MYCOBACTERIUM TUBERCULOSIS AND MULTIDRUG RESISTANCE (MDR): A REVIEW

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ABSTRACT
Background: Tuberculosis (TB) can be prevented through vaccination with the Bacillus Calmette-Guerin (BCG) vaccine, but it still poses a major global health threat, particularly in resource limited settings of the developing countries and resistance is a secondary compounder in the affected patients. The review highlights on *Mycobacterium tuberculosis* and multidrug resistance among other things.
Methodology: Previously published data were collected on things like: The causative agent, epidemiology of multidrug resistance (MDR), tuberculous treatment, Diagnosis and how resistance is developed.
Results: The review revealed that, **a**ntituberculous drugs are mainly divided into first-line drugs Isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA) and streptomycin (SM) and second-line drugs, which are sub Sub divided into fluoroquinolones, like ofloxacin (OFX) and injectables like Kanamycin (KAN). The review also revealed that MDR is a global threat and diagnosis could be done through absolute concentration method, resistance ratio method and proportions methods. It also revealed two principal pathways, for development of active drug-resistant TB namely acquired (secondary) drug resistance and primary drug resistance.
Conclusion: It could be concluded therefore that tuberculosis and drug resistance are complications that leads to serious situations among TB patients across the globe.
Keywords: *Mycobacterium tuberculosis*, MDR, tuberculosis, XDR

INTRODUCTION

Mycobacterium tuberculosis (*M. tuberculosis*) is an acid-fast bacilli that causes pulmonary tuberculosis (TB) in human, a devastating disease, in which one in four people in the world are infected (WHO, 2016). It only causes Tuberculosis when the infection becomes active; and activation occurs as a result of anything that can reduce the person's immunity, such as HIV, advancing age, diabetes or other immune compromising illnesses with common symptoms of cough, chest pain, weakness, weight loss, fever and night sweats (WHO, 2016).The global burden of TB remains alarmingly high, with 10.4 million incident cases and 1.5 million deaths reported by the World Health Organization in 2015 (WHO, 2016).

Multidrug-resistant *M. tuberculosis* strains are resistant to at least two of the most powerful first line anti-TB drugs, Isoniazid (INH) and Rifampin (RIF), with or without resistance to other first-line drugs. A subset of this strain is the Extensively Drug-Resistant (XDR) *M. tuberculosis* where there is also resistance to fluoroquinolones and at least one injectable second-line drug (such as Amikacin, Kanamycin or Capreomycin) in addition to resistance to Isoniazid and Rifampin (Nikalje and Mudassar, 2011).

Multidrug- resistant *M. tuberculosis* strains are a major global health concern because treatment of these cases requires second-line drugs, which are less effective, more expensive and more toxic, as well as sophisticated infrastructure for drug susceptibility testing not readily available in resource-limited settings. TB treatment success rates of cases caused by MDR variants of *M. tuberculosis* are alarmingly

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low, with only 54% of MDR cases resulting in cure, compared to 83% of drug-susceptible cases (WHO, 2016).

