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Review Article

Extensively Drug Resistant Tuberculosis: A Mini Review

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A B S T R A C T

Extensively drug resistant tuberculosis (XDR-TB) has recently emerged as a global health problem. XDR-TB is caused by *Mycobacterium tuberculosis* resistant to at least isoniazid (INH), rifampin (RIF), any fluoroquinolone (FQs) and any of the three second line injectable drugs [amikacin, kanamycin (aminoglycosides) or capreomycin (polypeptide)]. Approximately 9% of MDR-TB cases also have resistance to other classes of drugs i.e. XDR-TB cases. XDR-TB has been detected all over the world. As of October 2012, 84 countries had reported at least one XDR-TB case. Treatment outcomes in MDR-TB patients in high burden countries is poor, with only about 50% of patients successfully completing treatment. Drug susceptibility testing (DST) of second-line drugs is not as easy as DST for first-line drugs. The existing tests for second line drug susceptibility are not standardized and are less reproducible than tests for first line anti-TB drugs. In December 2010, the World Health Organization (WHO) endorsed *Xpert* for the rapid and accurate detection of TB, and recommends that it should be used as an initial diagnostic test in individuals suspected of having MDR-TB or HIV associated TB. Drug associated adverse events occur commonly during treatment of XDR-TB and often requires interruption of therapy.

K e y w o r d s

XDR-TB; *Mycobacterium tuberculosis* ; Drug susceptibility testing (DST); *Xpert*,

Introduction

Extensively drug resistant tuberculosis (XDR-TB) has recently emerged as a global health problem and pose a significant threat to TB control programmes. XDR-TB is caused by *Mycobacterium tuberculosis* resistant to at least isoniazid (INH), rifampin (RIF), any fluoroquinolone (FQs) and any of the three second line injectable drugs [amikacin, kanamycin (aminoglycosides) or capreomycin (polypeptide)]. Banerjee et al (2008).

Pre XDR-TB are those MDR-TB isolates which are resistant to a FQ or an injectable agent, but not both. These strains are essentially one mutation away from becoming XDR. (WHO report 2008, Daniel (2013).

Previously, XDR-TB was defined as TB caused by *M. tuberculosis* isolates resistant to isoniazid and rifampin i.e. MDR-TB plus at least three of the six

main classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicyclic acid).

The old definition was given by Centre for Disease Control and Prevention (CDC), in collaboration with the World Health Organization (WHO) in October 2005 at the 36th world conference on lung health in Paris, France and later published in 2006. However, the old definition was revised in October 2006, and current definition was proposed in view of the results that among second line drugs, drug-susceptibility testing to fluoroquinolones and second-line injectable drugs (i.e., amikacin, kanamycin or capreomycin) yields reproducible and reliable results, whereas drug-susceptibility testing to other second-line drugs is less reliable and resistance to these drugs (FQs and second-line injectable drugs) has been associated with poor treatment outcomes. (Shah, 2005; Morb Mortal Weekly Rep (2006).

Global epidemiology

In 2011, WHO estimated 12 million cases of tuberculosis worldwide, of which about 630000 (roughly 5%) were MDR tuberculosis and in 2012 WHO global tuberculosis report estimates that about 3.7% of new tuberculosis cases and 20% of previously treated cases in the world have MDR-TB. The highest caseloads of MDR tuberculosis are reported in India, China, Russia, and South Africa, accounting for more than 60% of cases worldwide. Zignol (2012).

Approximately 9% of MDR-TB cases also have resistance to other classes of drugs i.e. XDR-TB cases. XDR-TB has been detected all over the world. As of October 2012, 84 countries had reported at least one XDR-TB case. WHO report (2012). Data from a global survey conducted during years 2000-2004 involving 14 Supranational reference laboratories (SRLs) from 80 countries worldwide shows that among 17690 isolates, 3520 (19.9%) were MDR-TB isolates, out of which 347 (9.9%) met criteria for XDR-TB. Shah NS (2007). According to new definition, in the United States, U.S. National TB Surveillance System (NTSS) reported a total of 49 cases (3% of MDR-TB cases) during years 1993-2006. Of these, 17 (35%) were reported during 2000-2006. Morb Mortal Weekly Rep (2007). Around 40000 cases of XDR-TB are estimated to emerge globally each year. WHO report (2008). The proportion of XDR-TB among MDR-TB isolates varied among nations and the highest cases have been reported from South Africa, former Soviet Union, Korea and Japan. Banerjee et al (2008).

