Chapter 11

Epidemiology of Multidrug Resistant Tuberculosis (MDR-TB)

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Additional information is available at the end of the chapter

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1. Introduction

An understanding of the epidemiology of multidrug resistant tuberculosis (MDR-TB) and the extensively drug-resistant tuberculosis (XDR-TB) is critical for effective control of the global burden of tuberculosis (TB) which is caused by the organisms belonging to the *Mycobacterium tuberculosis* complex. Epidemiology of MDR-TB and XDR-TB will be reviewed here.

The history of tuberculosis treatment has observed sequential development of resistance to anti-tuberculosis drugs over the decades. Para amino salicylic acid (PAS) and isoniazid (INH) were introduced to reduce the development of streptomycin (SM) resistance, which heralded the era of combination treatment for tuberculosis [1]. Within 20 years, resistance to both INH and SM was already a challenge in the use of INH, SM and PAS as the standard anti-tuberculosis regimen. With the discovery of rifampicin (RMP) in 1966 [2] and the expansion of its use between 1970 and 1990, patients who were already carriers of isoniazid (INH) resistant *Mycobacterium tuberculosis* strains became resistant to RMP. This was the start of a progressively growing problem, multi drug resistant tuberculosis (MDR-TB), which has reached epidemic proportions in some countries. In the last two decades, with the misuse of other drugs with anti-tuberculosis action, in particular the fluoroquinolones (FQs), the most effective among the second-line drugs, resistance has dramatically increased to extensively drug-resistant TB (XDR-TB) which is defined as resistance to at least RMP and INH (the definition of multidrug-resistant tuberculosis (MDR-TB)), in addition to any fluoroquinolone, and at least one of the three injectable anti-tuberculosis (TB) drugs capreomycin, kanamycin and amikacin [3].

1.1. Epidemiology

John Last has defined epidemiology as "The study of the distribution and determinants of health-related states or events in specified populations, and the application of this

study to the control of health problems" [4]. Epidemiologists are concerned not only with death, illness and disability, but also with more positive health states and, most importantly, with the means to improve health [5]. Epidemiological studies are classified as either observational or experimental. Various methods can be used to carry out epidemiological investigations: surveillance and descriptive studies are used to study distribution while analytical studies are used to study determinants.

The two mostly common terms used in epidemiology are the 'prevalence' and the 'incidence'. The incidence of disease represents the rate of occurrence of new cases arising in a given period in a specified population, while prevalence is the frequency of existing cases in a defined population at a given point in time [5]. These are fundamentally different ways of measuring occurrence, and the relation between incidence and prevalence varies among diseases [5]. [For a comprehensive study on epidemiology, please refer the World Health Organization (WHO) manual on Basic Epidemiology].

1.2. Epidemiology of tuberculosis

Despite the availability of highly efficacious treatment for decades, TB remains a major global health problem. In 1993, WHO declared TB a global public health emergency, at a time when an estimated 7–8 million cases and 1.3–1.6 million deaths occurred each year. In 2010, there were an estimated 8.5–9.2 million cases and 1.2–1.5 million deaths from TB [6]. According to the newest report, has observed a gradual decline in the absolute number of TB cases since 2006 and also in the incidence rates of TB since 2002 [6].

2. Global epidemiology of MDR-TB

2.1. Global epidemiology of MDR-TB (global tuberculosis control: WHO report 2011)

Globally, around 50 000 cases of MDR-TB were notified to WHO in 2010, mostly by European countries and South Africa. This represented 18% of the 290 000 (range, 210 000–380 000) cases of MDR-TB estimated to exist among patients with pulmonary TB who were notified in 2010. The proportion of TB patients estimated to have MDR-TB that were actually diagnosed was under 10% in all of the 27 high MDR-TB countries outside the European Region, with the notable exception of South Africa where 81% of estimated cases were diagnosed. In, 15 high MDR-TB burden countries in the European Region, the proportion of estimated cases that were diagnosed ranged from 24% (in Tajikistan) to over 90% of cases (in Belarus and Kazakhstan); no data were reported from Lithuania. In Russian Federation, which ranks third in terms of estimated numbers of cases of MDR-TB at the global level, the proportion of estimated cases that were diagnosed was 44% in 2010. The numbers of patients diagnosed with MDR-TB and started on treatment with recommended second-line drug regimens in the high MDR-TB burden countries in 2010, at just under 40 000, was less than the number of cases notified [6]

