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REVIEW ARTICLE

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A REVIEW ON TB AND ITS ADVANCE DIAGNOSTIC TECHNIQUES

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ABSTRACT

In the Industrialized world, the times has gone when Laboratories need weeks to month to diagnose Tuberculosis from clinical specimens, Currently the Mycobaecteriology Laboratory is Experiencing more changes than ever before, Past decade, new methods for culture and susceptibility testing as well as molecular tools have been introduced which allow a rapid laboratory diagnosis of TB.so it is necessary to diagnose the disease with particular test prescribed by the specialist.

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KEY WORDS

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INTRODUCTION

Tb is a paradox of modern age, in an era of unprecedented it inequitably distributed wealth and scientific advancement, and nearly two million people die each year from infectious disease for which prone, cost effective treatments have been available for decades. Thanks to the discovery of antibiotics and better living standards, the epidemic of "galloping consumption". The catastrophic history of tb can be traced in human back to about 6000BC.In 1882 Robert Koch discovered as isolated mycobacterium as the a etiological agent of TB (Harries 2008), and correctly asserted 'Pulmonary TB cases are infectious, tubercle bacilli are killed by light and care need to be noticed by tuberculin test". Following the scientific breakthrough, human king discovered the chemotherapy, streptomycin, and all currently used first line anti- tuberculosis drugs in 1944 and put these in practice to cure tb. Over 25 years of use of these technologies, the industrialized world achieved a steady decline in the prevalence rates between the late 1970s, and the early 1980s (Harries 2008). It is argued that tb morbidity and mortality began decline in Europe long before the introduction of BCG chemotherapy, suggesting the medical interventions may have contributed little to controlling TB(Mckown 1976), Rather it has been ascribed to improved nutrition, housing, reduced overcrowding , lower family size, reduced poverty, and social equity (Meckown 1976). Nevertheless the low income countries like evidently failed to reach such benefits from the scientific breakthrough, nor could improve socio economics and environmental conditions during the period (Raviglione *et al* 2002). TB control program showed that treating TB is a cost-effective intervention. They further recommended additional effort to increase TB case finding and improve accessibility of service to the community in line with millennium development goals. Tuberculosis continues to be a great challenge all over the world. The cause of recent epidemic is multifactor, including economic social factors, synergism between the Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis* and spreading of multi drug resistant tuberculosis (MDR) (Siddiqui *et al.,* 1998), what emerged as an important problem by the end of XX century. The disease, tuberculosis (TB) killed many patients.

In the Industrialized world, the times has gone when Laboratories need weeks to month to diagnose Tuberculosis from clinical specimens ,Currently the Mycobaecteriology Laboratory is Experiencing more changes than ever before , Past decade , new methods for culture and susceptibility testing as well as molecular tools have been introduced which allow a rapid laboratory diagnosis of TB. Determining which assay will be most useful is a challenge for clinicians and laboratories who are both aiming at providing the best care for the patients.

Mycobacterium complex- A Multifaceted Group of Complex Organism

In Contrast to the Manu Non-TB Mycobacterium whish are capable of Replicating in Animal Environment, the Major ecological niche for MBT complex Organism are tissues of human and Warm-blooded animals. This group of obligate Pathogens include several closely related species which display >95% DNA/DNA Homology; M.tuberculosis, M.Bovis, Mycobacterium bovis Bacilli Calmette-Gurien (BCG), M.*Africanum*, M.*Microti* etc (1-4).Like the Mycobecteria members of MBT complex are aerobic,

nonspore-forming,non motile is slightly curved as straight rods(0.2×0.6×1.0×µm).The high content of complex lipids of the cell wall, its most important component being Mycol-arbinogalacton present a hydrophobic permeability barrier that prevents penetration of common anilines dyes, ones stains with special procedure, Mycobecteria are not easily decolorized, Not even with acid alcohol ,i.e. they are acid fast. Due to a very long generation time (16-18) to under go one cycle of replication, MBT yields visible colonies after growth of several weeks on solid media. Colonies of Mycobacterium TB complexes are non-pigmented to buff, colony morphology is predominantly flat and rough in Fig 1.most prominent member of the complex, globally among the leading case of infectious disease (5).

