EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

EPIDEMIOLOGICAL SURVEILLANCE OF *MYCOBACTERIUM TUBERCULOSIS*; DIAGNOSIS REVIEW AND UPDATE

*Olowe OA, Oyedeji JG, Akanbi OA and Olowe RA

Department of Microbiology and Parasitology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria**.**

*Correspondence for Author

Dr. Olowe OA

Department of Microbiology and Parasitology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria**.**

ABSTRACT

In past efforts to eradicate or curb the spread of any infectious disease it has been established that proper diagnosis is an important factor. The case with tuberculosis is not an exception. With proper diagnosis coupled with effective ongoing surveillance, new cases can be detected early and treated promptly, as such new cases can be prevented and TB related deaths reduced. Also, with advanced diagnostic methods, patient's response to drugs can also be properly monitored and the spread of resistant strains can be curbed and treatment failure reduced.

Diagnosing active tuberculosis based merely on signs and symptoms is difficult as is diagnosing the disease in those who are immunosuppressed. A diagnosis of *Mycobacterium tuberculosis* should, however, be considered in those with signs of lung disease or constitutional symptoms lasting longer than two weeks. The review present an update on important developments in TB diagnostics over the past decade, in both latent infection and active disease, briefly discuss.

KEYWORDS: *Mycobacterium tuberculosis,* surveillance, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is an important communicable disease that has been reasons for serious morbidity and mortality all over the world in the last decades of the 20th century.[1] In Nigeria, TB infection is a common occurrence with Nigeria ranking top in Africa with respect to TB burden.[2] The success of tuberculosis control programmes significantly depends on the quality of diagnostic services available. Within the framework of National

Tuberculosis Programmes, the first purpose of diagnostic services is to detect infectious cases of pulmonary tuberculosis, monitor treatment progress and document cure at the end of treatment by means of microscopic examination.[3]

Detection of cases may be active, involving a deliberate search for cases or passive, relying on patients with symptoms presenting for treatment. The latter approach requires much less investment in time and personnel but its success depends on public education and the availability of user-friendly facilities.[4]

A complete medical evaluation for tuberculosis (TB) must include a medical history, a physical examination, a chest X-ray and microbiological examination (of sputum or some other appropriate samples). Tuberculosis is diagnosed by finding *M. tuberculosis* bacteria in a clinical specimen taken from the patient. It may also include a tuberculin skin test, scans, X-rays and surgical biopsy. In the light of this, the diagnosis of tuberculosis can be divided into two; clinical and laboratory diagnosis.

Methods for Diagnosis of tuberculosis

Clinical diagnosis

Clinical diagnosis involves taking a patient's medical history and physical examination. Medical history includes obtaining the symptoms of pulmonary TB such as productive, prolonged cough of three or more weeks, chest pain, and haemoptysis. Systemic symptoms include low grade remittent fever, chills, night sweats, appetite loss, weight loss, easy fatigability, and production of sputum that starts out mucoid but changes to purulent [4]. Other parts of the medical history include prior TB exposure, past TB treatment, demographic risk factors for TB, and medical conditions that increase risk for TB disease such as HIV infection.[5]

Physical examination is also done to assess the patient's general health and find other symptoms suggestive of TB infection. The most common symptoms of pulmonary TB are: fever and sweating, weight loss, cough, haemoptysis, dyspnea, thoracic pain and hoarseness. Some of these symptoms however, are nonspecific and can be present in other diseases, therefore, clinical diagnosis alone cannot be conclusive in TB diagnosis.

Laboratory Diagnosis

Diagnosis of tuberculosis and monitoring of treatment progress relies greatly on bacteriological examination of clinical specimens. The usefulness, priority and scope of the various techniques used in tuberculosis bacteriology depend on the epidemiological situation prevailing in individual countries and on the resources available.[6] There exists several laboratory methods for identification of *M tuberculosis.*

1. Microscopy
2. Culture methods
3. Rapid Identification methods
4. Skin Tests
5. Immunological or Serological tests
6. Heamatological and Biochemical examinations
7. Molecular Techniques

1. Microscopy

Ziehl-Neelsen (zn) staining method

The principal method of pulmonary TB diagnosis is microscopic examination of Ziehl-Neelsen stained sputum samples. The technique utilizes basic fuchsin in ethanol for primary staining. Ziehl-Neelsen is a hot acid-fast stain because the slide has to be heated during incubation with fuchsin.The sputum specimens are smeared directly onto the slides (direct smears) and subjected to Ziehl-Neelsen (ZN) staining.[7]