India's rate is relatively high, lower than only Eastern Europe and Russia (14%) and South Korea (15%). Morb Mortal Wkly Rep (2006). The exact prevalence of XDR-TB in India is not known. In Kashmir valley of India, during a period of four years, 5.7% cases of MDR-TB were identified, among which eight (15.3%) were diagnosed as XDR-TB. Datta BS (2010). A total of 598 isolates of XDR-TB have been reported in the previous studies published since 2006 from the states of Kashmir, Delhi, Maharashtra, Uttar Pradesh, West Bengal, Gujarat, Tamil Nadu and Kerala. Michael JS (2012). In Mumbai, patients infected with tuberculosis that has become resistant to all the drugs used against the disease has emerged recently. This discovery makes India the third country in which a completely/Totally drug-resistant (resistant to all first and second line drugs) form of the disease has emerged, following cases documented in Italy in 2007 and Iran in 2009. Udwadia (2012).

The widespread emergence of XDR tuberculosis could lead to virtually untreatable tuberculosis. Epidemic of XDR-TB have been fueled by HIV coinfection. HIV patients because of their compromised immune systems are at increased risk for developing active tuberculosis caused by either a new *M. tuberculosis* infection or reactivation of latent TB and therefore could contribute significantly to the spread of MDR/XDR TB (WHO report (2006)).

Causes and spread of XDR-TB

Multiple factors are responsible for emergence of XDR-TB. Men made factors are prescription of inappropriate drug regimens, addition of a single drug to a failing regimen, supply of poor quality of drugs, poor patient adherence or interruptions of therapy in between, lack of cheap, rapid and accessible diagnostic methods to rapidly diagnose drug resistant tuberculosis as early as possible, poor implementation of the directly observed treatment short-course programme. A study from Mumbai, India showed that only 5 of 106 private practitioners could prescribe a correct prescription for a hypothetical patient with MDR tuberculosis (Udwadia, 2010). All these factors lead to acquired drug resistant tuberculosis and contacts of these resistant cases then develop primary drug resistant tuberculosis (Vareldzis, 1994; Weyer, 2012. Social factors such as living and working in crowded conditions put the person at higher risk of acquiring drug resistant TB. International travel has further facilitated the spread of drug resistant tuberculosis (Barry, 2012).

Like other forms of TB, XDR-TB also spreads through airborne route. Health authorities estimate that one patient with active TB can infect up to 15 others and thus resistant TB spreads. Health care-associated outbreaks have also been reported in both poor and wealthy nations which are responsible for rise in XDR-TB cases, typically among HIV infected patients. Edlin (1992), Narvskaya (2002). Therefore, a combination of infection control strategies such as use of respiratory masks, improved natural ventilation, reduced hospitalization with the provision of outpatient therapy, hospital based HIV testing with ARV therapy, isolation of TB patients and rapid drug susceptibility testing should be implemented in hospitals to control spread of drug resistant strains. A survey from South Africa shows that if all these interventions are implemented then it would prevent 48% of future XDR-TB cases (Basu, 2007).

Treatment of MDR-TB requires use of second line drugs which are costly, less potent, have frequent side effects, need to be given for long duration (18-24 months) as compared to conventional TB regimens. This results in noncompliance and loss of follow-up of patients. Treatment outcomes in MDR-TB patients in high burden countries is poor, with only about 50% of patients successfully completing treatment (Shean, 2008). Association between prior treatment with anti-TB drugs and development of drug resistance has been shown in a study from South Korea in which 65% of MDR-TB cases gave the history of prior treatment and out of which 16% were XDR-TB cases, while among patients who had received a new diagnosis, 10% were MDR TB, and none was XDR TB case. Jeon (2008). Other studies have also identified prior treatment for tuberculosis as a risk factor for development of drug resistance. Lomtadze N (2009), Espinal MA (2001), Zhao Y (2012).