2.2. Regimen surveys and definitions of patients registration groups for treatment of tuberculosis

'Regimen surveys' measure first-line and/or second-line drug resistance among a group of selected patients that cannot be considered representative of a patient population [7]. These surveys help to determine the predominant patterns of drug resistance, and are useful in providing guidance on appropriate regimens for MDR-TB treatment for particular patient groups. These include return cases after treatment failure, chronic cases and symptomatic contacts of MDR-TB cases. According to WHO, Regimen surveys should be conducted in the process of developing MDR-TB treatment programmes, or within selected centres or diagnostic units that regularly address high-risk cases.

The fourth edition of WHO *Guidelines for treatment of tuberculosis* defines patient registration groups by history of previous treatment [8]. [For a comprehensive study on definitions, please refer the document WHO/HTM/TB/2009.420].

2.2.1. New case

For the purpose of surveillance, a 'new case' is defined as a newly registered episode of TB in a patient who, in response to direct questioning denies having had any prior anti-tuberculosis treatment (for up to one month), and in countries where adequate documentation is available, for whom there is no evidence of such history. Determining the proportion of drug resistance among new cases is vital in the assessment of recent transmission.

2.2.2. Previously treated case

For the purpose of surveillance, a 'previously treated case' is defined as a newly registered episode of TB in a patient who, in response to direct questioning admits having been treated for TB for one month or more, or, in countries where adequate documentation is available, there is evidence of such history.

2.2.3. Primary resistance

Patients with TB resistant to one or more anti-tuberculosis drugs, but who have never been previously treated for TB, are said to have "primary resistance" (or "initial resistance") due to transmission of a drug-resistant strain.

2.2.4. Acquired resistance

Patients diagnosed with TB who start anti-tuberculosis treatment and subsequently acquire resistance to one or more of the drugs used during the treatment, are said to have developed "acquired resistance". In the past, resistance among previously treated cases (defined as cases with ≥ one month history of treatment) was used as a proxy for acquired resistance; however, this patient category is now known to also be comprised of patients who have been re-infected with a resistant strain, and patients who were primarily infected with a resistant strain and subsequently failed therapy or relapsed.

2.2.5. Cured

A patient who has completed a course of anti-TB treatment according to programme protocol and has at least five consecutive negative cultures from samples collected at least 30 days apart in the final 12 months of treatment. If only one positive culture is reported during that time, and there is no concomitant clinical evidence of deterioration, a patient may still be considered cured, provided that this positive culture is followed by a minimum of three consecutive negative cultures taken at least 30 days apart.

2.2.6. Failed

Anti-TB treatment will be considered to have failed if two or more of the five cultures recorded in the final 12 months of therapy are positive, or if any one of the final three cultures is positive. Treatment will also be considered to have failed if a clinical decision has been made to terminate treatment early because of poor clinical or radiological response or adverse events. These latter failures can be indicated separately in order to do sub-analysis.

3. Surveillance studies for the assessment of resistance rates and the detection of MDR-TB

MDR-TB poses a therapeutic challenge and is associated with increased mortality. Surveillance studies for the assessment of resistance rates and the detection of MDRTB are therefore crucial in order to optimize empiric drug therapy and to prevent the dissemination of resistant strains in a community [9]. The extent of the problem of MDR-TB has been examined by the WHO in cross-sectional surveys of drug resistance in either clinical series or whole-country cohorts [10]. Cross-sectional surveys almost certainly underestimate the burden and number of cases of MDR-TB because they do not take into account the numerical burden of TB in the high-burden countries [11]. When the exercise is repeated with a mathematical modeling design using drug-resistance estimates and the number of cases of TB, a more accurate picture of the global MDR-TB burden is claimed [12].