Kinyoun Staining

This is a modified method of Ziehl-Neelsen staining technique; for AFB diagnosis. Kinyoun staining is a cold acid-fast staining procedure and therefore does not require heating. Kinyoun stain has a greater concentration of phenol and basic fuchsin to allow penetration of the dye without applying heat. In both the Ziehl-Neelson and Kinyoun cases, AFB appears red after decolorization with acid alcohol.[7]

Lapeyssonie and Pause's method

Heating of smear in strong carbol fuchsin is not required. The method utilizes Teepol as well as a high concentration of basic fuchsin in phenol when compared with the carbol fuchsin of the traditional Ziehl Neelsen technique. The counterstain methylene blue is incorporated into the acid alcohol differentiator so that differentiation and counterstaining of smears are

simultaneously done. Results are similar to Ziehl Neelsen technique where acid fast bacilli are stained red.

Fluorescent microscopy method

The Fluorescent Microscopy Method uses fluorescent dyes to stain the mycobacterial cell: Two dyes Auramine 0 (Bright yellow) and Auramine 0-Rhodamine B (Yellow orange) are used in the fluorochrome procedure, primary staining is done with Auramine 0. The AFB fluoresce yellow against a counter stain of potassium permanganate when observed with a fluorescence microscope. While the reading of fuchsin-stained smears requires 1000x magnification, fluorochrome-stained smears are examined at 250x or 450x. The lower magnification used in this staining method allows the observation of a much larger area of the smear during the same period of time and thus, fewer fields are read. This procedure makes the method faster. Allegedly, fluorescent staining is more sensitive than Ziehl-Neelsen staining,[8] however, it has been claimed that both methods have comparable sensitivity, provided procedural standards are followed.[9] The method is very rapid and as a result, laboratories processing large numbers of specimens should adopt this technique, however, the fluorescence fades with time and so, the slides must be read within 24 hours. This staining method is also not often available in resource-limited areas due to the high cost of the fluorescence microscope and, especially, that of its maintenance.

2. Culture Method of TB Diagnosis

Isolation of *M. tuberculosis* complex in culture from the clinical specimens provides a definitive diagnosis. Cultivation, usually on solid media, is more sensitive than microscopy and increases the diagnosis yield by up to 50%. TB culture is required in order to isolate and identify the Mycobacterial species causing infection in an individual. It is also required in order to determine antibiotic susceptibility and when the number of bacilli in the clinical specimens is so low that microscopy may be negative, culture remains the gold standard for TB diagnosis. Culture can detect low AFB in specimens. Its disadvantage is the delay, usually 3-6 weeks, between receipt of the specimen and the emergence of visible growth.[10]

3. Rapid Identification Tests

TB antigen MPT64 is a rapid immune-chromatographic identification test for the *M. tuberculosis complex* that uses mouse monoclonal anti-MPT64. This test kit can be easily used for rapid identification of the *M. tuberculosis complex* in combination with culture system based on liquid media without any technical complexity in medical laboratories. The

kits produced by BD diagnostics and SD Bioline are examples of rapid test kits for the identification of *M. tuberculosis complex* from cultures.

4. Skin tests

Tuberculin Skin Test (TST)

The currently used tuberculin skin test (TST) is quite inexpensive and has been used worldwide for many years. The TST measures a delayed-type hypersensitivity response to purified protein derivative (PPD), a crude mixture of antigens from the members of the MTBC (and also NTM). This is usually positive in patients with tuberculosis although, due to genetic factors, a minority (up to 80% in some regions) fails to respond. The test does not clearly distinguish between active tuberculosis, past infection by tubercule bacillus and BCG vaccination. Also, the administration and reading of the TST require a certain amount of expertise that, when lacking, may result in erroneous interpretations.[11]

Mantoux test

An inter-cutaneous injection of 0.1 ml of tuberculin is given on dorsal aspect of the forearm. After 48 to 72 hours, the transverse diameter of any palpable induration, but not erythema, is measured. In both epidemiological and diagnostic work the criteria for a positive reaction will be determined by the national tuberculosis programme, taking into account the type and concentration of tuberculin Purified Protein Derivative (PPD) used and the degree of sensitization by environmental mycobacteria. In the UK and USA, responses of 5 ml or more to 1international unit (IU) and of 10 ml or more to 5 IU are respectively regarded as positive. In diagnostic work, smaller reactions are usually regarded as negative, although they do not exclude tuberculosis because of the conditions mentioned above which suppress the response and the small number of people who never react. Conversely, a positive reaction, for the reasons discussed above, does not necessarily indicate active disease. Reactions may be due to prior BCG vaccination but a reaction of 15 mm or more in diameter in a vaccinated child is a very likely indication of infection by the tubercle bacillus.[12]