Genetic mechanisms of drug resistance

Genetic resistance in *M. tuberculosis* is caused by random spontaneous chromosomal mutations at a frequency of 10^{-6} to 10^{-8} replications. The probability for rifampicin resistance is 10^{-8}, that for isoniazid, ethambutol, streptomycin, kanamycin and para aminosalicylic acid is 10^{-6}, and that for ethionamide, cycloserine and capreomycin is 10^{-3}. Rastogi (1993). These mutations are unlinked, therefore, if three drugs are used simultaneously, the probability of developing drug resistance becomes 10^{-18} to 10^{-20}. This high probability is virtually non-existent, so, it is advisable to use three or more drugs in combination for treatment. Unlike other bacteria, mobile genetic elements such as plasmids and transposons do not mediate drug resistance in *M. tuberculosis*. Zhang (2009). Clinical complications such as empyema, pulmonary cavities, solid caseous material, where penetration of antibiotics is difficult and pH is sufficiently low to inhibit the activity of most antibiotics permits the emergence of bacterial resistance. Elliott (1995), Iseman (1991), Gillespie (2002).

Isoniazid has a significant bactericidal activity against *M. tuberculosis*, therefore, it has become a critical component of the first line antituberculous regimens. Mutations in two chromosomal loci, katG and inhA have been found to cause isoniazid resistance. The primary target of INH inhibition is InhA enzyme (enoyl-acyl carrier protein reductase) which is involved in elongation of fatty acids in mycolic acid synthesis. Marrakchi (2000), Vilcheze (2000). Rifampin is considered to be a sterilizing agent as it kills persistent tubercle bacilli throughout the duration of therapy. Resistance to rifampin in *M. tuberculosis* occur most commonly as single point mutations in the rpoB gene, which encodes the β-subunit of RNA polymerase. However, a small percentage of rifampin-resistant isolates (<5%) do not contain any mutations in the rpoB gene, suggesting alternative resistance mechanisms, potentially including altered rifampin permeability or mutations in other RNA polymerase subunits. Pang (2013), Somoskovi (2001).

Injectable drugs such as kanamycin, amikacin, and capreomycin are the key SLDs for the treatment of MDR-TB. Streptomycin (SM) acts on the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA. Mutations in rpsL and rrs gene results in resistance to streptomycin in about 50% and 20% of SM- resistant strains respectively. rpsL gene encodes for S12 protein and rrs gene encodes for 16S rRNA. Cooksey (1996), Finken (1993). Approximately 70-80% of the amikacin resistant and 60% of the kanamycin resistant *M. tuberculosis* strains results from mutations in rrs gene which encodes for16S rRNA. Georghiou (2012). Capreomycin is a macrocyclic polypeptide antibiotic and resistance to this drug is caused by mutation in tlyA gene encoding a putative rRNA methyltransferase. Maus (2005).

Fluoroquinolone antibiotics (Ofloxacin, ciprofloxacin, and levofloxacin) are among the most potent second-line drugs

Table.1 Classes of anti-TB drugs and genes mutated in drug resistance

Anti-TB drugs	Genes involved in resistance	Mechanism of action
First line drugs		
Isoniazid	KatG-catalase-peroxidase, inhA-enoyl ACP reductase ahpC, oxyR, kasA, furA, ndh	Inhibition of mycolic acid biosynthesis
Rifampicin	rpoB-β subunit of RNA polymerase	Inhibition of RNA synthesis
Ethambutol	embB-arabinosyl transferase	Inhibition of arabinogalactan synthesis
Pyrazinamide	pncA-nicotinamidase/pyrazinamidase	Depletion of membrane energy
Second line drugs Fluoroquinolones	gyrA, gyrB-DNA gyrase subunit A and B	Inhibition of DNA gyrase
Injectables: kanamycin and amikacin Capreomycin Streptomycin	rrs-16S rRNA rrs, tlyA-2'O-methyltransferase rrs, rpsl, gidB	Inhibition of protein synthesis
Cycloserine	alrA, ddl	
Ethionamide	etaA/ethA-Flavin monooxygenase inhA	Inhibition of mycolic acid synthesis
Para-aminosalicylic acid	Unknown	
Third line drugs Amoxicillin-clavulanate Imipenem Clarithromycin Linezolid Clofazimine		
Agents in clinical development: TMC207(diarylquinolone) OPC67683(nitroimidazole) PA824(nitroimidazole) LL3858(pyrrole) SQ109(diamine)		