Collection and Pretreatment of Specimens for Smear and Culture

Many different types of clinical specimens may be collected for Mycobecteria analysis (12-14).Most of the specimens originate from respiratory tract, but urine,gastricfluid,biopsies are normally sterile fluids are also common, generally as much specimens as possible should be submitted to the laboratory. Transports Media and Preservatives are usually need owing to robust nature of the Mycobecteria. Also it is important that Fixatives to be added. Swabs are, in general, not optimal for the recovery of AFB Since the amount of material to be analyzed is limited and hydrophobic cell envelope of Mycobecteria comprises a transfer of the organism from swab to media. If processing delayed for more than in specimen should be refrigerated at 4c, except blood and bone marrow. Normally sterile specimen such as biopsies or body fluids other than gastric juices usually do not require pretreatment if collected aseptically (12-14)Tissues and biopsies should be ground in sterile physiological saline before inoculation and body fluids concentrated to maximize the yield of Mycobecteria. To check for possible contaminates, any specimens may first be inoculated on a chocolate agar plate. The majority of specimens submitted for mycobacterial culture, however consist of complex organic matrix contaminated with a variety of microorganisms such specimen need to homogenized and decontaminated by adequate procedures (12-14).Prior to inoculation to the media pretreated specimens have to be concentrated. Since harsh decontamination can skill 20-90% of Mycobecteria present in clinical specimens, it is important to apply pretreatment which is effect in removing unwanted organism, but at the same time, guarantees maximum survival of mycobacterium. Excellent results are achieved by decontamination with N-acetyal-L-Cysteine/NaOH.One specimen are pretreated ,the contamination rate of media should be around 5%.If it is lower pretreatment it to harsh, if it is high pretreatment was either too weak or digestion incomplete.

Direct Detection of Acid Fast Microscopy

Smear Microscopy is still the most rapid inexpensive ways diagnose .TB and a rapid means to identify the most contagious patients while MBT may be invisible by Gram stain or only faintly stains ‘’ghost’’, special acid fast staining procedure are necessary to promote the up take of dyes (12-14) Phenol and high temp as used in the classical Ziehl-Neelson staining allow penetration to the carbol-fuchsin stain more easily. When counter stained with methylene blue, as red to pink stained AFB are highlighted against a blue black background which facilities the microscopic recognition. Mycobecteria are also capable of forming stable complexes with some aryl methane dyes such as Auramino.The later is used for Fluorescence microscopy which allows a more rapid screening of the slides because they can be read at a lower power, As shown recently Kin-Youns Carbol-Fuchsin method is inferior to both the Ziel-Neelsen and Flurochrome techniques (17).The reliability of Smear microscopy is high dependent on the experience of Laboratory personal and on throw number of organisms present in the

specimens.10^6 AFB/ml usually result in a positive smear. However only about 60%of the smears are positive if 10^6 AFB/ml are present. The overall sensitivity of the smears range from 22to 80% (18). In follow up specimens of patients under treatment, the diagnostic value of the smear is limited such patient may have dead bacteria for a long period of time while culture shows no growth. Specifying of the smear for detection of mycobacterium is very assessing AFB morphology for presumptive identification of Mycobecteria at the species level. Is however dangerous. Sputum smears should be done from pretreated, concentrated specimens to increase sensitivity. It is best to confirm the smears by having them reviewed by another, experienced techniques Good lab practice include the confirmation of a Flurochrome the smear by a Carbol-Fuchsin staining method(Ziehl-Neelson) and reporting the result with in 24hrs upon arrival of the specimen in the laboratory (21).