Heaf test

This method employs a spring-loaded 'gun' which drives 6 needles into the skin of the dorsal aspect of the forearm through a drop of undiluted PPD. The method is technically easy but it is necessary to autoclave the gun between use in order to avoid transmission of HIV and other viruses. Some guns have detachable magnetic heads which can be autoclaved separately. The

practice of dipping the head in alcohol or flaming it is unsafe. The test is read at 48-72 hours (although a strong reaction will still be visible at 7 days.[13]

Tine test

This is similar, except that PPD is dried onto four spikes (tines) on a small, single-use, disposable unit. The device is pressed firmly onto the skin so that the tines penetrate the skin and held in place for 10 seconds so that the dried PPD dissolves in the tissue fluids. Results are more variable than with the other test methods but it has some advantages when very few people are tested.[14]

5. Immunological and Serological Tests

There have been numerous reports of elevated level of antibodies to a range of antigens of the tubercle bacillus in tuberculosis, but the low sensitivity and specificity of serodiagnostic tests limit their usefulness. Despite several descriptions of enzyme-linked immunosorbent assay tests for tuberculosis, no universally applicable test with acceptable sensitivity and specificity is available.

ELISA- Techniques

The Enzyme Linked Immunosorbent Assay (ELISA) is a useful serological tool for TB diagnosis. In the ELISA technique, several antigens must be combined as a cocktail mixture. Fusion proteins incorporating several antigens using the standard recombinant DNA technology can be produced.

Quantiferon-TB Test: QuantiFERON-TB® and Bovigam® are two registered products which measure the release of interferon-gamma in whole blood from human subjects and cattle infected with *M. tuberculosis* and *M. bovis* respectively, in response to stimulation by PPD. The IFN secreted by T-cells into the plasma is measured by ELISA to indicate the likelihood of TB infection. Different studies demonstrated that the QuantiFERON-TB test was comparable to tuberculin skin test (TST) in its ability to detect latent TB infection.[15]

Interferon-Gamma Assay (Interferon-gamma determination): Interferon-Gamma Assay (Interferon-gamma determination). This is one of the most significant developments in the diagnostic armamentarium for TB, especially in pediatric TB testing in the last hundred years, the assays are based on IFN-y determination. The assays stem from the principle that T

cells of sensitized individuals produce IFN- when they re-encounter the antigens of *M. Tuberculosis.*[15]

Enzyme-Linked Immunospot for interferon-gamma (ELISPOT): The ELISPOT assay for diagnosis of *M. tuberculosis* infection is based on the rapid detection of T cells specific for *M. tuberculosis* antigens. IFN-y released in vitro from these cells can be detected by the extremely sensitive ELISPOT.[15] Such T cells give rise to a dark spot and the readout is the number of spots. The T cells enumerated by the ELISPOT assay are effector cells that have recently encountered antigen invivo and can rapidly release IFN-y when re-exposed to the antigen. In contrast, the long-life memory T cells, which persist long after clearance of the pathogen, are relatively quiescent and less likely to release IFN-y during the short period of exposure to antigen in the in vitro ELISPOT assay.

LAM assay (for urine, CSF): LAM assay (for urine, CSF) Very useful in pediatrics and TB testing in patients who could not produce sputum samples. The principle is based on the detection of lipoarabinomannan (LAM) in urine or Cerebro spinal fluid (CS F). LAM is a major and specific glycolipid component of the outer mycobacterial cell wall. Studies have shown that LAM is excreted in the urine of mice injected intraperitoneally with a crude cell wall preparation of *Mycobacterium tuberculosis*. Lam detection in urine or CSF is highly sensitive. LAM at concentrations of 1ng/ml and5 pg/ml can be detected.[16]

Other serological tests includes; 1gM and lgG Assays.

6. Haematological and Biochemical examinations

Haematological and biochemical changes in tuberculosis are rather non-specific. Blood examination may reveal a raised lymphocytes count, a raised erythrocyte sedimentation rate (ESR), elevated C-reactive protein levels and a mild anaemia. These changes resolve on effective treatment. Serum albumin levels are sometimes low and there may be mild abnormalities of liver function. Elevations in the levels of various enzymes in cerebrospinal, pleural, pericardial and peritoneal fluids have been described but their diagnostic significance has not been clearly established.

