MOLECULAR GENOTYPING OF *Mycobacterium tuberculosis* ASSOCIATED WITH EXTRAPULMONARY TUBERCULOSIS

by

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LIST OF ABBREVIATIONS

AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
AD	Anno domini
BCG	Bacilli Calmette-Guerin
Bp	Base pair
BACTEC	Becton Dickinson Diagnostic System
BC	Before century
Cm	Centimetre
CAS	Central Asian
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXR	Chest X – ray
CT	Computed tomography
C	Cytosine
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
DOTS	Direct observed treatment short course
DR	Direct repeat
dH_20	Distilled water
DST	Drug susceptibility testing
EAI	East African Indian
ECL	Enhanced chemiluminescence
E	Ethambutol
EDTA	Ethylenediamine tetra acetic acid
XDR	Extensive multidrug
EPTB	Extrapulmonary tuberculosis
G	Gram
G	Guanine
H	Haarlem
HUSM	Hospital USM
HIV	Human immunodeficiency virus
IS	Insertion sequence
IGRA	Interferon-gamma release assay
IC	Internal control
H	Isoniazid
LIS	Laboratory Information System
LTBI	Latent TB
LJ	Lowenstein-Jensen
$MgCl_2$	Magnesium chloride
MRI	Magnetic resonance imaging
µl	Microlitre
µM	Micromolar
ml	Millimetre

mM	Millimolar
Min	Minute
MDR	Multidrug resistance
MGIT	Mycobacterial Growth Indicator Tube
MIRU	Mycobacterial interspersed repetitive unit
MA	Mycolic acid
Ng	Nanogram
NPHL	National Public Health Laboratory
OR	Odd ratio
PET	Paraffin embedded tissue
PBS	Phosphate buffer saline
Pmole	Picomole
PCR	Polymerase chain reaction
PTB	Pulmonary TB
PPD	Purified protein derivative
Z	Pyrazinamide
RD	Region of difference
RFLP	Restriction fragment length polymorphism
RPM	Revolution per minute
R	Rifampicin
SSPE	Saline sodium phosphate EDTA
SPSS	Statistical Package for Social Sciences
Sec	Second
SDS	Sodium dodecyl phosphate
SPSS	Statistical Package for Social Science
NaOH	Sodium hydroxide
Spoligotyping	Spacer oligonucleotide
X	Times or multiple
TBE	Tris borate EDTA
TB	Tuberculosis
UV	Ultraviolet
U	Unit
USM	Universiti Sains Malaysia
VNTR	Variable number of tandem repeat
VF	Visual field
WHO	World Health Organization
ZN	Ziehl-Neelsen

LIST OF SYMBOLS

≈	Almost equal to
° C	Degree Celsius
%	Percentage

PENGETIPAN MOLEKUL *Mycobacterium tuberculosis* YANG BERKAIT DENGAN JANGKITAN TUBERKULOSIS LUARPULMONARI

ABSTRAK

Kemajuan terkini dalam pengetipan molekul menyediakan teknik-teknik yang mantap untuk menganalisis kepelbagaianstrain *Mycobacterium tuberculosis* dan juga pola transmisi jangkitan. Dalam kajian percubaan pertama dari Kelantan, Malaysia, kekerapan dan kepelbagaian *M. tuberculosis* genotip yang dikaitkan dengan tuberkulosis luar pulmonari (EPTB) telah dijalankan menggunakan teknik spoligotyping. Keputusan genotip dibandingkan dengan SITVIT Web, pangkalan penanda molekul data antarabangsa terbesar untuk *M. tuberculosis*yang mengandungi 7104 corak spoligo di seluruh dunia. Hasil kajian menunjukkan bahawa 74.6% (n = 44) daripada pencilan telah dikategorikan di bawah " shared international type"(SIT) yang telah ditakrifkan sebelum ini manakala 25.4% (n = 15) yang tidak mempunyai nombor SIT telah dikategorikan sebagai strain "orphan". Keturunan terbesar terdiri daripada 23 pencilan (38.9%) yang dimiliki oleh keturunan Beijing. Kajian ini mencadangkan kekerapan *M. tuberculosis* lebih berkait dengan tetapan geografi dan bukan pada tempat-tempat jangkitan. Satu bahagian strain yang berkaitan dengan EPTB dalam populasi kajian ini juga disebabkan oleh strain baru yang tidak terdapat dalam pangkalan data SITVIT Web itu. Kajian ini boleh berfungsi sebagai bahan rujukan bagi kajian pengetipan masa hadapan untuk penganggaran sebenar kelaziman *M. tuberculosis* yang berkaitan dengan EPTB di Malaysia amnya.

MOLECULAR GENOTYPING OF *Mycobacterium tuberculosis* ASSOCIATED WITH EXTRAPULMONARY TUBERCULOSIS

ABSTRACT

Recent advances in molecular typing provide a robust tool to analyse the heterogeneity of *Mycobacterium tuberculosis* strains as well as their transmission patterns. In the first attempt study from Kelantan, Malaysia, the frequency and diversity of *M. tuberculosis* genotypes associated with extrapulmonary tuberculosis (EPTB) were performed using spoligotyping. Genotyping results were compared with the SITVIT Web, a largest *M. tuberculosis* molecular markers international database containing 7104 spoligotype patterns worldwide. The results showed that 74.6 % (n = 44) of the isolates were grouped under previously defined shared international type (SIT) numbers while 25.4 % (n = 15) did not have any SIT numbers, thus designated as orphan strains. The largest lineage comprised of 23 isolates (38.9 %) belonging to the Beijing lineage. Present study suggested frequency of *M. tuberculosis* lineages are related more on geographical settings rather than sites of infection. Bulk of the strains associated with EPTB in the study population also caused by new strain that is not available in the SITVIT Web database. The study may serves as a reference material for further genotyping studies on estimating the actual prevalence of *M. tuberculosis* associated with EPTB in Malaysia generally.