History of Antimycobacterial Drug Resistance

Antimycobacterial drug resistance emerged with the use of the first effective anti-TB agent Streptomycin was discovered in 1944. Many individual TB patients receiving streptomycin improved during the first months of treatment, only to relapse again as treatment continued. It was soon understood that this was due to the evolution of streptomycin-resistant MTB strains. As a result, streptomycin monotherapy was quickly abolished, and the first combination therapy was established by adding para-amino salicylic acid to the treatment (Gygli et al., 2017). During the subsequent years, different anti-TB agents were introduced and added to the growing multidrug anti-TB regimen. Nowadays, the treatment regimen, recommended by the WHO, compromises a 2-month initiation phase with a cocktail of four first-line anti-TB agents, i.e., isoniazid, rifampicin, ethambutol, and pyrazinamide, followed by a 4-month continuation phase with isoniazid and rifampicin. Despite the early establishment of anti-TB combination therapy, antimycobacterial drug resistance continues to emerge. A crucial factor is patient adherence, which is negatively impacted by the lengthy treatment duration, the complexity of the regimen, and the association of many adverse drug effects. Furthermore, differences in the quality of public health systems and the availability of high-quality anti-TB drugs also contributed to the spread of drug-resistant MTB strains (Gygli *et al.*, 2017). Over the years, drug-resistant TB alarmingly rose and even progressed to extensively drug-resistant (XDR), and, most recently, to total drug-resistant (TDR) TB. The former encompasses strains that are not only resistant to at least isoniazid and rifampicin, but are also resistant to second-line anti-TB drugs, i.e., at least one of the fluoroquinolone drugs and one of the injectable aminoglycosides (WHO, 2018). In case of TDR TB, resistance to a number of drugs beyond the XDR TB definition, including resistance to all clinically recommended drugs, occurs. Due to uncertain defining criteria, the WHO has not officially recognized these TDR strains yet. Nonetheless, the available treatment options for MDR and XDR TB are limited, more expensive, toxic and less effective than those for drug susceptible TB (WHO, 2018).

Historical Background of Antibiotic Treatment for Tuberculosis

Liem, 2016 stated that tuberculosis (TB), which once known as "consumption" or phthisis, was one of the deadliest diseases to humanity for millennia. Until the late eighteenth century, it was almost a death sentence to patients diagnosed with this disease as there was no effective treatment for tuberculosis. The discovery of *Mycobacterium tuberculosis* as the causative agent by Dr. Robert Koch in 1882, followed by the sanatorium movement in Europe and the USA, began to bring better treatments to TB. However, it only became curable with the later discovery of antibiotics, which have brought on a real revolution in TB chemotherapy. Begining with streptomycin and p-aminosalicylic acid (PAS) in 1945, many drugs were developed for TB treatment during this so-called golden age of antibiotics (1940s–1960s). The introduction of these drugs, with isoniazid, ethambutol, rifampicin and pyrazinamide being of most significance, to TB treatment immediately led to a sharp and continuous decline of TB incidence throughout the world (Leim, 2016). In the 1960s, it was commonly thought that TB was no longer a public health concern as it would soon be eradicated. However, the disease abruptly came back in the 1980s in association with the rising epidemic of the acquired immune deficiency syndrome (AIDS) and the emergence of drug-resistant forms (Leim, 2016).

Antituberculosis drugs are mainly divided into two parts:

1. First-line antituberculosis drugs- Isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA) and streptomycin (SM).

2. Second-line antituberculosis drugs- Sub divided into two

i. Fluoroquinolones- Ofloxacin (OFX), levofloxacin (LEV), moxifloxacin (MOX) and ciprofloxacin (CIP).

ii. Injectable antituberculosis drugs- Kanamycin (KAN), amikacin (AMK) and capreomycin (CAP).

iii. Less-effective second-line antituberculosis drugs- Ethionamide (ETH)/Prothionamide (PTH), Cycloserine (CS)/Terizidone, P-aminosalicylic acid (PAS) (Hum and Sungweon, 2013).

Epidemiology of MDR- Tuberculosis

According to the World Health Organisation (WHO) global report 2017, among 10 million incident TB cases worldwide, 3.5% are estimated to have rifampicin- or multidrug-resistant tuberculosis (MDR/RR-TB) in 2017 (WHO, 2018). In addition, 18% of previously treated TB cases were estimated to have had MDR/RR-TB in the same year (WHO, 2018).The acquisition or emergence *M. tuberculosis* resistance may occur from previous exposures to quinolones (Deutschendor*f et al*., 2012), use of inferior regimens (van der Werf, 2012) poor adherence to anti-TB drug, previous TB treatment (Zhao *et al.,* 2012), and high human immunodeficiency virus (HIV) co-infection (Jindani and Enarson, 2004). In Africa, reports of MDR TB based on continuous surveillance as in South Africa show progressively increasing MDR rates despite overall decreasing numbers of TB cases (Sarita *et al*., 2017).