7. Molecular Techniques

Traditionally,*Mycobacterium tuberculosis* complexstrains were defined according to their phenotypic traits and geographic origin. Nuclei acid amplification tests and adenosine

deaminase testing may allow rapid diagnosis of TB.[17] However, based on genetic definitions some strains are now regarded as phenotypic variants of *M. tuberculosis.*[13] As a result, strains are defined on the basis of particular genotypic characteristics that place them in the *M. tuberculosis* complex; hence, genomic deletions, or regions of difference (RDs), can effectively distinguish the different strains. Molecular epidemiology generally aims to investigate whether naturally occurring strains differ in epidemiology. For instance, do specific clinical strains differ in their infectiousness, severity of disease, or susceptibility to anti-tuberculosis agents? Molecular epidemiology can serve to better inform routine TB control activities. Successful molecular epidemiological investigations have sought to estimate the fraction of cases attributable to recent transmission or reactivation, distinguish between endogenous reactivation and exogenous re-infection, investigate properties and patterns of drug resistance with specific populations or groups of strains, and better understand transmission dynamics within specific populations.

Molecular methods have a lot of advantages over the traditional methods such as; more specific results can be obtained, it can be used for epidemiological studies, it can be used for contact tracing, it can be used to detect infection of an individual with more than one strains of the organism and the results are obtained faster than in traditional methods of culture and identification.

Target Genes Used in Molecular Diagnosis of TB

Below is a list of target genes that can be used in molecular diagnosis of TB. 16S ribosomal DNA - Cannot distinguish between many atypical species of Mycobacteria (e.g., *M. kansasli* from *M. gastri)* 16S-23S ITS region - Differentiates only the rapidly growing Mycobacterial species.

The 65 kDa heat shock protein gene, e.g., rec A, (involved in DNA repair), sodA (encoding for superoxide dismutase), and rpoB (encoding for the beta-subunit of RNA-polymerase), used as the gold standard only for the differentiation of rapidly growing Mycobacteria Xpert MTB-RIF gene - Used for detection of MTB and resistant gene to rifampicin.

Genetic Sequencing Variability

Genetic sequencing is nowadays the reference identification method, not only for mycobacteria but for all microorganisms, and the *16S* ribosomal DNA is still the most important target sequence. Sequencing of the 5' end (about 500 bp) provides final results for

the vast majority of members of the genus mycobacterium. The determination of the full gene is needed to distinguish *M. peregrinum* from *M. septicum*, *M. murale* from *M. tokaiense, M. marinum* from *M. ulcerans*, and *M. novocastrense* from *M. flavescens.*[18] The only species that cannot be distinguished from each other on the basis of 16S ribosomal DNA are *M. kansasii* from *M. gastriM. mucogenicum* from *M. phocaicum*, *M. fluoranthenivorans* from *M. hackensackense,*[7] and lastly, *M. abscessus* from *M. massiliense* and *M. bollettii.*[19] Due to its wider variability (its length ranges from 270 to 400 bp).

ITS gene can be usefully sequenced to differentiate the rapidly growing species,[20] which are more closely related to each other than the slow growers. Rapid growers have two copies of the ribosomal operon (except for *M. chelonae* and *M. abscessus* which have one) and a single organism may possess two different ITS copies. This may make the interpretation of the electropherograms problematic because of the presence of overlapping peaks. To obviate this problem, cloning of the genetic region is required before sequencing. Another increasingly used genetic target for identification purposes is a 440 bp sequence of the 65 kDa heat shock.

Xpert MTB/RIF Test

Xpert MTBIRIF is an example of an automated, cartridge-based nucleic acid amplification assay for the simultaneous detection of TB and rifampicin resistance, directly from sputum in under two hours. This means that the equipment performs PCR, detection of the product, sequencing, and data comparison of the sequenced product with rifampicin resistance. WHO endorsed the technology in December 2010 and is monitoring the global roll-out of the technology to promote coordination.[21]

Conventional diagnosis of drug resistant TB relies on bacterial culture, identification and drug susceptibility testing, a slow and cumbersome process. During this time, patients may be inappropriately treated, drug resistant strains may continue to spread, and resistance may become amplified. The new Xpert MTB/RIF test is rapid, fully-automated and therefore not as susceptible to human error. It provides a highly accurate diagnosis in a single test that identifies both the presence of TB and drug resistant TB. This equipment lets people be offered the proper treatment immediately.[21]

It is also recommended that an additional sputum specimen should be collected from sputum smear-negative individuals who are still suspected to have TB and referred for further testing at a facility where Xpert MTB/RIF testing is available.[21]

ACKNOWLEDGEMENT

REFRENCES

1. Murray CJL, Dejongbe E., Chum HJ., Nyangulu DS., Salamd A., Styblo K. Bacterial infection, cost effectiveness of chemotherapy for pulmonary TB in three sub-saharan African countries, infect. Dis. Lancet, I992; 85-88.