Direct Detection of MBT Complex by Nucleic Acid Amplification Base Method

Direct detection of MBT complex in clinical specimens by Nucleic acid amplification (NAA) - based assay represent one of the most significant improvement in mycobecterial diagnostics. Theoretically TB can be diagnose on the day of arrival of a specimen in the laboratory Home brew PCR protocols amplifying a large variety of chromosomal DNA element,(22). This technique together with other method e.g., Transcrption-meadited amplification (TBA), Ligase Chain Reaction(LCR) and strand displacement amplification (SDA), have been developed are marketed in user friendly , kit based formats, targeting either DNA or RNA to guarantee a high degree of reproducibly and to facilite and to facilitate its application in a clinical Mycobaecteriology laboratory. Although NAA based techniques have the potential to detect Mycobecteria other than MBT Complex, Current experiences largely based on the diagnostics of TB with emphasis on respiratory specimens. Only a few studies have addressed the use of NAA assay for extra pulmonary specimens (23). Most often, these specimens certain small no of AFB only. AS a consequence, smears are often negative and culture yields MBT after several weeks only if at all. Molecular test should be requested by the clinician when a rapid confirmation of suspected TB patient is important, because of high perspective value of the positive smear of respiratory system of MBT (79%)(16),such specimens do not necessity have to be tested with NAA based method. With a smear such test may however be welcome a chest a) X-ray is abnormal b)the patient is iummnosupressed(HIV infected) c) there are epidemiological concerns d) there are clinical symptoms but culture remains negative. In the smear negative situation, NAA based test can helpful in TB is differentially diagnosis (24)

Culture the back bone of the diagnosis of TB

Despite the advance in direct detection of MBT complex in clinical specimens of molecular methods at present culture is indispensable for a number of reason since it a) Enhance diagnostic certainly, particularly when NAA based results are available b)Provide biomass for further identification and antimicrobial susceptibility test and c) detect NTM, in addition to MBT complex. For detection of Mycobecteria in clinical specimens, to cement ‘’gold standard’’ consist a combination of solid and liquid media. Solid semi solid media have been known for decades, more attractive and liquid media which offer significantly shorter turn and times for detection of mycobecteria.In detecting as 10^{-1}-10^{-2} viable organism/ml specimen, cultural is more sensitive then smear, Also it is the only reliable means to monitoring the effectiveness of therapy in TB patient (26)

SOLID MEDIA Eggs based media such as Lowenstein-Jenson (LJ), Ogawa or stone brink have the capability to bind and neutralize toxic compounds encountered in clinical specimens, usually, and they contain Malachite green, a

dye which inhibits growth of containing organisms. Eggs bared media support growth on average, 18-25 days, since they non synthetic the quality of the ingredients may vary considerably may affect the reproducibility of results. Agar based media e.g. Middle rook7H10 or7H11 are chemically better defined and show growth of MBT complex colonies with in 10-14 days. Visual examination of the colonies is much easier. Negative aspects of agar media are limited shelf –life higher costs of preparation and requirement for CO2 enriched atmosphere.