3.1. Global project of drug resistance surveillance

The WHO and the IUATLD (International Union against Tuberculosis and Lung Disease) have established a global project of drug resistance surveillance that is based on standard epidemiological methods and quality control through an extensive network of reference laboratories. The Global Project has served as a common platform for country, regional and global level evaluation of the magnitude and trends in anti-tuberculosis drug resistance quantified the growing global burden of MDR-TB and started to document the spread of XDR-TB. Since its launch in 1994, the Global Project has collected and analyzed data on drug resistance from surveys of sampled patients and from national surveillance systems from an ever increasing number of settings around the world [7].

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The review of Cohn et al, 1997 represented a comprehensive description of worldwide drug resistance surveys performed during the 1990s. According to the study, resistance to multiple drugs varied by geographic region and was more common when resistance was acquired rather than primary. The rate of multidrug resistance (and occasionally other drugs) was low in most surveys of primary resistance, ranging from 0 to 10.8% (median rate, 0.5%); however, for acquired resistance, the rate of multidrug resistance ranged from 0 to 48.0% (median rate, 12.2%). For surveys that did not distinguish between primary and acquired resistance, the range was 0.5% to 14.3% (median rate, 2.3%). In terms of antituberculous drug resistance, they found a great deal of variability between different countries, and within some countries, differences between regions or cities [13].

The review of Caminero et al of 2010 [14], broadly discuss the epidemiological data of the global report, issued in 2008. The report included drug susceptibility data from 90 726 patients in 83 countries and territories from year 2002 to 2007. The median prevalence of resistance in new cases of TB was 11.1% for any drug and 1.6% for MDR-TB. The prevalence of MDR-TB in new TB cases ranged from 0% in eight countries to 22.3% in Baku, Azerbaijan, and 19.4% in the Republic of Moldova. Of the 20 settings with the highest proportion of MDR-TB in new cases, 14 were located in countries of the former Soviet Union (between 6.8% and 22.3% in nine countries, including Moldovia and Azerbaijan) and four in China (7% in two provinces in China) [15, 16], A trend analysis of the 2008 report shows that between 1994 and 2007 the prevalence of MDR-TB in new cases (initial resistance) increased substantially in South Korea and two Russian Oblasts, Tomsk and Orel. By contrast, the prevalence remained stable in Estonia and Latvia, both of which have high rates of initial MDR-TB. The prevalence of MDR-TB in all TB cases decreased in Hong Kong and the United States [14].

Of 37 countries and territories that reported representative data on XDR-TB, five countries, all from the former Soviet Union, each reported 25 or more cases of XDR-TB, with MDR-TB prevalence ranging from 6.6% to 23.7% [15, 16], data from Eastern Mediterranean countries showed that the prevalence of initial MDR-TB was higher than previously estimated, with the exception of Morocco and Lebanon, with rates of respectively 0.5% and 1.1%. Initial MDR-TB rates in Jordan and Yemen were respectively 5.4% and 2.9%. The Americas, Central Europe and Africa reported the lowest rates of initial MDR-TB, with the notable exceptions of Peru, Rwanda and Guatemala, which reported rates of respectively 5.3%, 3.9% and 3.0%. [15, 16]. Data on previously treated cases from the WHO/ Union 2008 report were available for 66 countries and two regions of China [15]. Drug susceptibility testing (DST) results were available for 12 977 patients. Resistance to at least one anti-tuberculosis drug ranged from 0% in three European countries to 85.9% in Tashkent, Uzbekistan. The highest proportions of MDR-TB were reported in Tashkent (60.0%) and Baku, Azerbaijan (55.8%). data from Gujarat State, India, providing the first reliable descriptions of previously treated cases in India, showed 17.2% MDR-TB in this group [15].

The 2008 WHO/Union report also included a global estimation of the MDR-TB problem [14]. Based on drug resistance data from 114 countries and two regions of China reporting to this project, combined with nine other epidemiological factors, the proportion of MDR-TB among new, previously treated and combined cases was estimated for countries with no survey

information available. The estimated proportion of MDR-TB for all countries was then applied to incident TB cases (also based on indirect estimates). It was calculated that 4 89 139 (95% confidence limits [95%CL] 455 093–614 215) cases emerged in 2006, and that the global proportion of MDR-TB among all cases was 4.8% (95%CL 4.6–6.0). India, China and the Russian Federation were estimated to have the highest number of MDR-TB cases: India and China have approximately 50% of the global burden and the Russian Federation a further 7%. Twenty seven countries accounted for 86% of the world's MDRTB burden [14].