2. WHO Global Tuberculosis Control: Epidemiology, Strategy, Financing: WHO report 2009.

3. Collins CH, Grange J M and Yates M D. 1997; *Tuberculosis Bacteriology*: organization and practice second edn. London: butterworth- Heinemann.

4. Olowe OA, Famojuro HB. Laboratory Identification of TB using simple Rapid Techniques in Osogbo South Western Nigeria. Res. J Med. Sc 2010; 4(3): 227-230.

5. Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson; & Mitchell, Richard N. *Robbins Basic Pathology* (8th ed.). Saunders Elsevier. 2007; 516-522.

6. WHO 1998, Services in TB control part 3.Whqlibdoc.Who.int/hq/1998/WHO-TB-98.258-(part 3)

7. Tortoli, E., Nanetti, A., Piersimoni, C., Chichero Farina, C., Mucignat, G., Scarparo, C., Bartolini, L., Valentini, R., Nista, D. & other authors. Performance assessment of new multiplex probe assay for identification of mycobacteria. J Clin Microbiol 2001; 39: 1079–1084. Steingart et al 2006.

8. Steingart K. R., et al. 2006. Fluorescence versus conventional sputum smear microscopy for tuberculosis: asystematic review. Lancet Infect. Dis. 6:570–581.[PubMed]

9. Somoskovi A., Parsons L. M., Salfinger M. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. Respir. Res. 2001; 2: 164–168.

10. Keating L. A., Wheeer P.R., Mansoor H., Inwald J. K., Dale J., Hewinson R. G., Gordon S. V., Mol. Microbiol. 2005 Apr, 56 *(Adekambi, T., Colson, P et al., 2003)*: 163-74 [PubMed]

11. Huebner R. E., Schein M. F., Bass J. B., Jr. The tuberculin skin test. Clin. Infect. Dis. 1993; 17: 968–975. [PubMed]

12. CDC - Guidelines for Using the QuantiFERON-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States, 2000.

13. Grange J, Winstanley P A and Davies P D O. Clinically significant drug intkeractions with anti tuberculosis agents. *Drug Safety* 1994; 11: 242-251.

14. Pai M., Riley L. W., Colford J. M., Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: asystematic review. Lancet Infect. Dis. 2004; 4: 761–776.[PubMed]

15. Lalvani A., Richeldi L., Kunst H. Interferon gamma assays for tuberculosis. Lancet Infect. Dis. 2005; 5: 322–324. [PubMed] 109.

16. Daley P., et al. Blinded evaluation of commercial urinary lipoarabinomannan for active tuberculosis: a pilot study. Int. J. Tuberc. Lung Dis. 2009; 13: 989–995. [PMC free article] [PubMed]

17. Bento, J; Silva, AS; Rodrigues, F; Duarte, R. "[Diagnostic tools in tuberculosis]". *Acta medica portuguesa* Jan–Feb 2011; 24(1): 145–54. PMID 21672452.

18. Kirschner P., Springer B. et al,. Genotypic Identification of Mycobacteria by nucleic acid sequence determination: Report of a two year experience in aclinical laboratory. J of clin microbiol. 1993; 31: P 2882-2889

19. Adekambi, T., Colson, P. & Drancourt, M. rpoB -based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J Clin Microbiol, 2003; 41: 5699–5708.

20. Roth, A., Reischl, U., Streubel, A., Naumann, L., Koppenstedt, R. M., Habicht, M., Fischer, M. & Mauch, H. Novel diagnostic algorithm for identification of mycobacteria using genus- specific amplification of the 16S–23S rRNA gene spacer and restriction endonucleases. J Clin Microbiol, 2000; 38: 1094–1104.

21. World Health Organization 3 February 2011, accessiondate Indicator. World Health Organization,Geneva,Switzerland:
http://www.stoptb.org/wg/tb_hiv/assets/documents/cgd1/IRUM%20Draft%20WHOPEPFAR%20Indicator%2004%2018%2008.doc