In developing countries with limited access to TB drug sensitivity tests, prevalence of MDR TB is dependent on special national surveys and hospital-based clinical researches as found in Nigeria (Fawcett and Watkins, 2015). Most of the hospital-based reports in Nigeria indicate that, there is some level of MDR TB, which though not documented on a regular basis, it show progressive increase over time. In Nigeria, as well as other high-burden countries such as South Africa and India, it has been noted that the increasing TB prevalence may be driven by HIV co infection (Adegboyega *et al.*, 2014). According to the WHO, the estimated incidence of TB in Nigeria is 322 per 100 000 population with only 15% of the total burden of the disease in the country being notified in 2015. The WHO estimates that the proportion of patients with MDR/RR-TB is 4.3% among new cases and 25% among previously-treated cases in Nigeria (WHO, 2016).

Epidemics of MDR TB

During the late 1980s and early 1990s, epidemics of MDR TB occurred in North America and Europe killing about 80% of those who were infected. Today, the greatest number of cases is in India and China (Udawadia *et al*., 2011). Although smaller epidemics have been described due to migrations (DeVries *et al.,* 2005). Multi Drug Resistant TB epidemics especially that of XDR TB: High TB burden, high HIV prevalence, suboptimal TB control practices, and introduction of second-line TB drugs into low- and middle-income countries have contributed immensely to this menace (Gandhi *et al.,* 2006).

In the XDR TB epidemic reported in South Africa (Gandhi *et al.,*2006), there was prominence of associated HIV co infection in most patients who were transmission cases. Another feature was poor observance of infection control precautions such as inadequate patient isolation and airflow regulation within wards, which made the wards conducive for transmission between patients in contact with MDR TB cases.

There was also notable direct transmission from patients to health care workers, which was evident by Tuberculin Skin Test (TST) conversion as well as later linkage mappings that correlated the strains in the patients' samples with those of the health workers (Adeola, 2015).

Diagnosis of MDR-TB

The different types of MDR-TB diagnosis include; conventional methods (Absolute concentration method, the resistance ratio method and The proportions method) which require 6-8 weeks' time before sensitivity results are known. Usually, Lowenstein-Jensen (LJ) culture media is used for drug sensitivity testing using:

(I) Absolute concentration method

In absolute concentration method, the Minimal Inhibitory Concentration (MIC) of the drug is determined by inoculating the control media and drug containing media with inoculums of *M. tuberculosis.* Media containing several sequential two-fold dilutions of each drug. Resistance is indicated by the lowest concentration of the drug which will inhibit growth (defined as 20 colonies or more at the end of four weeks) (Nikalje and Mudassar, 2011).

(II)The resistance ratio method

In resistance ratio method, MIC of the isolate is expressed as a multiple of the MIC of a standard susceptible strain, determined concurrently, in order to avoid intra and inter-laboratory variations. These two methods require stringent control of the inoculums size and hence are not optimal for direct sensitivity testing from concentrated clinical specimens (Nikalje and Mudassar, 2011; Himasree *et al*., 2017).

(III) The proportions method

In the proportions method, the ratio of the number of colonies growing on drug containing medium to the number of colonies growing on drug free medium indicates the proportion of drug resistant bacilli present in the bacterial population. Below a certain proportion called critical proportion, a strain is classified as susceptible and above that as resistant (Himasree *et al.*, 2017).