Caminero et al, divided the world into four large regions according to the influence of the three factors i.e., past and present management of TB and transmission of MDR-TB.

1. Countries with an epidemic: high prevalence and incidence of MDR-TB.

2. Countries with high MDR-TB prevalence but low or decreasing incidence.

3. Countries with low prevalence and incidence of MDR-TB and

4. Countries with low prevalence but an increasing incidence of MDR-TB.

The fourth edition of WHO *Guidelines for surveillance of drug resistance in tuberculosis* is an updated version of earlier editions published in 1994, 1997 and 2003. These guidelines incorporate the 2007 WHO *Interim recommendations for the surveillance of drug resistance in tuberculosis* and the conclusions of an Expert Committee Meeting on Anti-Tuberculosis Drug Resistance Surveys held in Geneva in September 2008. In addition experience gained from 15 years of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance is also included [7].

Given below are some of the updates and clarifications in surveillance methodology that have been incorporated into the 4th edition:

1. At a minimum, surveillance should evaluate susceptibility to the following drugs:

a. Isoniazid and rifampicin;

b. If resistance is detected to rifampicin, then susceptibility to the fluoroquinolones and second- line injectable agents most often used in the setting have be tested. Testing for susceptibility to the first-line drug ethambutol should also be considered.

2. Statistical and epidemiological methodology is a fundamental aspect of designing surveys that sample patients, and appropriate technical assistance should be received in the early stages of planning. In particular, for surveys that use cluster-based sampling methods, results should be adjusted to correct for biases introduced by these sampling techniques. Missing values should also be accounted for, e.g. using multiple imputation techniques when possible.

3. MDR-TB management is a component of the Stop TB Strategy and WHO Member States have committed themselves to achieve universal access to diagnosis and treatment by 2015. Therefore, all drug resistance surveillance activities should be linked to patient treatment and care. Planning a comprehensive treatment programme for patients identified during a survey as having drug-resistant TB should run in parallel to planning the survey itself.

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3.2. MDR-TB and immigration

Gilad et al [9] assessed the incidence of TB in Southern Israel in the period between 1992 and 1997, and studied the prevalence of resistance to anti-TB drugs and its distribution among the various subpopulations inhabiting that region, with the intention of tailoring the empirical anti-TB treatment guidelines to those subpopulations. This study described the unique epidemiology of drug-resistant TB in Southern Israel, a region inhabited by both native and immigrant populations. Significant differences in age, gender, and resistance rates were found among the four distinct subpopulations inhabiting the Negev region. They attributed the observed differences to immigration from countries of high prevalence of drug-resistant TB. According to an earlier 10-year survey (1978- 1987) of TB in the Negev, Ethiopian immigrants and Bedouin Arabs comprised 76% of TB cases and 33% of them were extrapulmonary TB [17]. However the study of Gilad et al [9], recorded only 20% of isolates as extrapulmonary, and Ethiopian immigrants and Bedouin Arabs comprised only 40% of the cases. These differences demonstrate how dynamic this disease might be, tremendously influenced by immigration, and demonstrate the importance of continued surveillance in such a setup [9].

A worldwide survey of drug resistance rates by the WHO [18] demonstrated high rates of resistance among isolates in the former Soviet Union, with the highest rates detected in Latvia. The Resistance rates observed rom the study for the immigrants from the former Soviet Union (IFSU) were much higher than those encountered in the Russian republic for every drug or drug combination [9]. These rates were very similar to those found in Latvia, and were even higher overall (50% and 41.5%, respectively). In this current era, import of infectious diseases across international borders occurs much readily [9]. The studies done in Germany and Canada also had reported an increased incidence of multidrug resistance due to the immigration [19-21].

These studies have shown the impact of immigration on the incidence and distribution of drug-resistant TB in a particular country and the importance of continuous surveillance and immediate therapeutic decisions to prevent the dissemination of such resistant strains to their general populations [9].