CHAPTER 1: INTRODUCTION

Tuberculosis (TB) remains as a major public health problem which has caused significant morbidity and mortality worldwide (WHO, 2014). This airborne disease is caused by *Mycobacterium tuberculosis*, the most common strain of mycobacteria that cause pulmonary TB (PTB) in human. It may also infect other parts of the body causing extrapulmonary TB (EPTB).

1.1 History of tuberculosis

For centuries long, TB has been known to a mankind as devastating plague. It was inferred to present as early as prehistory period of time and continued to kill people until today. Archaeological evidences had showed TB was present in Egypt more than 5000 years ago (Daniel, 2006). Typical skeletal abnormalities of TB which include Pott's deformities was found in Egyptian mummies and were clearly depicted in early Egyptian art. Similarly, archaeological evidence of early TB was also found in a Columbian prehispanic mummy (200 BC – AD 100) in Sinu River, Colombia demonstrating spinal TB (Palomino *et al*., 2007). In China and India, TB has been documented as early as 2300 and 3300 years ago, respectively.

The much older name for TB originally came from the ancient Greek as phthisis which means consumption (Daniel, 2006). Hippocrates (460 – 377 BC), a Greek physician has recognized phthisis as the most widespread disease during the time. In his writing, he described it as a fatal disease that attacked people between the age 18 and 35 years. Aristotle (384-322 BC) had considered TB as contagious disease though most of authors at that time believed it to be a hereditary disease or due to individual's mental and moral weaknesses. Clarissimus Galen (131-201 AD), the

most renowned physician after Hippocrates had suggested phthisis as ulceration of lungs, chest or throat that comes along with cough and low fever (Pease, 1940).

The Great White Plaque marked the beginning of TB epidemic in Europe. It last for more than 200 years and probably started in early 17th century. Death was considered inevitable at that time and by 1650, TB was recognized as the leading cause of the mortality. It was deduced that the dissemination is due to high population density and poor sanitary in waxing cities of Europe and North America, provide crucial environment for the spread of the disease. The TB epidemic ultimately spread gradually to other countries through exploration, immigration and colonization (Daniel, 2006). Meanwhile, TB in Asia hit the highest point in China and India toward the end of 19^{th} century.

Franciscus Sylvius (1614-1672) was the first to use the term tubercle to describe consistent lesions in lungs and other viscera of consumption patients that could progress to ulcers and cavities (Palomino *et al*., 2007). Later, Richard Morton (1637-1698) confirmed tubercles were always present in patients of lung consumption and Laurent Bayle (1774-1816) had proved the tubercles were not a consumption product but rather the cause of the illness. In 1839, Johan Lukas Schonlein brought in the name ‘tuberculosis’ to describe the disease replacing the words phthisis and consumption in TB history. On March 24, 1882, Robert Koch disclosed that he had identified and isolated the causative agent for TB, a rod-shaped bacterium known as tubercle bacillus to Berlin Physiological Society (Gradmann, 2006).

Koch’s postulates brought a new paradigm in TB era as it led to subsequent findings of tuberculin, a substance isolated from tubercle bacilli which is initially regarded as a TB cure. Although Koch failed to demonstrate it, his findings become a

cornerstone to create a diagnosis for TB. Clemens Freiherr vor Pirquet introduced a term “latent tuberculosis” in 1907 as he noted a positive reaction to tuberculin among child who did not manifested the disease (Gideon and Flynn, 2011). Subsequently, Charles Mantoux introduced a cannulated needle and syringe used to inject the tuberculin intracutaneously in 1908. During 1930s, Florence Serbeit established purified protein derivative (PPD), an active component of tuberculin for diagnosing TB that is still used until today (Daniel, 2006).

Sanatorium regimen which started by Herman Brehmer (1826-1889) was the first broadly implementation of TB treatment throughout Europe and United States. The idea of the regimen was to protect the public by isolating ill person and help TB patients in healing process by providing them adequate bed rest, exercise, fresh air and good nutrition (McCarthy, 2001; Warren, 2006). This approach was then gradually replaced by the drug therapy. The discovery of streptomycin in 1934 followed by para-aminosalicyclic acid, nicotinamide, conteben, isoniazid, rifampicin, pyrazinamide, ethambutol, cycloserine and ethuonamide gave a new hope in treating TB disease. In 1921, the BCG (Bacillus Calmette-Guerin) vaccine against TB developed by Albert Calmette (1863-1933) and Camille Guerin (1872-1961) was administered in human. The vaccine is still vastly applied until today (Daniel, 2006).

1.2 Etiologic agent of tuberculosis

TB is usually caused by *M. tuberculosis* complex, a group of closely related species that shared 99.9 % similarity at nucleotide level and identical 16S rRNA gene sequences (Brosch *et al*., 2002; Whitman *et al*., 2012). Regardless of their genetic homology, each of the members is associated with specific primary host, although infection can also occur in various alternative hosts. *M. tuberculosis* complex members also differ in phenotypic characteristics.