Modern Methods

(I) Ligase chain reaction

Ligase Chain Reaction (LCR) involves the use of an enzyme DNA ligase which functions to link two strands of DNA together to continue as a double strand. This can occur only when the ends are complementary and match exactly and this method facilitates the detection of a mismatch of even one nucleotide. It is based on the gene coding for luciferase, an enzyme identified as the light producing system of fireflies. In the presence of adenosine triphosphate (ATP), it interacts with luciferin and emits light. The luciferase gene is placed into a mycobacteriophage. Once this mycobacteriophage attaches to *M. tuberculosis*, the phage DNA is injected into it and the viral genes are expressed. If *M. tuberculosis* is infected with luciferase reporter phage and these organisms are placed in contact with antituberculosis drugs, susceptibility can be tested by correlating the generation of light with conventional methods of testing. This technique has the potential to identify most strains within 48 hours (Bardarov *et al*., 2003 as cited in Nikalje and Mudassar, 2011).

(II) DNA Line Probe Assays

Line probe assays (LPAs) are basically DNA–DNA hybridization assays that allow the simultaneous detection of different mutations by using multiple probes (Makinen *et al.*, 2006). After DNA extraction and target amplification, amplicons are hybridized to specific oligonucleotide probes that are complementary to the target sequences and are immobilized on the surface of a strip. After several post-hybridization washes to remove non-specific binding, the amplicon-probe hybrids are visualized by eye as colored bands on the strip.

The turnaround time of the whole assay is 5–7 h (Makinen *et al.*, 2006: Mitarai *et al*., 2012). Although several LPAs have been developed, most of them focus only on the hotspot regions of drug-resistance and different assays target different genes. For instance, the INNO-LiPA Rif TB LPA (Innogenetics, Zwijndrecht, Belgium) analyzes only the rpoB hotspot region (codon 509 to codon 534; Asp516Val, His526Tyr, His526Asp, and Ser531Leu mutations) for MTB identification and RIF resistance screening (WHO, 2008). The AID TB Resistance LPA includes three modules to detect first-line and second-line anti-TB drug resistance in culture and clinical specimens (Ritter *et al.,* 2014). LPAs are rapid, simple and easy to perform. Result analysis (manually or automatically) is simple. However, LPAs require complex laboratory infrastructure and expensive equipment that is normally only available in reference laboratories. The number of un interpretable results is high, and LPA target coverage is limited to the main mutations. Thus, their sensitivity and specificity vary according to the mutation prevalence in the area under study (WHO, 2017).

(III) Real-Time PCR Assays

Real-time PCR is now broadly applied for the development of rapid diagnostic tests. Two main approaches are commonly used in real-time PCR:

(i) the use of non-specific fluorescent dyes to detect any double-stranded DNA generated by PCR amplification, and (ii) the use of sequence-specific probes tagged with a fluorescent reporter for the specific detection of the hybridization between probes and amplicons. Each probe has a specific melting temperature (Tm), and a Tm change reflects the presence of mutations in the target. This feature has been used to develop real-time PCR tests for drug resistance screening (Bunsow *et al*., 2014).

An example of real-time PCR-based assay for DR-TB detection is;Xpert MTB/RIF.

Xpert MTB/RIF; a fast molecular-based test, is endorsed by WHO for the detection of the MTB complex and RIF resistance screening in suspected cases (WHO, 2018). This test was first recommended in 2010 for the diagnosis of pulmonary TB in adults from sputum specimens. Since 2013, it has been recommended also for the diagnosis of TB in children and of some specific extra-pulmonary forms. The Xpert MTB/RIF assay uses semi-quantitative nested real-time PCR to amplify a fragment containing the 81 bp hotspot region of the rpoB gene (codons 507–533) that is then hybridized to five molecular beacon probes (Bunsow *et al*., 2014). Each probe covers a separate sequence and is labeled with a fluorescent dye. The whole experiment is performed in a self-contained cartridge, like a mini-laboratory, to minimize cross-contamination between samples. Sensitivity and specificity for smear-positive samples can reach 100 and 99%, respectively, and for smear-negative samples are 67 and 99%, respectively, compared to the standard culture-based DST. It significantly decreases the detection time of RIF resistance from 4 to 8 weeks (culture and DST) to 2 hours. It has immediately a good impact on patients because it allows starting rapidly the MDR-TB treatment (Bunsow *et al*., 2014).

