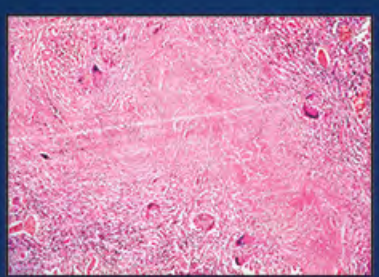
ISSN 2319-1260

January 2014 Volume 3 Number 1

World Clinics

PULMONARY & CRITICAL CARE MEDICINE

Tuberculosis







Editor-in-Chief
Surinder K Jindal

Guest Editor
Digambar Behera

JAYPEE

Contents

Editorial xi
Digambar Behera

Abbreviations xiii

Epidemiology of Tuberculosis **1**
Digambar Behera

Mycobacteriology, Pathogenesis, and Pathology of Tuberculosis **6**
Vinaya S Karkhanis, Gayathri P Amonkar, Jyotsna M Joshi

Clinical Features and Complications of Tuberculosis **21**
Parvaiz A Koul, Nargis K Bali

Radiology of Pulmonary Tuberculosis **46**
Mandeep K Garg, Anmol Bhatia

Laboratory Diagnosis of Tuberculosis: State-of-the-art **66**
Madhukar Pai

Treatment of Tuberculosis **79**
Surinder K Jindal

Newer Antituberculosis Drugs **86**
Alladi Mohan, J Harikrishna, D Prabath Kumar

Smoking and Tuberculosis **97**
Raj Kumar

Tuberculosis and Pregnancy **104**
Jai B Sharma, Prerna Gupta

Tuberculosis and Liver Diseases **110**
Tarana Gupta, Swastik Agrawal, Radha K Dhiman

Drug-resistant Tuberculosis **125**
Rajesh N Solanki

Tuberculosis Emergencies **141**
Gautam Ahluwalia

Tuberculous Pleural Effusion **149**
Hitesh Batra, Veena B Antony

Revised National Tuberculosis Control Program in India **167**
Prahlad Kumar

International Standards of Tuberculosis Care **193**
Prasanta R Mohapatra, Sourin Bhuniya

World Clin Pulm Crit Care Med. 2014;3(1):1-5.

Epidemiology of Tuberculosis

Digambar Behera MD DNB FCCP FAMS FICP

Department of Pulmonary Medicine, WHO Collaborating Center for Research and Capacity Building in Chronic Respiratory Diseases, Postgraduate Institute of Medical Education and Research, Chandigarh, India

JAYPEE BROTHERS

ABSTRACT

Tuberculosis is a common problem in the world particularly in the developing nations. About a third of the world population is infected with tuberculosis, which in numerical terms will be around 2 billions of people. About 955 of deaths due to tuberculosis occurs in the developing world. The burden of HIV infection and disease has further complicated the situation. Despite the availability of highly efficacious treatment for many years and most nations having a TB control program in place, TB continues to remain a major global health problem. The disease affects the poor and mostly the young adults in their productive years causing ill-health and death. The latest World Health Organization (WHO) estimates suggest that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths (just under 1.0 million among HIV-negative people and 0.3 million HIV-associated TB deaths). Although most of these TB cases and deaths occur among men, the burden of disease among women is also high. In that year, there were an estimated 2.9 million cases and 410,000 TB deaths among women, as well as an estimated 530,000 cases and 74,000 deaths among children. This number of TB deaths is unacceptably large given that most such morbidity and mortality are preventable if people can access health care for a diagnosis and the right treatment is provided. Short-course regimens of first-line drugs that can cure around 90% of cases have been available for decades. Although it typically affects the lungs (pulmonary TB, in more than 80% of cases), it can also affect other sites as well (extrapulmonary TB) like pleura, lymph nodes, bone, central nervous system, kidney, gastrointestinal tract, skin, etc. The disease is spread through droplet nuclei from pulmonary TB during coughing and sneezing. A small proportion of people infected with *Mycobacterium tuberculosis* will develop TB disease with a lifetime risk of about 10% but the probability of developing TB becomes higher among people infected with the HIV and some other specific situations including immune suppressed states,