LIQUID MEDIA a part from the biphasic septi-chek system (Beckton-Dickinson microbiology system, sparks MD, USA) the radiometric, semi automated BACTEC460TB system (Becton D.M.S) represented the most efficient and rapid technique to culture mycobecterial for more efficient and rapid techniques to culture Mycobecteria for more two decades is still used by many laboratories to date by this mechanism 14CO2labeled Palmitic acid as a carbon sources in medium is metabolized by microorganism to 14CO2 which is monitored by the instrument . The amount of 14CO2 and the rate at which the gas is produced are directly proportional to the growth rate of the organism in the medium for MBT. An average diction time of 8 days was found for smear. Positive specimens, compared to 19 days in non radioactive, conventional solid media (27). For smear negative specimen, an average recovery time of MBT between 14 days in the BACTEC 460TB and 26 days in conventional solid media has been reported(28).Although utilizing different detection principle, the new culture concept have in common that they are based on Non –radiometric liquid media. New development range from manual systems utilizing simple tubes (MB red ox, HEIPHA Diagnostic a Biotech, Heideberg,Germany;Mycobectera growth Indicator Tube(MGIT), Becton Dickinson microbiology system)to fully automated system BACTEC MGIT 960, Becton Dicksion microbiology system;MB/BACTEC Alert3D, Bio-meriax,Marcy-Etoile,France;ESP CULTURE system 11,Westlaka,Ohio,USA).The non automated MB redox technique is based on a modified Kirchner medium which contain a colorless tetrazolium salt as redox indicator which is reduced to colored formazan by activity growing Mycobecteria. AFB can than detected visually as pink-to purple pin head size particle. Another manual culture method is represented by the MGIT (Becton Dickinson microbiology system) which contain a modified 7H9 broth in conjunction with fluorescence quenching based oxygen sensor. Growth of mycobecterial or other microorganisms in the broth depletes the oxygen, and the indicator fluorescence brightly when tubes are illuminated with UV at 365nm.Currently there are fully automated systems which allow contain of monitoring of mycobecterial cultural allows a) Bactec MGIT 960 based on MGIT technology b) The Mb/Bact Alert 3D utilizing a colorimetric co2 sensor in each bottle detect growth of Mycobecteria and c) The ESP culture system!! Which is based on the diction of pressure changes head space above the broth medium resulting from gas production or confirmation due to the growth of microorganism? All the new non labeled system has similar performance characteristics. In clinical evaluation, recovery rates and time to detection were similar to those of the BACTEC 460TB system and superior to those of conventional solid media (mb redox (29); Manual MGIT (29); BACTEC MGIT960 (30); ESP Culture system!! (32).

IDENTIFICATION OF MBT COMPLEX

The use of Non radioactively labeled nucleic acid probe kits were a big step forward towards a more rapid identification of Mycobecteria 10 years ago. For MBT complex probe technology has a sensitivity of nearly 100% provided that approximately 10^5AFB/ml is present. Result of culture confirmation is available with in 2hrs. Specificity of the MBT complex probe is high as well. It is however, impracticise test are precisely done as recommended by the manufacturer .Otherwise

cross-section with Mycobecteria other than MBT complex may occur, as documented in rare instance (33).Nevertheless gene probes, are unable to differentiate within MBT complex. Identification of the species level is, However, is justified for epidemiologic, public health and therapeutic reasons. Most strains of MBT complex is encountered ia a clinical Mycobaecteriology laboratory can easily be identified by physiological and biochemical criteria Table. 1.For strains with phenotype which do not match fully with the type strains or do not grow additional tests and procedure, mainly molecular once, have to be considered M.Canettii and most strains of M.microtti, for instance can be confirmed by spacer oligonucleotode typing (Spoligotyping)other markers are OxyR and PncA genes which show intraspecific sequence polymorphisms and are useful for distinction between MBT and *M.Bovis* (35). PCR test for the RD sequence may contribute to a differentiation of *M.africanum*, MBT and M.*microtti*(36) Recently a combination of selected conventional method and comparative genomic based PCR detection analysis have been suggested for rapid identification of the member of MBT complex.(37).

Table 1 (a)
Test Commonly Used For Identification of MBT Samples

Species'	No3 Reduction	Niacin Production	LebekTest	T2H Inhibitor (b)	Susceptibility to Pyrazamide
MBT	+	+	-	R	S
MBT Asian	+	+	-	S	S
MBTspp					
carprae	-	-	-	S	S
M.*Bovis*	+	-	+	S	R/S
M.*Bovis*					
BCG	-/+	-/+	-	S	R
M.*Africanum*					
Leonean Type	+/-	+/-	+	S/R	S/R
M.*Africanum*					
Ugandan	?	+/-	+/-	R/S	S
M.*Microtti*	?	?	?	?	?
M.*Microtti.*					
Cainettii	+	-	-	S	S/R