How Drug Resistant TB Develops

There are two principal pathways leading to the development of active drug-resistant TB: (i) acquired (secondary) drug resistance and (ii) primary drug resistance.

(i) Acquired Drug Resistance

Acquired drug resistance is the result of inadequate, incomplete or poor treatment quality that allows the selection of mutant resistant strains. If drug-susceptible TB is treated with a regimen exclusively based on a single effective TB medicine, there is a risk that bacteria with drug-resistant mutations will be selected and multiply further during the course of treatment, eventually becoming the dominant strain.

If a person infected with a strain, initially resistant to a specific medicine is treated with that medicine plus a new additional medicine, then there is a risk of developing resistance to the additional medicine. Step-wise additions of drugs may eventually lead to more severe patterns of drug resistance and eventually to untreatable forms of TB (WHO, 2014).

(i) Primary drug resistance.

Primary or initial drug resistance means that a person has been infected with a drug-resistant TB strain. Transmission of drug-resistant TB occurs exactly in the same way as transmission of drug susceptible TB. High prevalence of drug-resistant TB in the community increases the risk of drug-resistant TB exposure in the community. Undiagnosed, untreated, or poorly treated drug-resistant TB contributes to sustained high drug-resistant TB prevalence, as well as high proportions of infectious drug-resistant TB cases among the community. Environments conducive for TB transmission (such as crowding, poor ventilation and poor infection control practices in health facilities and other congregate settings), also contribute to transmission of drug-resistant TB. Similar to drug susceptible TB, drug-resistant TB only progresses to active disease in a minority of those infected, and drug-resistant TB infection can remain latent for long periods of time. A poorly functioning immune system increases the risk of progression, and therefore factors that can impair the immune system (e.g. HIV, under-nutrition, diabetes, silicosis, smoking, alcohol abuse, a wide range of systemic diseases and treatments with

immune suppressant) are also risk factors for developing drug-resistant TB disease (WHO, 2014).

Interventions to Prevent Drug-Resistant TB

There are five major ways of preventing drug-resistant TB which includes:

Early Detection and High-quality Treatment of Drug-susceptible TB

Ensuring early detection of TB involves the introduction or strengthening of interventions to improve access and utilization of high-quality TB services established across the health system including the private sector. Specific interventions include: suitable diagnostic methods to ensure early detection of TB comprising screening of risk groups and inclusion of household contacts of infectious TB patients; placing patients on effective treatment with treatment follow-up; and minimizing barriers to health care access. In every setting, specific challenges for treatment adherence should be assessed and support packages designed accordingly (WHO, 2014).

In settings where treatment of drug-susceptible TB is of sufficient quality, i.e. where the rate of acquired resistance is relatively low, most incident drug-resistant TB cases may still be generated through the transmission of drug-resistant TB from the pool of prevalent drug-resistant TB cases. In such settings, the most important element of drug resistant TB prevention is to ensure proper diagnosis, as well as treatment and management of drug-resistant TB. Early diagnosis and prompt, effective treatment is among the strongest actions to curb the drug-resistant TB epidemic. The proportion of drug-resistant TB in new cases is typically lower than in retreatment

cases. However, the absolute number of new TB cases may be much higher than the number of retreatment cases. Therefore, many countries have a higher absolute number of drug-resistant TB in new cases, rather than in retreatment cases. Presently, in most low- and middle-income countries, drug susceptibility testing (DST) is done only in a fraction of all cases. If done routinely, it is often only done in retreatment cases. This leads to a large number of undetected drug-resistant TB cases, or severely delayed diagnosis of the majority of drug-resistant TB cases (WHO, 2014).