3.3. HIV and MDR-TB

The epidemiological impact of HIV on the epidemic of drug-resistant TB is not known and may depend on several factors. HIV-positive TB cases are more likely to be smear negative. In addition, delayed diagnosis of drug resistance and unavailability of treatment (particularly in previous years) have led to high death rates in people living with HIV. Both of these factors (smear negativity and short duration of disease due to mortality) may suggest a lower rate of general transmission. However, HIV-positive cases progress more rapidly to disease and in settings where MDR-TB is prevalent (either in the general population or in the local population such as a hospital or a district), this may lead to rapid development of a pool of drug-resistant TB patients or an outbreak [7].

According to Cohn et al, 1997 [13], though the association of MDR-TB with AIDS has been well documented during outbreaks [22-24], the role of HIV infection as a risk factor for the development of drug-resistant TB in other settings was not clear [25]. In Kenya, Malawi, Tanzania, COte d'Ivoire, and France, drug resistance was not associated with HIV infection [26-30]. In contrast, in a survey of eight metropolitan areas of the United States, HIV infection was associated with resistance to antituberculous drugs, both within and outside the New York City area [31]. The acquired MDR-TB also occurs in largely immunocompetent hosts, which was seen in India, Korea, Nepal, and Bolivia [32-35].

The studies by Borrell and Gagneux [36] pointed out that, from a scientific point of view, the actual evidence for primary transmission of MDR -TB in HIV-negative individuals that has been confirmed by molecular methods is very limited, and that more studies including molecular data are needed to know the true extent of primary MDR-TB & XDR –TB in a general population.

3.4. Inadequate treatment and development of MDR and XDR-TB

Multidrug-resistant tuberculosis (MDR-TB) is a major challenge for TB control worldwide. Inadequate treatment of MDR-TB inevitably results in high mortality and the development of XDR-TB [37]. The study of Jeon et al, 2011 [38], shows how inadequate treatment has contributed to the high prevalence of MDR and XDR-TB in Korea. According to Jeon et al, the three TB referral hospitals in the public sector are responsible for the management of MDR-TB in the public sector of Korea. This study showed poor outcome for patients with MDR-TB at the 3 TB hospitals in Korea: low treatment success rate (37.1%), high default rate (37.1%), and high all-cause mortality rate (31.2 %) during the 3-4 yr after treatment initiation. Since the National Tuberculosis Program (NTP) of Korea has focused on new cases, there have been limited nationwide data about the incidence and prevalence of MDR-TB and its treatment outcomes. Treatment success rate of their study was the lowest ever reported among MDR-TB cohorts in Korea [38].

4. Molecular epidemiology of MDR-TB

4.1. Molecular epidemiology

Many different definitions of molecular epidemiology have been published and all mention the use of molecular tools, but not all explicitly mention epidemiology. Molecular epidemiology is not just molecular taxonomy, phylogeny, or population genetics but the application of these techniques to epidemiologic problems [39]. Epidemiology attempts to identify factors that determine disease distribution in time and place, as well as factors that determine disease transmission, manifestation, and progression. Further, epidemiology is always motivated by an opportunity or possibility for intervention and prevention [39]. What distinguishes molecular epidemiology is both the “molecular,” the use of the techniques of molecular biology to characterize nucleic acid- or amino acid-based content, and the “epidemiology,” the study of the distribution and determinants of disease occurrence in human populations [39].

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Molecular epidemiology makes use of the genetic diversity within strains of infectious organisms to track the transmission of these organisms in human populations and to evaluate the host and parasite -specific risk factors for disease spread.

Therefore molecular epidemiologic techniques can be incorporated into almost any epidemiologic assessment to improve exposure and outcome measures

4.2. Molecular epidemiology of TB

The molecular epidemiologic approach to studying tuberculosis epidemiology has identified several new observations that could not have been obtained by conventional epidemiologic or laboratory approaches [39]. Mycobacterial strain typing by means of molecular methods has become an important instrument for tuberculosis surveillance, control and prevention [40]. Among DNA fingerprinting methods which restriction fragment length polymorphism (RFLP) typing is the most common method used has permitted novel investigations of the epidemiology and pathogenesis of tuberculosis. The use of IS*6110*, an insertion sequence which is present in *Mycobacterium tuberculosis*, is generally considered to be the gold standard for tuberculosis molecular epidemiology studies [41], but other molecular typing techniques could be used as adjuncts in selected circumstances [42].