Email: dirlrsi@bol.net.in

© 2014 Jaypee Brothers Medical Publishers. All rights reserved.

certain occupations, malnutrition, overcrowding and in certain associated disease states. TB is also more common among men than women, and affects mostly adults in the economically productive age-groups; around two-thirds of cases are estimated to occur among people aged 15–59 years. Without treatment, mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% will die within 10 years; among culture-positive (but smear-negative) cases, 20% will die within 10 years. Treatment using combinations of anti-TB drugs developed in the 1940s and 1950s can dramatically reduce mortality rates. In clinical trials, cure rates of above 90% have been documented; the treatment success rate among smear-positive cases of pulmonary TB reported to WHO reached 87% at the global level in 2009.

INTRODUCTION

About one-third of the global population (nearly 2 billion population) is infected with tuberculosis (TB) and 95% of deaths due to TB are in the developing world. It is the second leading cause of death from an infectious disease worldwide after human immunodeficiency virus (HIV). Despite the availability of highly efficacious treatment for many years, TB continues to remain a major global health problem. The disease affects the poor and mostly the young adults in their productive years causing ill-health in millions of people. The latest World Health Organization (WHO) estimates suggest that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths (just under 1.0 million among HIV-negative people and 0.3 million HIV-associated TB deaths). Although most of these TB cases and deaths occur among men, the burden of disease among women is also high. In that year, there were an estimated 2.9 million cases and 410,000 TB deaths among women, as well as an estimated 530,000 cases and 74,000 deaths among children.[1] The number of TB deaths is unacceptably large given that most are preventable if people can access health care for a diagnosis and the right treatment is provided. Short-course regimens of first-line drugs that can cure around 90% of cases have been available for decades. Although it typically affects the lungs (pulmonary TB, in more than 80% of cases), it can also affect other sites as well (extrapulmonary TB) like pleura, lymph nodes, bone, central nervous system, kidney, gastrointestinal tract, skin, etc. The disease is spread in the air through droplet nuclei when people who are sick with pulmonary TB expel bacteria during activities like coughing and sneezing, etc. In general, a relatively small proportion of people infected with *Mycobacterium tuberculosis* will go on to develop TB disease with a lifetime risk of about 10%. However, the probability of developing TB is much higher among people infected with the HIV and some other specific situations. TB is also more common among men than women, and affects mostly adults in the economically productive age-groups; around two-thirds of cases

JAYPEE BROTHERS

are estimated to occur among people aged 15–59 years. Without treatment, mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear-negative) cases, 20% died within 10 years.[2] Treatment using combinations of anti-TB drugs developed in the 1940s and 1950s can dramatically reduce mortality rates. In clinical trials, cure rates of above 90% have been documented; the treatment success rate among smear-positive cases of pulmonary TB reported to WHO reached 87% at the global level in 2009.

GLOBAL SCENE

According to the WHO in its 2013 publication of the 17th report, in 2012 there were an estimated 8.6 million new cases/incidence of TB (range = 8.3–9.0 million) globally (incidence is defined as the number of new and relapse cases of TB arising in a given time period, usually 1 year). This is equivalent to 122 cases per 100,000 population, although there is uncertainty about all estimates about the burden of TB. It also reported that there is a fall in the absolute number of incident cases, although slowly. Most of these estimated number of cases were reported from Asia (58%) followed by the African region (27%). A smaller proportion of these cases occurred in the Eastern Mediterranean region (8%), the European region (4%), and the American region (3%). Nearly 81% of all estimated incident cases worldwide are accounted for by the 22 high-burden countries (HBCs) since the year 2000. The largest number of incident cases in 2012 occurred in five countries as follows:

1. India (2.0–2.4 million)
2. China (0.9–1.1 million)
3. South Africa (0.4–0.6 million)
4. Indonesia (0.4–0.5 million)
5. Pakistan (0.3–0.5 million).