Effective Implementation of Infection Control Measures

TB infection control is a combination of measures aimed at minimizing the risk of TB
transmission within populations. Infection control policies should be well formulated and implemented at every level of health delivery (public and private), in congregate settings, such as correctional facilities, military barracks, homeless shelters, refugee camps, boarding schools and nursing homes, and at the household level. Community campaigns can focus on how to minimize the exposure of TB in general and how households that have a TB patient within them can help prevent transmission. Societal level infection control involves improved general living and working conditions, thus creating environments that are less conducive to TB transmission (WHO, 2014).

Strengthening and Regulation of Health Systems.

Assessing health system barriers and opportunities is an essential part of planning TB control interventions. Such assessments should identify bottlenecks that could be addressed both through TB-specific programmatic interventions and interventions that need to be pursued beyond the purview of then NTPs (National TB control programme). Opportunities for integration of service delivery, sharing of resources and joint actions to improving human resource development, diagnostic capacity, and drug management with other public health programmes should be explored. TB-specific interventions that risk disrupting or distorting overall health system operations and prioritizations should be avoided to the extent possible (WHO, 2014).

Addressing Underlying Risk Factors and Social Determinants

The most critical and immediate social intervention for prevention of drug-resistant TB is to assess social and financial barriers to access and adhere to health-care services and to address them accordingly. While this includes providing all TB diagnostic and treatment services free
of charge to the patients, it must also minimize the cost to patients for other related clinical services (such as managing co morbidities, notably HIV infection which may have a negative impact on TB treatment outcomes), as well as minimize the indirect costs of care (for example, those related to transport and loss of income). Indirect costs of TB care are often catastrophic, even when TB diagnosis and treatment is provided free of charge. Access to available social protection schemes, including sickness/disability funds and other cash transfers, should be ensured for people with TB. If such schemes are not fully developed or if people with TB are not
eligible, the NTP should advocate for the development/adaptation of social protection schemes. Such interventions are critical for both drug-susceptible and drug-resistant TB patients in order to reduce the risk of poor treatment outcomes leading to acquired/amplified resistance and transmission of drug-resistant TB. They may also contribute to poverty alleviation on a household level, with medium- to long-term preventive effects, to improving human resource development, diagnostic capacity, and drug management with other public health programmes should be explored (WHO, 2014).

Why MDR-TB is a Public Health Threat

Loss of sensitivity to both isoniazid and rifampin even without resistance to additional drugs has major effects on outcome of the treatment of the disease (Ormerod, 2005). Numerous controlled trials have shown that a 6-month regimen of rifampicin and isoniazid, supplemented by pyrazinamide and streptomycin or ethambutol for the first 2 months, will provide a cure in >95% of cases if the medication is taken correctly. Such a regimen also renders infectious cases non-infectious in 2 weeks (Ormerod, 1997).

Each drug varies in its ability to kill tubercle bacilli (bactericidal ability), to deal with persistent organisms which are only occasionally metabolically active (sterilizing ability) and to prevent the emergence of drug resistance (Ormerod, 1997). Isoniazid is the best bactericidal drug and if mono resistance to this occurs, treatment with rifampin and ethambutol has to be extended for 9–12 months, in addition to 2 months initial pyrazinamide. Rifampin is the best sterilizing drug, and mono resistance to this drug requires treatment with isoniazid and ethambutol for 18 months, with 2 months initial pyrazinamide (Iseman, 2015). Therefore loss of response to both the main bactericidal drug and the main sterilizing drug means that patients remain infectious for much longer, both in the community and in hospital, that treatment is required for at least 12 and possibly more than 24 months, and that less effective and more toxic second-line drugs have to be used (Ormerod, 2005).

CONCLUSION

Drug resistance is a worldwide problem that threatens to undermine effective control of TB. As shown by the recent report of WHO/ IUALTD, hotspots of MDR-TB have appeared in regions with weak TB-control programmes and misuse of anti-TB drugs. Prevention of drug resistance depends on appropriate treatment of all patients with TB with combination drug regimens and early detection of resistance followed by tailored treatment with second-line agents and most recent, the newly introduced drugs.

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