a) c) =? = Unknown

Susceptibility testing of MBT complex

Drug susceptibility testing is a mandatory an initial isolates of MBT and released from all patients. If the culture remains positive over a longer period susceptibility testing should be repeated to monitor a possible development of drug resistance The new guidelines be National committee of clinical laboratory standards (NCCLS) currently recommendation to repeat susceptibility testing at least every three month (38).Most experience is available on testing primary antibiotic i.e. isonizied (INH) Rifampin (RMP), Ethambutol (EMB) and Pyrazinamide (PZA). By culture based method, although there are three accepted method for drug susceptibility testing of MBT (i.e. the absolute concentration method, the resistance ratio method, the proportion method (39), the later is widely used in the western hemisphere. By this method for most of the antituberculosis drug, resistance of MBT is defined as presence of resistant organism in>1% of a given population of MBT cells. Therapy will ultimate be successful in the critical proportion of organisms and critical concentration of drug (i.e. the concentration which inhibit the wild type but not the resistant mutant) are strictly defined and accordingly, the strain is fully susceptible to front-live drugs in vitro. Despite more rapid culture based method tested(38).Susceptibility testing results for the primary antituberculosis drugs can be rapidly generated by the BACTEC 460TB.Once resistance to more drug being detected it is good lab practice to conform it either by a second method(Agar proportion method)since the impact of resistance strains on patient management obvious (21).Although susceptibility test on solid media e.g. Middle brook 7H10 agar or LJ) for confirmation of resistance take quite time it is important since it can indicate false resistance due to mixed population of MBT. If resistance to one several primary drugs i.e. being detected an extended spectrum of drugs may be tested, either by radiometric procedure (40).Different methods shows in table 2.

Table 2

A Summary of Lab Methods Available in Diagnosis of TB In clinical specimens

Method	Turn and Time	Comments	Advantage	Disadvantage
AFB Smear	24hrs	+ Smear requires 5000-10,4AFB/ml	Rapid, cheap high +tive predictive value.	No species diagnosis
NAA Based Assay	6-48hrs	Req, appropriate lab Equipments	Rapidity of Diagnose	
Culture	Days to week	req, 10-100AFB/ml	good for morphology To observe spp.	Take long time
By Gene Probe	few weeks	for culture not for clinical Specimens	Specifics and close to 100%	
By Bio-Chemistry	several weeks	Nitrate, Niacin catalase		Long time
16Sr DNA Seq.	24-48hrs	does not identify MBT Complex	-	-
Susceptibility Method	several days	Req BACTEC 460TB, Radiometric	-	-

Based				
Molecular	1-2 days	Gene sequencing,Line probe assay	rapid detection	expensive

Agar proportion method.

In last decades antimicrobial susceptibility testing has become dynamic field spawing may new technologies that may one day prove successful in a clinical Mycobaecteriology laboratory. Among the leading novel strategies are, again several of the new growth based , no radiometric methods preliminary studies which include susceptibility testing of MBT to INH,RMP,EMB and SM by the BACTEC MGIT 960 (41) the MB/Bact Alert 3D (42) and the ESP culture system(43). Show excellent overall agreement with the proportion method on solid media and liquid media as well as promising turn around and time comparable to those by radiometry. Susceptibility tasting of MBT to PZA is more difficult due to the requirement of a more acidic Ph value in the medium.

Although molecular approaches would provide the clinician with susceptibility results with in 1-2 days most of these protocols yet no ready for the clinical Mycobaecteriology lab. Resistance in MBT complex organisms arises from mutagenesis which are confined to chromosomal DNA and do not involve mobile genetic elements such as plasmids. Molecular research has shown that often more than a single mutation is responsible for drug resistance making the whole issues very complex, particularly since different genes can be invented. Resistance to INH for instance appears to be results of one single as of multiple mutations in the KatG, OxyR-anPc and KasA genes. RMP resistance will appear to be best understood. Owing the fact that >79% of all RMP resistant strains carry a mutation in aseptic core region of the rpogene (48), resistance to the drug can easily and reliably be detected by molecular method. This may be achieved by probe assay and gene sequencing.

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