The group comprises *M. tuberculosis*, which is the main etiologic agent of human TB worldwide; *Mycobacterium africanum,* a common cause of human TB in West African countries (Mostowy *et al*., 2004)*; Mycobacterium bovis* which predominantly causes bovine TB, yet can produce zoonotic TB in human (Kubica *et al*., 2006); *Mycobacterium bovis* BCG, a live attenuated strain vaccine that is used to immunize people against TB (Mahairas *et al*., 1996); *Mycobacterium microti* which typically causes TB in rodent, but have been reported to cause infection in both immunocompromised and immunocompetent patients (van Sooligen *et al*., 1998); *Mycobacterium canetti*, a smooth tubercle bacilli variant that was first isolated in 1969 from a native Frenchman (van Soolingen *et al*., 1997); *Mycobacterium caprae*, a causative agent of caprine TB that was firstly isolated from goat in Spain (Aranaz *et al*., 2003); *Mycobacterium pinnipedii* which primarily infects seals (Cousins *et al*., 2003) and *Mycobacterium mungi*, a TB causative agent in banded mongoose that were found to live near human population in Botswana (Alexander *et al*., 2010). In addition, another two distinct branches, dassie and oryx bacilli, which are found to have phylogenetic similarities yet are not completely described, were also included in the group (van Ingen *et al*., 2012). Dassie and orxy bacilli were primarily isolated from hyrax and bovidae family, respectively.

1.2.1 General characteristic of *M. tuberculosis* complex

All members of *M. tuberculosis* complex are found to be aerobic, non-motile, non-sporulating and slow-grower (Warren & Body, 1995). The shaped are rod with straight to slightly curved, ranging in size from 0.2 – 0.6 x 1.0 – 10 µm. They are considered to be gram-positive organism due to lack of an outer cell membrane.

Generally, morphological colonies of mycobacteria are being classified as either having a smooth and soft or rough and crumbly appearance (Lehman *et al*., 2007). The mycobacteria colonies colour is typically buff. For example, *M. tuberculosis* colonies are raised, non-pigmented and appear to be dry with rough appearance. The colonies often present with cording pattern, a curved strand of bacilli appearance resulting from tight cohesion of the bacilli.

As for growth requirement, mycobacteria need a carbon source, a nitrogen source and essential metal ions such as iron, magnesium and zinc. Fatty acids have been found to stimulate the growth, yet high concentration can lead to inhibitory effect. The most important source for growth is oxygen. Some of the species are microaerophilic, which require oxygen but at lower level for instance *M. bovis* and *M. africanum* while *M. tuberculosis* is a strict aerobe (Niemann *et al*., 2000).

1.2.2 Overview of mycobabacteria cell envelope structure

Generally, mycobacteria cell envelope encompasses a cytoplasmic membrane, a cell wall and a capsule-like outermost layer. The cytoplasmic membrane is a typical bilayer membrane and has thickness of approximately 4 to 4.5 nm. It is surrounded by a unique cell wall that has high lipid content (Rom & Garay, 2004). Due to this property, the cell wall prevents accurate gram staining, allows only staining by acid-fast dyes and is known as acid-fast organism.

The mycobacteria cell wall is made up of peptidoglycan, arabinogalacatan and mycolic acids (MA). Peptidoglycan, the innermost layer confers rigidity to help mycobacteria maintain its shape (Hett & Rubin, 2008). It is linked to arabinogalactan, the major polysaccharide in cell wall constituents. The arabinogalactan in turns are covalently linked to MA, a long chain series of fatty acids that represent more than 50 % of cell wall mass. These MA are responsible for thick waxy lipid coat characteristic of mycobacteria which contributes to impermeability of the cell wall and virulence (Glickman *et al*., 2000; Kieser & Rubin, 2014).

The capsule-like outermost layer consists of lipids, glycolipids and proteins that vary between species and some, like lipomannan, lipoarabinomannan and cord factor are contributing to mycobacteria virulence (Brennan, 2003). Figure 1.1 shows a schematic illustration of the main components of mycobacteria cell envelope and their distribution.

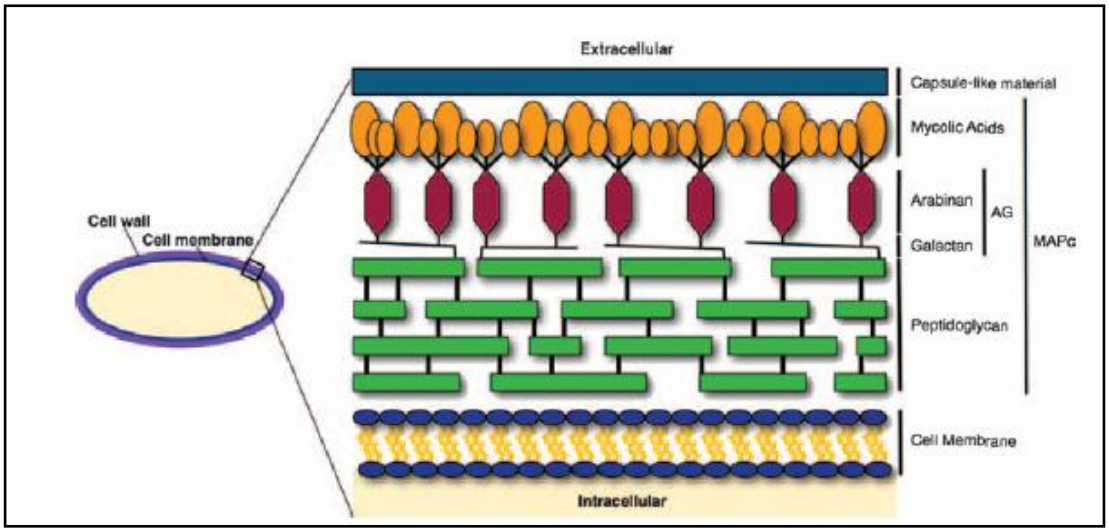

Figure 1.1: The schematic illustration of mycobacterial cell envelope. (Adapted from Hett & Rubin, 2008).