While India alone accounted for 26% of all such cases, China contributed another 12% of global cases. Of all the new cases in the world in 2012, about 0.5 million were children and 2.9 million (range = 2.7–3.1 million) were women.

The incidence rates vary considerably amongst countries. While the lowest rates are observed in high-income countries like the United States of America, Western Europe, Japan, Canada, Australia, and New Zealand. The incidence rate per 100,000 population in these countries is less than 10 cases per 100,000. Many countries situated in the region of the Americas have incidence rates below 50 per 100,000 and this is the region with the lowest burden of TB on average.

Incidence rates in the most HBCs have been of around 150–300 per 100,000 populations, although some countries within this have markedly lower rates

JAYPEE BROTHERS

like Brazil and China. Some other countries like Mozambique, South Africa, and Zimbabwe have rates of more than 500 per 100,000 population. The global incidence rate was relatively stable from 1990 up to around 2001, and then started to fall, achieving the Millennium Development Goal (MDG) target ahead of the 2015 deadline. The rate of decline was 2% between 2011 and 2012.

All the incident of TB cases in 2012 included 1.1 million (13%) with a range of 1.0–1.2 million (12–14%) among the HIV population. The proportion of TB-HIV coinfection was the highest in the African region. About 37% of TB cases were coinfected with HIV in this region that accounted for 75% of all TB cases among people living with HIV in the world. In some parts of Southern Africa, more than 50% of TB cases were coinfected with HIV.

There were an estimated 12.0 million prevalent (prevalence is defined as the number of cases of TB at a given point of time) cases (range = 11.0–13.0 million) of TB in 2012. This is equivalent to 169 cases per 100,000 population. The prevalence of TB can be directly measured in nationwide population-based surveys (the best method of estimation); WHO has recently published comprehensive theoretical and practical guidance on how to design, implement, analyze, and report such surveys.[3] There was a 37% fall globally in the prevalence by 2012 since 1990. However, current forecasts suggest that the Stop TB Partnership target of a 50% reduction in TB prevalence by 2015 compared with a baseline of 1990 will not be met worldwide. When repeat surveys are conducted, trends in TB prevalence can be directly measured as well. If survey data are not available, prevalence can be indirectly estimated as the product of incidence and the average duration of disease, but with considerable uncertainty.

MULTIDRUG-RESISTANT TUBERCULOSIS

The updated best global estimates of the burden of multidrug-resistant tuberculosis (MDR-TB) in 2013 showed that there were 450,000 (range = 300,000–600,000) new cases of this form of the disease worldwide in 2012 (both primary and acquired MDR-TB). About 3.6% of all new TB cases and 20% of the previously treated cases of TB had MDR-TB in 2012. The highest levels of MDR-TB are found in Eastern Europe and Central Asia, where in some countries more than 20% of new TB cases and more than 50% of those previously treated for TB have MDR-TB.

The 27 MDR-TB and extensively drug-resistant tuberculosis (XDR-TB) HBCs representing approximately over 85% of the world's estimated number of incident MDR-TB and XDR-TB cases are Armenia, Azerbaijan, Bangladesh, Belarus, Bulgaria, China, Democratic Republic of Congo, Estonia, Ethiopia, Georgia, India, Indonesia, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Myanmar, Nigeria, Pakistan, Philippines, Republic of Moldova, Russian Federation, South Africa, Tajikistan, Ukraine, Uzbekistan, and Vietnam.