Spoligotyping is a technique based on the polymorphism of the direct repeat (DR) locus present in *M. tuberculosis* DNA. The DR sequences are composed of multiple 36bp copies, interspersed by short non repetitive sequences [43]. The direct-repeat locus in *M. tuberculosis* contains 10 to 50 copies of a 36-bp direct repeat, which are separated from one another by spacers that have different sequences. However, the spacer sequences between any two specific direct repeats are conserved among strains. Because strains differ in terms of the presence or absence of specific spacers, the pattern of spacers in a strain can be used for genotyping (spacer oligonucleotide typing, or "spoligotyping"). Spoligotyping has two advantages over IS*6110*-based genotyping. As small amounts of DNA are required, it can be performed on clinical samples or on strains of *M. tuberculosis* shortly after their inoculation into liquid culture. In addition the results of spoligotyping, which are expressed as positive or negative for each spacer, can be expressed in a digital format. However, spoligotyping has less power to discriminate among *M. tuberculosis* strains than does IS*6110*-based genotyping.

Mycobacterial interspersed repeat units (MIRU) genotyping categorizes the number and size of the repeats in each of 12 independent MIRUs, with the use of a polymerase-chain-reaction (PCR) assay, followed by gel electrophoresis to categorize the number and size of repeats in 12 independent loci, each of which has a unique repeated sequence. Two to eight alleles are at each of the 12 loci, yielding approximately 20 million possible combinations of alleles. The discriminatory power of MIRU genotyping is almost as great as that of IS*6110*-based genotyping. Unlike IS*6110*-based genotyping, MIRU analysis can be automated and can thus be used to evaluate large numbers of strains, yielding intrinsically digital results that can be easily catalogued on a computer data base.

The PGRS, the DR and the GTG repeated sequences have mainly been used for sub typing strains for which differentiation by IS*6110* finger printing appeared insufficient. This

is useful when *M. tuberculosis* strains contain no or lesser than six copies of IS*6110.* According to a recorded study in Sri Lanka, 68% of the isolates had less than five copies which were similar to that of other countries in the Asian region, such as India, Malaysia, Oman and Hong Kong [44].

The study by Ghebremichael et al [45] determined the transmission pattern of TB strains in Sweden. By MIRU-VNTR 31 (45%) of the 69 patients with Beijing strains were found in altogether 7 clusters (2–11 per cluster), yielding 45 different patterns. Thus the MIRU-VNTR typing, with fewer and larger clusters, was less discriminatory than IS*6110* RFLP. The two strains where a possible epidemiological linkage was established differed in one allele and thus did not cluster in MIRU-VNTR. All strains that clustered by MIRU-VNTR were identical also by RD deletions, mutT gene polymorphism and Rv3135 gene analysis, but not by spoligotyping and IS1547. Four of the IS*6110* RFLP clusters contained isolates that differed by MIRU-VNTR. The combination of MIRUVNTR with RFLP resulted in the disappearance of two clusters, and a reduction of the number of isolates in two clusters, compared to the clustering observed with IS*6110* RFLP clustering alone. In this study they found that patients with DR Beijing strains have been diagnosed for more than a decade in Sweden. The majority of the patients were foreign born, and their country of origin reflects areas where the Beijing genotype is prevalent [45].

4.3. Molecular epidemiology of MDR-TB

A study by Calver et al [46], investigated an outbreak of tuberculosis using a molecular epidemiologic approach and clinical and epidemiologic data to identify inadequacies in the implemented DOTS-plus strategy that lead to the emergence of pre–XDR TB and XDR TB in South Africa. They genotyped the drug-resistant *M. tuberculosis* isolates using molecular techniques including insertion sequence (IS) *6110* RFLP, spoligotyping and MIRU typing (12-loci format). Genotyping results indicated an on-going transmission of drug-resistant TB, and contact tracing among case-patients in the largest cluster demonstrated multiple possible points of contact. Phylogenetic analysis demonstrated stepwise evolution of drug resistance, despite stringent treatment adherence. These findings suggested that existing TB control measures in South Africa were inadequate to control the spread of drug-resistant TB in their HIV co-infected population. Diagnosis delay and inappropriate therapy facilitated disease transmission and drug resistance.