1.2.3 Mycobacterial genome

Mycobacteria genome is encoded in a single circular deoxyribonucleic acid (DNA) with high guanine and cytosine (G + C) content ranges from 64 % to 70 % of the genome. In 1998, a first complete genome sequence was elucidated for *M. tuberculosis* H37Rv, a virulent laboratory reference strain by Cole *et al*., (1998). It was revealed that the strain posses a high G + C content (65.5 %) with a genome size of 4 411 529 base pair (bp). Analysis of the genome revealed that the pathogen has all genes for essential catabolic and anabolic metabolic pathways in which a large fraction of the genes was found to be devoted to lipid and polypeptide metabolism (Saunders & McFadden, 2003).

Comparative genomic studies between *M. bovis* BCG-Pasteur, an attenuated strain used for BCG vaccination had revealed absence of a few regions (region of difference, RD 1-14) in relative to *M. tuberculosis* H37Rv. According to Gordon *et al*., (1999), RD 1 until RD 3 were found to be specific deletion to *M. bovis* BCG while RD 4 until RD 7 were also found to be absence on *M. bovis*. It is postulated that amongst these specific deletion regions contributed to the attenuation of the live tuberculosis vaccine of *M. bovis* BCG (Pym *et al*., 2002).

1.3 Transmission of tuberculosis

TB transmission occurs when a person inhale droplet nuclei containing TB bacilli ranges from 1 to 5 µm in diameter. These droplet nuclei were generated when infected persons cough, sneeze, spit or shout. Depending on the environment, these tiny particles can remain suspended in air for several hours (CDC, 2008). Once inhaled, the droplets transverse the mouth, upper respiratory tract and bronchi before settle down in alveoli of the lungs. Figure 1.2 (a) illustrated the transmission of TB, spreading from person to person.

1.4 Pathogenesis of tuberculosis

Infection occurs once TB bacilli deposited in alveoli, where they are surrounded and engulfed by alveolar macrophages. The bacilli continued to multiply in the macrophages, initiate a recruitment of inflammatory cells to the area, which in turns forming an early tubercle. In the meantime, dendritic cell presentation of tubercle bacilli antigens leads to T cell priming and trigger an adaptive immune response. The accumulation of activated macrophages, T cells, B cells and other host cells lead to the formation of the granuloma, which can contain the bacilli (Nunes-Alves *et al*., 2014) as illustrated in Figure 1.2 (b).

The granuloma formed limits the replication and spread of the bacilli. Destroyed macrophages released the bacilli and formed a caseous center in the lesion, restricted further growth of bacilli. The infection progression is halted, yet, some of them are able to survive in this deleterious condition and enter a dormancy state. Apart from that, the lesion may become calcified or continued to liquefy and eventually ruptured causing the bacilli to be released in neighbouring vessels and airways (Nunes-Alves *et al*., 2014). In this state, bacilli can be easily spread when the infected person cough, sneeze or speak.

