slant culture.

Conflict of Interest

The authors declared absence of conflict of interest.

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