JAYPEE BROTHERS

TUBERCULOSIS OR HUMAN IMMUNODEFICIENCY VIRUS COINFECTION

About 2.1 million TB patients knew their HIV status in 2010 compared with 1.6 million in 2009, with the highest HIV testing rates of TB patients in Europe (80%) and Africa (59%). In 68 countries, including 22 in Africa, at least 75% of TB patients knew their HIV status. Forty six percent of HIV-positive TB patients were enrolled on antiretroviral drug therapy and 77% started on co-trimoxazole preventive treatment in 2010. About 4.1 million people enrolled in HIV care were reported to have been screened for TB, in 2012 which is up from 3.5 million in 2011. Of the reported 1.6 million people newly enrolled in HIV care in 2012, about 0.5 million (31%) were provided with isoniazid (INH) prophylaxis. Globally, 46% of TB patients knew their HIV status in 2012 (up from 40% in 2011). In the African region that has the highest TB or HIV burden, 74% of TB patients knew their HIV status (up from 69% in 2011). Among the 41 countries with the highest TB or HIV burden, more than 85% of TB patients knew their HIV status in 15 countries and in 7 of these countries over 90% of patients knew their HIV status. The coverage of antiretroviral therapy (ART) among TB patients who were known to be HIV-positive reached 57% in 2012, up from 49% in 2011. As in the past few years, about 80% of HIV-positive TB patients were treated with co-trimoxazole preventive therapy.

Editor's Comment

Tuberculosis continues to pose a major challenge to the global health scene. There were an estimated 8.6 million new cases in 2012, which were equivalent to 122 cases per 100,000 global population. Indian alone accounted for 26% of all such cases. The total overall burden including both the new and the old cases was an estimated 12 million cases. There is a trend toward a decline in the TB burden in the occurrence of new cases. Some of the important issues of concern include the high prevalence of patients with MDR-TB and HIV-TB coinfection.

Surinder K Jindal

REFERENCES

1. World Health Organization. (2013). Global Tuberculosis Report 2013. [online] Available from: apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf. [Accessed April, 2014].
2. Tiemersma EW, van der Werf MJ, Borgdorff MW, Williams BG, Nagelkerke NJ. Natural history of tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: a systematic review. *PLoS One.* 2011;6:e17601.
3. World Health Organization. (2011). Tuberculosis PREVALENCE SURVEYS: a handbook. (WHO/HTM/TB/2010.17). [online] Available from: whqlibdoc.who.int/publications/2011/9789241548168_eng.pdf. [Accessed April, 2014].

JAYPEE BROTHERS

World Clin Pulm Crit Care Med. 2014;3(1):6-20.

Mycobacteriology, Pathogenesis, and Pathology of Tuberculosis

[1]Vinaya S Karkhanis MD, [2]Gayathri P Amonkar MD DNB, [3,*]Jyotsna M Joshi MD

[1,3]Department of Pulmonary Medicine, T.N. Medical College, B.Y.L Nair Hospital, Mumbai, Maharashtra, India
[2]Department of Pathology, T.N. Medical College, B.Y.L. Nair Hospital, Mumbai, Maharashtra, India

JAYPEE BROTHERS

ABSTRACT

Mycobacterium is a genus of actinobacteria from Mycobacteriaceae family. *Mycobacterium tuberculosis* species is a causative agent of tuberculosis in humans, discovered by Robert Koch in 1882. It is characterized by its aerobic nature, acid fastness, intermittent growth, and ability to undergo selective mutation. High lipid containing mycolic acid in its cell wall is responsible for its acid fastness, resistance and virulence. Mitchison hypothesized that the tubercle bacilli in lesions consist of at least four different subpopulations, which vary in metabolic activity and multiplication, which is the basis for multidrug anti-tuberculous therapy. Staining with Ziehl Neelsen or the fluorescent auramine-rhodamine stain show rod-shaped, straight, or slightly curved organisms as "serpentine cords". Mycobacterial culture is considered as the gold standard for identification of the bacilli. On culture, it produces rough, raised, and irregular colonies on incubation up to 8 weeks. Genome of mycobacterium tuberculosis studied using the strain MTBH37Rv contains about 4,000 genes. Genetic resistance to an anti-TB drug is due to spontaneous chromosomal mutations at frequency of 10^{-6}–10^{-8} mycobacterial replications. Host response to tuberculous bacillus may vary. Virulence of the organism and immunological status of the host are important determinants in pathogenesis of the disease. It ranges from no infection, latent infection, primary pulmonary tuberculosis, post-primary tuberculosis to reactivation tuberculosis.