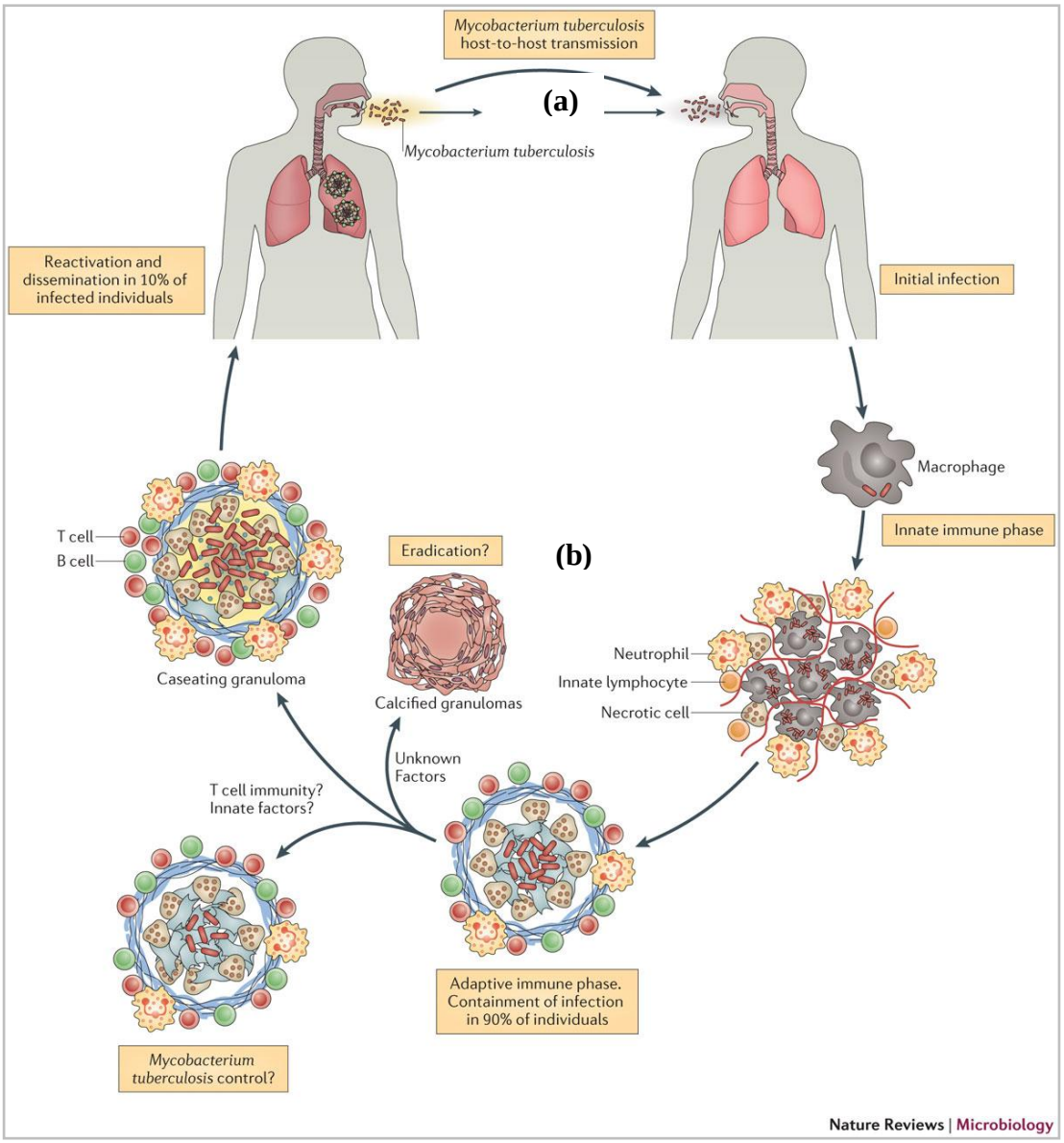

Figure 1.2: Transmission (a) and pathogenesis (b) of TB infection. (Adapted from Nunes-Alves*et al*., 2014).

1.5 Active tuberculosis

A person who is infected with TB may not necessarily develop the disease. Most people developed adequate immunity to keep the infection at bay. In fact, healthy people who are infected with TB only have 5 % to 10% chance of converting to active disease over their lifetime (WHO, 2002).

1.5.1 Pulmonary tuberculosis

PTB is the most common clinical manifestation of active TB. Symptoms may include chest pain and prolonged cough that last for 3 weeks or longer, which may be accompanied with the present of blood or sputum. Shortness of breath usually indicates extensive TB disease with widespread involvement of lung and parenchyma (Rossman & MacGregor, 1995). According to previous study, about 25 % of people may remain asymptomatic in the early stages of the disease (Lawn & Zumla, 2011).

1.5.2 Extrapulmonary tuberculosis

TB infection may spreads outside lung causing EPTB, representing about 15 % to 20 % of all TB cases in immunocompetent patients (Sharma & Mohan, 2004). Though disseminated form may also occur, EPTB is usually confined to a single site. According to Betts *et al*., (2003), symptoms and signs are localized with minimal or absent constitutional symptoms. Those with constitutional symptoms, usually have more widespread disease or pulmonary involvement.

Apart from that, the diagnosis of EPTB is often complicated due to atypical clinical manifestation, difficulty in obtaining specimens for laboratory confirmation and paucibacillary characteristic of the specimens (Sharma & Mohan, 2004; Norbis *et al*.,

2014). Hence, only a few proportions of cases have positive microbiological confirmation for *M. tuberculosis*. Delayed presentation, inaccurate diagnosis and inappropriate treatment might worsen the scenario in EPTB cases.

1.5.2 (a) Lymph node tuberculosis

Lymph node TB is the most common form of EPTB, often involve cervical lymph nodes, with or without associated disease with other lymphoid tissue. Numerous studies have found that lymph node have predilection for women, approximately 2:1 and is more common in younger patients (Thornton, 1995; Eshete *et al*., 2011). It usually presents with gradually increasing painless swelling lymph nodes of weeks to month duration. Some patients may have systemic symptoms.

1.5.2 (b) Pleural tuberculosis

Pleural TB occurs when ruptured subpleural infected foci of *M. tuberculosis* spread into pleural space (Thornton, 1995). Symptoms may include pleurisy, chest pain, cough and fever. A chest x-ray (CXR) often show a unilateral effusion with commonly small to moderate in size. Bilateral pleural TB effusions are rare and associated with disseminated disease. A person with pleural TB may have underlying pulmonary infection that is masked on CXR due to compression of effusion fluid to the lung.

1.5.2 (c) Central nervous system tuberculosis

Central nervous system (CNS) infection is the most devastating form of TB. It constitutes approximately 5 % to 15% of the EPTB cases, carries a high morbidity and mortality despite of given anti-TB treatment (Marais *et al*., 2010). According to Norris & Buckley (1995), in early stages of infection, tuberculous foci developed in

meninges, spinal cord or brain parenchyma following haematogenous seeding. Later, the foci ruptured into subarachnoid space manifest primarily as tuberculous meningitis and less commonly as tuberculous encephalitis, tuberculoma and brain abscess.

1.5.2 (d) Skeletal tuberculosis

Skeletal TB refers to TB infection of the bones and/or joints. It usually involves weight-bearing bones and joints such as spine, hip, knee, wrist and shoulder (Sankaran, 1993). Spine TB, often accompanied with back pain is the commonest form of skeletal TB. It was responsible for about 40 % to 50% of the cases (Agrawal *et al*., 2010). Prompt diagnosis is often difficult due to failure of consider TB in differential diagnosis.

1.5.2 (e) Genitourinary tuberculosis

Genitourinary TB commonly present only after a long latent period ranges from 5 to 25 years. It often involves men more than women at nearly a 2:1 ratio (Rom & Garay, 2004). Any part of genitourinary systems such as kidney, bladder, fallopian tube, prostate and epididymis may be involved. Patients may normally present with symptoms referred to organ involved or may have unexplained urological symptoms.

1.5.2 (f) Abdominal tuberculosis

Abdominal TB is defined as mycobacterial infection in the gastrointestinal tract, peritoneum, or intra-abdominal solid organs. According to Grange & Zumla (2008), abdominal TB can be acquired due to swallowed infected sputum of active PTB, haematogenous dissemination from a focus of active PTB or military TB or spread of

bacilli from infected adjacent organs. Symptoms and signs of infection are nonspecific, often lead to difficulty in diagnosis. They include weight loss, abdominal pain and tenderness, anorexia, diarrhoea, fever and night sweats.