Hsu et al, 2010 [47], investigated the transmission and predominant genotypes of MDR- TB in Eastern Taiwan using both spoligotyping and MIRU-VNTR. Of the tested MDR isolates of 73 (94%) Spoligotyping, identified the Beijing strain as the predominant genotype (n = 48, 66%), followed by Haarlem H3 (n = 15, 21%), T1 (n = 3, 4%) and East-African Indian 2 MANILLA (n = 1, 1%). Six (8%) isolates did not match any spoligotype in the SpolDB4 database. Using MIRU-VNTR typing, they observed a unique pattern in 27 isolates, and 46 had clustered pattern strains (10 clusters). According to them by MIRU-VNTR they observed an isolate in cluster 9, however from spoligotyping, it had a unique pattern and therefore they did not considered it as a clustered pattern strain. By considering both spoliotyping and MIRU-VNTR into account, 28 (38.4%) isolates were judged to have a unique pattern and 45 (61.6%) were clustered pattern

strains (classifying into 10 clusters). Assuming that there was one source case in each cluster and the rest in the cluster were due to transmission, Hsu et al, concluded that 47.9% ([45 − 10]/ 73) of the patients had MDR-TB due to recent transmission [47].

To better understand the epidemiology of MDRTB, the New York City Tuberculosis Control Program began DNA genotyping of MDRTB strains from new cases in 1995 [48]. The objectives of the study were to provide descriptive molecular epidemiology of MDRTB cases in the city during 1995–1997 and to identify predominant MDR strains present during the three years, as well as the extent and risk factors for clustering among the tested cases. Genotyping results were available for 234 patients; 153 (65.4%) were clustered, 126 (82.3%) of them in eight clusters of >4 patients. Epidemiologic links were identified for 30 (12.8%) patients; most had been exposed to patients diagnosed before the study period. From the analysis, the largest cluster observed was from the "W" strain (59 patients) representing almost 25% of the 241 MDRTB patients during the 3 years. This strain caused a well-documented multi-institutional outbreak in New York City from 1990 through 1993 [49-53]. Strain "W1", which was isolated in seven patients, is a variant of the W strain. It had an additional IS*6110* copy and was a part of the W strain outbreak [52, 53]. Forty percent (12 of 30) of the epidemiologic links in this cohort were to patients with these two strains. According to Munsiff et al [48] these strains were likely transmitted in the early 1990s when MDRTB outbreaks and tuberculosis transmission were widespread in New York.

To analyze the molecular epidemiology of *M. tuberculosis* strains at a hospital in Buenos Aires, Argentina, and mutations related to MDR and XDR-TB, Gonzalo et al [54], conducted a prospective case –control study. Spoligotyping identified predominance of the Haarlem family among the MDR TB cases (family responsible for the 1990s [55] outbreak) as well as the LAM and T families. A similar strain family distribution was reported for the French Departments of the Americas [56] and Turkey [57]. The Beijing family was seldom encountered in these areas, which is in line with recent observations in 7 countries in South America, including Argentina [58]. According to them [54] the MDR TB Haarlem2 strain appears to be more successful than other circulating MDR- TB strains and also than its susceptible counterpart (of 25 Haarlem2 strains, 20 were MDR TB).

By genotyping all isolates and combining with the mutational results, Perdigão et al [59] were able to assess the isolates' genetic relatedness and determine possible transmission events. According to their study strains belonging to family Lisboa, characterized several years ago, were responsible for the majority of the MDR-TB. Even more alarming was the high prevalence of extensive drug-resistant tuberculosis (XDR-TB) among the MDR-TB isolates, which was found to be 53%.

4.4. Transmission of MDR-TB and XDR-TB

Mathematical models predict that the future of the multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) epidemic will depend to a large extent on the transmission efficiency or relative fitness of drug-resistant *Mycobacterium tuberculosis* compared to drug-susceptible strains. Molecular epidemiological studies comparing the spread of drug-

resistant to that of drug-susceptible strains have yielded conflicting results: MDR strains can be up to 10 times more or 10 times less transmissible than pan-susceptible strains [36].