*Corresponding author
Email: drjoshijm@gmail.com

© 2014 Jaypee Brothers Medical Publishers. All rights reserved.

INTRODUCTION

Mycobacterium is a genus of actinobacteria, from Mycobacteriaceae family. The genus includes pathogens known to cause serious diseases in mammals, such as *Mycobacterium tuberculosis* (MTB) and leprosy.[1] Members of this genus have the distinct property of acid-fastness, i.e., ability when once stained, to resist decolorization by mineral acids staining.[2] The name *Mycobacterium* means fungus bacterium that refers to shape of the bacillus when it is grown on liquid medium in a mold-like fashion. When seen through a microscope it forms heaps of small rods with protective layers around them and thus looks like a fungus.

CLASSIFICATION OF MYCOBACTERIA

Numerical or Adansonian taxonomy is used for classifying mycobacteria.[3] A large number of characteristics for each bacterial strain are determined and the strains are then clustered according to the degree of similarity.[1-9]

Classical and Asian Variants of Mycobacterium Tuberculosis

These two variants belong to the original species of mycobacteria. They are rod-shaped, thin, and commonly straight, slightly curved 2–4 microns in length and 0.2–0.8 microns in breadth. They appear in the form of microscopic "serpentine cords". They are nonmotile, noncapsulated, and do not form spores. These are obligate aerobes, weakly Gram-positive organisms. They use carbon compounds as a source of energy. The optimum temperature is 37°C. Growth is inhibited by heat or pasteurization and increased by 5–10% carbon dioxide (CO_2). Growth does not occur below 25°C and above 40°C. Doubling time is 18 hours. They are resistant to drying, many chemicals, dyes, and many antimicrobial agents. They resist decolorization by 25% sulfuric acid and absolute alcohol. Hence they are also known as acid and alcohol fast.[4] MTB is most easily identified by its special colony morphology (eugonic growth), by nitrate reduction, and by niacin accumulation.

Variants and Species of Tubercle Bacilli

Mycobacterium bovis: the properties of *M. bovis* are in general similar to those of MTB. It is the principal agent responsible for tuberculosis (TB) in domestic and wild mammals. It also infects humans, causing zoonotic TB through ingestion, inhalation, and less frequently, by contact with mucous membranes and broken skin.[5]

Mycobacterium africanum: it is a member of MTB complex. In contrast to MTB and *M. bovis*, *M. africanum* strains show higher degrees of variability in their phenotypic attributes, which comprise characteristics of MTB and *M. bovis*.[6]

JAYPEE BROTHERS

Since its first description in 1968, *M. africanum* has been found in several regions of Africa, where it represents up to 60% of clinical strains from patients with pulmonary TB.[7]

Bacillus Calmette-Guérin (BCG): Calmette and Guérin obtained a strain, which has lost its virulence in experimental animals.

Mycobacterium microti: *M. microti* was named by Reed in 1957. It is indistinguishable from MTB in morphology, staining, biochemical, and cultural characteristics, hence included in the MTB complex. Cultural strains have characteristic of the African-1 variant. *M. microti* is not pathogenic for men.