1.5.2 (g) Miliary tuberculosis

Miliary TB refers to TB infection involving many different organs, including the lung. It occurs when tubercle bacilli enter the bloodstream and disseminate to all parts of the body, where they grow and cause disease in multiple sites (Thornton, 1995). The term "miliary" refers to the radiograph appearance of millet seeds, usually less than 2 mm in diameter scattered throughout the lung. This condition is rare, accounts for 2 % from all TB cases, yet has high mortality rate despite of availability of effective TB treatment (Sharma *et al*., 2012). It is more common in older age groups and in human immunodeficiency virus infection/ acquired immune deficiency syndrome (HIV/AIDS) patients.

1.6 Latent tuberculosis

People who are diagnosed having a latent TB infection do not feel sick and do not have any symptoms. Most of them are healthy with a normal chest x-ray and a negative sputum test. Generally, latent TB is defined solely on a positive result of particular tests namely tuberculin skin test (TST) and interferon-gamma release assay (IGRA) with the absence of clinical symptoms (National Clinical Guideline Centre, 2006). People with latent TB are not infectious and cannot spread TB infections to others. However, they may develop to active TB diseases, often when their immune system becomes weak.

1.7 Epidemiology of tuberculosis

TB disease remains a major public health threat worldwide. It is a second most common cause of death globally after HIV/AIDS. It was estimated 9 million of incidence cases corresponding to 126 cases per 100 000 population and 1.5 million of TB death globally in year 2013 (WHO, 2014). Yet, the newly infected cases were observed to be decreased 0.6 % between 2012 and 2013 while mortality rate has decreased 45 % since 1990.

Out of 9.0 million of people that newly fell ill with TB, 1.1 million were co-infected with HIV. The occurrence was highest in African region with estimation of 34 % from the total TB cases locally. These cases represented 78 % of the TB cases co-infected with HIV globally. Apart from that, 3.5 % of new TB cases and 20.5 % of previously treated cases are estimated to have multidrug resistance TB (MDR-TB). Overall, 9 % of these MDR-TB cases were reported to be extensive multidrug resistance TB (XDR-TB). It has been reported in 100 countries with most of them were notified from Ukraine, South Africa, India and Kazakhstan (WHO, 2014).

Most of the TB cases were found in Asia, 56 % and African Region, 29 %. Small proportion of the cases occurred in Eastern Mediterranean Region, 8 % followed by European Region, 4 % and Region of America, 3 %. India and China alone had contributed 24 % and 11 % of global cases respectively. 82 % of incidence cases majorly were reported from high-burden countries while the lowest incidence rates were observed to be predominantly in high-income countries such as United State, Canada and Japan. Figure 1.3 showed world map distribution of estimated TB incidence rate for year 2013 (WHO, 2014).