Experimental work performed with model organisms has highlighted a level of complexity in the biology of bacterial drug resistance that is generally not considered during standard epidemiological studies of TB transmission. However, much more work is needed to understand the detailed molecular mechanisms and evolutionary forces that drive drug resistance in this pathogen. Such increased knowledge will allow for better epidemiological predictions and assist in the development of new tools and strategies to fight drug resistant TB [36].

In infectious disease epidemiology, the relevant measure that reflects the reproductive fitness of a pathogen is the number of secondary cases generated; this measure is also known as the basic reproductive rate, R_0 [60]. In addition to the absolute number of secondary cases (i.e., absolute fitness), an often more useful measure is that of 'relative fitness', where the success of a particular pathogen variant is compared to the success of another. For example, the fitness of a drug-resistant bacterial strain can be expressed relative to the fitness of a drug-susceptible strain. In addition to epidemiological measures of relative fitness, differences in relative fitness can be measured experimentally [36].

The results of experimental studies performed with strains resistant to INH, SM or RMP suggested that, in clinical settings, there was a strong selection pressure for drug resistance-conferring mutations that cause minimal fitness defects [61]. Although these findings support the notion that virulence and competitive fitness assays can be predictive of the epidemiology of drug-resistant TB, they do not capture the overall complexity of the life cycle of *M. tuberculosis* [36]. Although several mechanisms of compensatory evolution have been described in other bacteria [62] little work has been done on this topic in *M. tuberculosis.*

Various molecular tools have been developed to genotype *M. tuberculosis* strains [63]. These tools have been applied to molecular epidemiological investigation of TB transmission for many years. According to the standard concept, patient isolates sharing a particular genotype or DNA 'fingerprint' can be considered epidemiologically linked and represent cases of active TB transmission (i.e., they are clustered TB cases), whereas strains with distinct or 'unique' DNA patterns are thought to reflect reactivation of latent infections. They compared molecular epidemiological fitness estimates from two previous reviews and more recent studies [60, 64]. Overall, the relative fitness estimates for MDR-TB vary dramatically, ranging from an almost 10-fold increased fitness compared to fully drug-susceptible strains found in a study from Russia [65] to about 10-fold lower fitness in Mexico [66] other studies have reported that MDR strains do not cause any secondary cases at all [67]. The reasons for this high variability in relative fitness of MDR strains have likely to do with the differences in study design and setting, differences in sample size and different methodologies and also to the variation in the quality of the TB control programmes [36]. According to Borrell and Gagneux, in addition to methodological, socio-economic and environmental factors, the variation in MDR fitness also reflects biological heterogeneity. Current epidemiological evidence for transmission of MDR- and XDR-TB, particularly compared to pan-susceptible TB, is very inconclusive. This can be partially explained by the fact that *M. tuberculosis* is more genetically diverse than is often

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appreciated [68] and because drug-resistant strains can exhibit heterogeneous fitness compared to drug-susceptible strains [36].

5. Conclusion

An understanding of the epidemiology of multidrug resistant tuberculosis (MDR-TB) and the extensively drug-resistant tuberculosis (XDR-TB) is critical for effective control of the global burden of tuberculosis (TB). For a comprehensive study on epidemiology of multidrug resistant tuberculosis (MDR-TB), please refer the reviews in the reference list.

6. Future studies

Future Studies on Epidemiology

In all epidemiological studies it is essential to have a clear definition of a case of the disease being investigated by delineating the symptoms, signs or other characteristics indicating that a person has the disease. A clear definition of an exposed person is also necessary. This definition must include all the characteristics that identify a person as being exposed to the factor in question. In the absence of clear definitions of disease and exposure, it is very difficult to interpret the data from an epidemiological study.

Future Studies on Transmission of TB

Future epidemiological studies on the transmission of drug-resistant TB should incorporate more comprehensive strain data, including specific drug resistance-conferring mutations and information on the strain genetic background. These variables, as well as their interaction, could play an important role in the transmission success of particular drug-resistant variants.

Future Studies on HIV/TB

The investigators who conduct the studies on HIV/ TB need to consider other possible risk factors for drug resistance such as demographics; prior therapy, socioeconomic status, and quality of TB control programs, etc.

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