Cell Wall of *Mycobacterium tuberculosis*

The cell wall structure of MTB deserves special attention because it is unique among prokaryotes, and it is a major determinant of virulence for the bacterium. The cell wall complex contains peptidoglycan, but otherwise it is composed of complex lipids. Over 60% of the mycobacterial cell wall is lipid. The lipid fraction of MTB's cell wall consists of three major components: (i) mycolic acids, (ii) cord factor, and (iii) wax-D. The cell envelope is composed of three major constituents: (i) the plasma membrane, (ii) the cell-wall core, and (iii) the extractable, non-covalently linked glycans, lipids, and proteins. External to the membrane is peptidoglycan in covalent attachment to arabinogalactan, which in turn is attached to the mycolic acids with their long meromycolate and shorter alfa-chains. This portion is termed as the cell-wall core—the mycolylarabinogalactan-peptidoglycan complex (MAPc). The extractable material is composed of free lipids including the phthiocerol-containing lipids, the phosphatidylinositol mannosides (PIMs), lipomannan (LM), lipoarabinomannan (LAM), trehalose dimycolate (cord factor), trehalose monomycolate, and the diacyl- and polyacyltrehaloses presumably intercalating with the alfa and meromycolate chains of the mycolic acids. Interspersed are the cell-wall proteins; the PIMs, the phthiocerol-containing lipids, LM, and LAM. The unique structure of the mycobacterial MAPc makes it an ideal target for specific antimycobacterial drugs.[10]

Cord Factor

In 1884, Robert Koch described MTB bacilli grown in culture as rope-like structures. More than half a century later, in 1947 Middlebrook found that the ability to form cords under specific conditions is an "essential accompaniment of virulence".[11] In 1950, Bloch extracted the substance responsible for cord formation from virulent organisms and identified it as a "toxic substance".[12] The removal of substance with petroleum ether resulted in somehow avirulent organisms, but did not affect the growth of the bacilli. This suggested that the substance was located at the surface and since it was obtained only from "cord forming" organisms it

JAYPEE BROTHERS

was called "cord factor". Six years later, the cord factor was finally identified as trehalose 6,6′-dimycolate (TDM) by Noll.[13] TDM is the most abundant glycolipid produced by virulent MTB.[14]

Genome Structure

Mycobacterium tuberculosis has circular chromosomes of about 4,200,000 nucleotides long. The G+C content is about 65%.[15] The genome of MTB was studied generally using the strain MTB *H37Rv*. The genome contains about 4,000 genes. Genes that code for lipid metabolism are a very important part of the bacterial genome, and 8% of the genome is involved in this activity.[16] The different species of the MTB complex show a 95–100% deoxyribonucleic acid (DNA) relatedness based on studies of DNA homology, and the sequence of the 16S ribosomal ribonucleic acid (rRNA) gene are exactly the same for all the species. Plasmids in MTB are important in transferring virulence because genes on the plasmids are more easily transferred than genes located on the chromosome. One such 18 kb plasmid in the MTB H37Rv strain was proven to conduct gene transfers.

Staining Properties

A Gram stain is not able to reliably detect *Mycobacteria* which can appear as non-stained "ghosts" or as beaded Gram-positive bacilli. Therefore, acid-fast stains, such as the Ziehl-Neelsen stain or the fluorescent auramine-rhodamine stain, are recommended for mycobacteria. The acid-fast stain forms a complex between the unique mycolic acids of the mycobacterial cell wall and the dye (e.g., fuchsin). Complex formation makes the *Mycobacteria* resistant to destaining by acid-alcohols providing the basis for the "acid-fast" terminology.[17]

Culture Characteristics

The growth of MTB in culture is considered as the gold standard for identification of a case of TB. The sensitivity of culture is much better than an acid-fast smear with only 10–100 viable organisms/mL of specimen required for a positive culture. Solid media utilized are egg-based represented by Ogawa and Lowenstein, such as the Löwenstein-Jensen (LJ) medium.[18] The second type is agar-based, such as Middlebrook 7H9 and 7H10 medium. Other media, such as Petragnani and Dorset egg medium, blood-containing media (Tarshis medium), serum-containing medium (Loeffler's serum slope), and potato-based media (Pawlov's medium), also can be used. Of these media, LJ medium is most widely used and is also recommended by the International Union against TB.