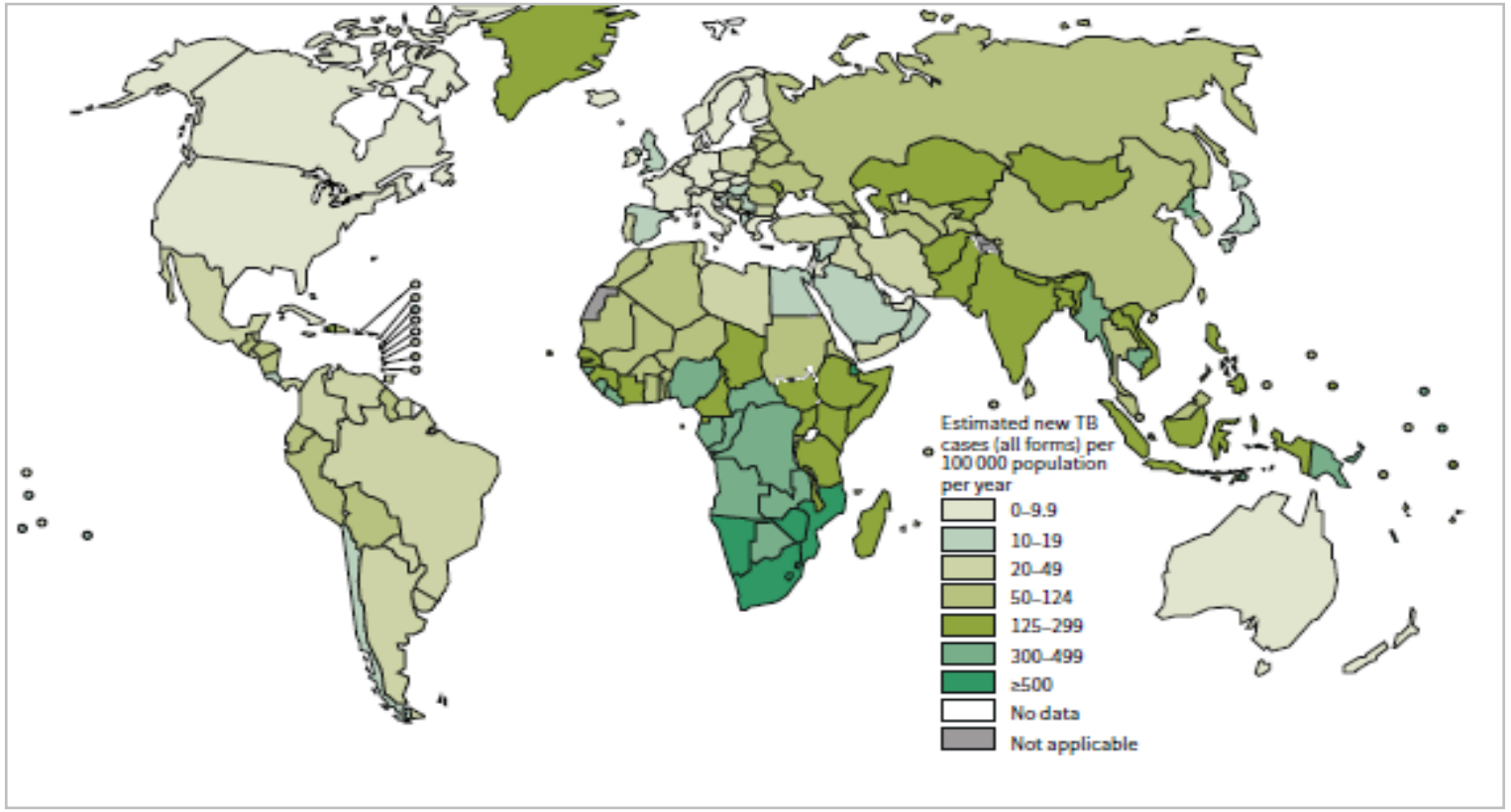

Figure 1.3: Estimated TB incidence rates in 2013. (Adapted from WHO, 2014).

1.8 Factor affecting tuberculosis

There are a number of risk factors for TB reported in former studies. It relies upon both the risk of being infected, depending on the incidence of TB in particular populations and the risk of infection leading to active disease, depending on many factors affecting on the individual genetically and environmentally.

1.8.1 Human Immunodeficiency Virus

HIV infection has resulted in increasing numbers of TB cases and TB mortality in many countries especially in populations with high HIV prevalence (D'Ambrosio *et al*., 2012). People living with HIV are estimated to develop TB 29 times greater than those who are HIV negative. Co-infection with HIV may cause increment in TB progression following primary infection, re-infection of TB or reactivation of latent TB infection. The extrapulmonary involvement can be seen in more than 50 % of TB and HIV co-infected patients while only 12 % to 20 % for immunocompetents patients. Development of TB in turns accelerates HIV replication led to more rapid progression of HIV disease. The co-infection act synergistically to magnify the burden of the diseases which ultimately causes death (Kwon and Ernst, 2011).

1.8.2 Diabetes mellitus

Globally, about 10 % of TB cases are linked to DM, a chronic metabolic disorder that weakens body immune systems. It is reported that people with DM have 2 to 3 times higher risk on developing TB than non-diabetic people. DM may worsen TB patients' outcome as it can alter the immune system responses necessary to control TB infection and reduce bactericidal activity by interfering TB drugs pharmacokinetic (Jeon *et al*., 2012). Apart from that, patient might confront a greater

probability death during TB therapy due to DM implications, such as renal failure heart disease and renal impairment.

1.8.3 Immigration

A proportion of TB cases among immigrants population are steadily increased in many countries, for example United State and Europe. It is postulated that immigration portrays a critical role in spreading TB disease due to existing TB infection or reactivation of latent TB (Gilbert *et al*., 2009). The immigrants are usually poor workers that come from high TB burden countries and carry the infection with them. Majority of them live in overcrowded places, suffer from malnutrition and have limited access in healthcare system. These conditions are favourable for developing active TB, initiates a local transmission of the disease (Barnett & Walker, 2008).

1.8.4 Smoking

A strong relationship has been demonstrated in previous studies between TB and smoking. Reasons for the relationship may be explained by the adverse effects of smoking on pulmonary host defences causing smokers inefficiently combat TB infection (Wen *et al*., 2010). Smoking also reduces the effectiveness of TB therapy, which in turns prolonged the infection that may lead to severe TB forms. Active smokers are more likely to develop latent TB infection, progress to active TB and increase the risk of death than non-smokers. In addition, current population-based study in Taiwan has found smoking of more than 10 cigarettes a day for continuous period had a higher risk of TB recurrence (Yen *et al*., 2014).

1.8.5 Malnutrition

Association between TB and malnutrition is known for a long time. It has been reported malnutrition profoundly affects cell mediated immunity which play a major role in body defense against TB (Cegielski & McMurray, 2004). Altered immunity, by this means increases the burden of primary infection or initiate latent TB progression to active TB. These malnourished active TB patients tend to have delayed recovery and higher mortality compared to well-nourished patients.

1.8.6 Poverty

TB is often known as a disease of poverty. Prevalence of TB is noted to be higher in poor countries with the lowest national products particularly in Sub-Saharan African regions (Palomino *et al*., 2007). In comparison to wealthy countries, poor countries are lacking a good public health care system which is expected to keep TB endemic under control. Apart from that, poor people might live in unfavourable conditions that increase TB infection susceptibility and spreading of the disease, for instance, crowded living condition, lack of sanitary and poor nutrition.

1.9 Tuberculosis in Malaysia

TB is one of the top five communicable diseases in Malaysia. In 1961, National Tuberculosis Control Programme was conducted with the aim to control morbidity and mortality causes by the disease. As a result, a significance number of reported TB cases had reduced from 350 cases per 100 000 population to less than 100 cases per 100 000 population in 1980's (Ministry of Health, 2011a).

Generally, about two thirds of the TB patients in Malaysia were between 15 to 54 years age group with male predominant. Small percentages of TB cases were also found among immigrants, whom usually came from high TB burden neighbouring countries (Iyawoo, 2004). Influx of the immigrants was noted to influence the number of TB cases particularly in several states in Malaysia. According to Dony *et al.*, (2004), immigrants contributed more than 24 % of the incidence cases in Sabah. Currently, the highest number of TB cases was found to be in Sabah with 4426 cases. Figure 1.4 shown the number of TB cases by Malaysia's states in 2012.

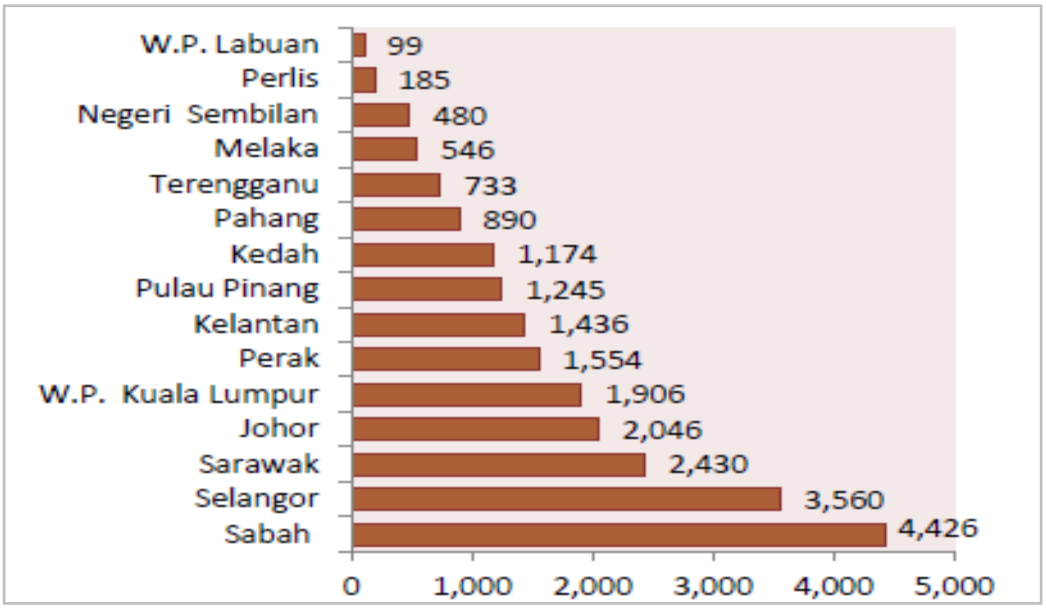

Figure 1.4: Number of TB cases according to Malaysia's states in 2012. (Adapted from Department of Statistic, 2013).

In the past few years, TB incidence rate in Malaysia was noticed to be gradually increased. As shown in Figure 1.5, increment of the incidence rate began in 2008 with 77 per 100 000 populations and continue to increase until 99 per 100 000 populations in 2013. In spite of that, TB mortality rate among HIV-negative patients remained constant with 5.8 per 100000 population for those years (WHO, 2014).

A total of 24071 TB cases which has been notified in 2013 encompasses 23417 (97.3 %) of new and relapse cases and 654 (2.7 %) of previously treated cases. Of the total number new and relapse cases, 14317 (61.1 %) cases were found to be pulmonary bacteriological confirmed and 5947 (25.4%) cases were pulmonary clinically diagnosed. EPTB cases were found to be 13.5 % (3153 cases) of the total TB cases (WHO, 2014).

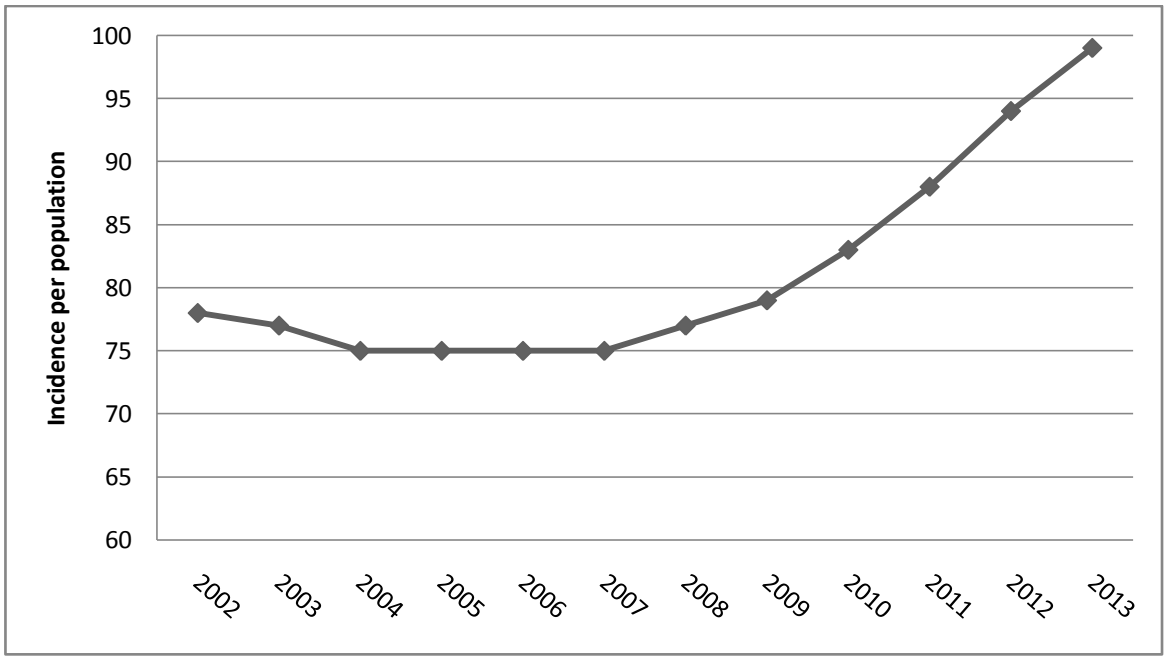

Figure 1.5: TB incidence rate per 100 000 populations in Malaysia, 2002-2013 (Adapted from WHO, 2014)