JAYPEE BROTHERS

The LJ medium consists of coagulated whole egg, asparagines, mineral salt, and glycerol. Malachite green inhibits growth of bacteria other than MTB. Addition of 0.75% glycerol enhances growth of MTB, but is inhibitory to growth of *M. bovis*. On culture, MTB produces rough, raised, and irregular colonies with a wrinkled surface. The colonies usually appear in almost 2 weeks, sometimes require incubation up to 8 weeks. Substantial improvement in the time of detection and the recovery rate was realized by using broth-based culture system, such as the BACTEC™ 460TB, Septi-Chek™ AFB, Mycobacteria Growth Indicator Tube (MGIT), and BACTEC™ 9000. The Septi-Chek™ AFB is a biphasic medium which combines broth and solid media.[18] The liquid media are usually for preparation of mycobacterial antigens for vaccines and antibiotic sensitivity of MTB. The commonly used culture media include Soloac's, Dubos, Middlebrook's, and Beck's medium.

BIOCHEMICAL TEST OF IDENTIFICATION

The important biochemical test of identification are discussed below.[19]

Niacin Test

Niacin is part of the energy metabolism of mycobacteria in redox reactions. All mycobacteria produce niacin, but MTB accumulates it as a result of the major activity of nicotinamide adenine dinucleotide and the inability to process the resulting niacin. The test demonstrates the presence of cyanogen chloride formed through the reaction of chloramine-T and potassium thiocyanate in the presence of citric acid. The cyanogen chloride breaks the pyridine ring of niacin, forming the aldehyde gamma-carboxyglutamate that binds with the aromatic amine producing a yellow color.

Nitrate Reduction Test

Nitrate reductase, an enzyme capable of reducing nitrates to nitrites, appears in the cellular membranes of mycobacteria; the bacteria can utilize this enzyme as a source of nitrogen. The test detects the presence of nitrate reductase in a medium that contains sodium nitrate. The enzyme reduces nitrate to nitrite that appears through the addition of sulfanilamide and dihydrochloride-N-naphthylethylenediamine, forming a complex of diazonium chloride with a fuchsia color.

Catalase Test

This is an antioxidant enzyme responsible for eliminating molecules of hydrogen peroxide from the cells that are produced during respiration. The reaction results

JAYPEE BROTHERS

in the release of water and free oxygen. Two classes of catalase, thermolabile and thermostable, appear in mycobacteria. In MTB and *M. bovis*, the enzymatic activity is inhibited at 68°C; this contrasts with the remaining species of mycobacteria that maintain enzymatic activity following the increase in temperature. Oxygen in the form of bubbles, caused by the activity of the enzyme in a solution of perhydrol 30% and Tween 80 at 10%, appears during the test. The height of the column of bubbles can be measured, thus quantifying the enzymatic activity.

Urease Test

The urease enzyme is coded by the *ureABC* genes and is able to hydrolyze urea, forming two molecules of ammonium; this is used by the mycobacteria in the process of biosynthesis. The presence of urease, the enzyme that hydrolyzes urea into ammonia and CO_2, can be detected in mycobacterial species by incubating an actively growing culture to a urea broth for up to 7 days at 35–37°C in a non-CO_2 incubator, with readings done at days 1, 3, and 7. MTB is positive for urease and will produce a dark pink to red color following incubation in urea broth.

Pyrazinamidase Test

This is an intracellular enzyme codified by the *pncA* gene which is able to hydrolyze pyrazinamide (PZA) in pyrazinoic acid. Some strains present a mutation in the *pncA* gene that generates resistance to PZA, the principal mechanism of resistance in MTB to this drug. The method of transport of PZA into MTB is completed by passive diffusion, where it is converted into pyrazinoic acid through the action of the pyrazinamidase enzyme. Its usefulness as a test of identification is based on the differentiation of MTB (positive pyrazinamidase) from other species of the MTB complex (negative pyrazinamidase), with the exception of *M. canetti* which is also positive.

MYCOBACTERIAL ANTIGENS

Mycobacterial antigens can be broadly classified according to their: (i) soluble or particulate nature, (ii) their chemical structure as polysaccharides, lipids or proteins, (iii) to the nature of the immune response evoked by them, and (iv) by their distribution within the genus-soluble antigens.[20]

For antigenic analysis, diffusion in gel techniques has been used. Stanford and his colleague demonstrated four groups of soluble antigens in ultrastructurally disintegrated preparations of mycobacteria.

Recently, the structure of an antigenic glycolipid isolated from cell walls of various strains of MTB was investigated by mass spectrometry and nuclear

JAYPEE BROTHERS

magnetic resonance spectroscopy. The material proved to be a complex mixture of closely related components which are indeed 2,3-di-*O*-acyltrehaloses. However, no sulfate group was found in the antigen. The lipid substituents comprise an estimated 20% of C_{14}-C_{19} fatty acids, including tetradecanoic (myristic), 9-tetradecenoic (myristoleic), 9-hexadecenoic (palmitoleic), 9-octadecenoic (oleic), 10-methylhexadecanoic (tuberculopalmitic), and 10-methyloctadecanoic (tuberculostearic) acids in addition to hexadecanoic (palmitic) and octadecanoic (stearic) acids, and approximately 80% of higher, methyl-branched acids.[21]

DRUG RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis strains that were resistant to streptomycin (SM) appeared soon after the introduction of the drug for treatment of TB in 1944. Genetic resistance to an anti-TB drug is due to spontaneous chromosomal mutations at a frequency of 10^{-6}–10^{-8} mycobacterial replications. Mutations in the genome of MTB that can occur spontaneously are with an estimated frequency of 3.5×10^{-6} for isoniazid (INH) and 3.1×10^{-8} for rifampicin (RMP). Because the chromosomal loci responsible for resistance to various drugs are not linked, the risk of a double spontaneous mutation is extremely low: 9×10^{-14} for both INH and RMP.[22] The probability of developing bacillary resistance to three drugs used simultaneously becomes 10^{-18}–10^{-20}. In theory, the chance of drug resistance is thus virtually nonexistent when three effective drugs are used in combination for TB treatment.[23] Amplification of genetic mutation through human error results in clinically drug-resistant TB. These include monotherapy, inappropriate doctor prescription, and poor patient adherence to treatment.[24] Drug resistance in TB is classified as acquired resistance when drug-resistant mutants are selected as a result of ineffective treatment or as primary resistance when a patient is infected with a resistant strain. Extensive studies on the *rpoB* gene in RMP-resistant isolates of MTB identified as a variety of mutations and short deletions in the gene. A total of 69 single nucleotide changes; 3 insertions, 16 deletion, and 38 multiple nucleotide changes have been reported.[25] More than 95% of all mutations are located in a 51-bp core region. RMP resistance determining region of the *rpoB* gene is between codons 507–533 with the most common changes in codons *Ser531Leu*, *His526Tyr*, and *Asp516Val*. These changes occur in more than 70% of RMP-resistant isolates. Mutations in the *pncA* gene correlate well with phenotypic resistance to PZA. However, PZA-resistant isolates without *pncA* mutations were also observed suggesting that another mechanism may be involved in conferring PZA resistance. Various studies have identified five mutations in codon 306 [(ATG-GTG), (ATG-CTG), (ATG-ATA), (ATG-ATC), and (ATG-ATT)], which result in three different amino acid substitutions in ethambutol (EMB)-resistant isolates.[26] These five mutations are associated with 70–90% of all EMB-

JAYPEE BROTHERS

Available at all medical bookstores
or buy online at www.jaypeebrothers.com

JAYPEE BROTHERS
Medical Publishers (P) Ltd.
www.jaypeebrothers.com

Join us on facebook.com/JaypeeMedicalPublishers

ISBN 978-93-5152-544-8