used for treatment of MDR-TB. However, FQ resistant *M. tuberculosis* strains results from injudicious use of this class of drugs either in the management of MDR-TB or by excess use of these drugs in the treatment of lower respiratory tract infections, urinary tract infections, gastrointestinal as well as other types of community acquired infections, increasing the burden of selective pressure and compromising their efficacy in the treatment of TB. Ginsburg (2003), Malik (2012). High level FQ resistance in *M. tuberculosis* occurs due to acquisition of point mutations in the quinolone resistance determining region (QRDR) of the gyrA gene of DNA gyrase. Mutations in this region account for 42–100% of FQ resistance in *M. tuberculosis.* Mutations in *gyrB* have been less commonly found in *M. tuberculosis, however,* many studies have recently reported clinical isolates resistant to FQs with *gyrB* mutations. Devasia (2012), PitaksajjakulP (2005), Duong (2009), Aubry (2006).

Second line drug susceptibility testing

The accurate estimation of XDR-TB burden requires laboratory based diagnosis that relies on first and second line drug susceptibility testing. However, DST of second-line drugs is not as easy as DST for first-line drugs. The existing tests for second line drug susceptibility are not standardized and are less reproducible than tests for first line anti-TB drugs. There is limited quality control of SLD testing, although efforts to expand proficiency testing of laboratories are underway. The critical concentrations for second line drugs are not clear cut and there have been no commercially available test kits for second-line drugs, making these tests more error-prone due to procedural inaccuracies. Richter (2009), Fattorini (2008), Laszlo (2002).

The TB Supranational Reference Laboratory Network (SRLN) was created in 1994 in order to support the WHO-IUATLD Global Project on TB drug resistance surveillance. SRLs collaborate with national reference laboratories (NRLs) and participates in quality culture and drug susceptibility testing, proficiency testing, provide external quality assurance during drug resistance surveys and provide training on culture and DST in relevant countries as needed. Between 1994 and 2009, the SRLN was expanded to 29 laboratories on six continents. WHO report (2010), MMWR (2006). In India, National Institute for Tuberculosis Research (NITR) in Chennai is RNTCP's Supranational Reference Centre (designated by WHO) and it participates in international external quality assessment for microscopy, culture and DST and monitors the quality of laboratories in the country on behalf of RNTCP. Michael (2012), Laszlo (2002).

TB is mainly a disease of poor countries where lack of necessary equipments and laboratory facilities to accurately diagnose drug resistance in *M. tuberculosis* make it difficult to estimate true burden of XDR-TB. In these countries even drug susceptibility testing (DST) for first line drugs are not performed routinely because of limited resources. Hence, many cases remain undetected. Anthony SF (2008). It has been estimated that in many countries, only 3% of new TB cases have access to DST. Young DB (2008). According to WHO, only 7% of the estimated global burden of MDR-TB patients are being detected. WHO report (2013).

Conventional diagnosis of drug-resistance in *M. tuberculosis* strains relies heavily upon mycobacterial culture and drug susceptibility testing in liquid or solid

media by which results are only obtained after weeks to months of incubation. The results are available too late to the clinicians and therefore, the patients are continue to undergo treatment with drugs to which they are resistant, remain contagious, and those with XDR-TB and HIV often die before they are even diagnosed. Shah (2011), Georghiou (2012). Ninty six well microtitre plate method is easy to set up and has faster turn around time (14 days) compared with traditional agar proportion (21 days) method for the susceptibility testing of second line drugs. Wengenack (2010), Hall (2011). MGIT 960 (Becton Dickinson Diagnostic System, Sparks, MD) provides reliable and rapid results in the detection and recovery of mycobacterium from clinical specimens and also the drug susceptibility testing (DST) for the first line drugs isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide. Many laboratories have also validate DST of the second-line drugs by BACTEC MGIT 960 system (Kim, 2013;, Gerdes, 2006).

Molecular assays for rapid detection of genotypic resistance to SLDs can be employed to reduce result time. The most common molecular strategies which are used in various studies are DNA sequencing, quantitative PCR by amplification of the *M. tuberculosis* 16S rRNA gene, line probe assays, microarray technique, SNP analysis, molecular beacons. Campbell PJ (2011), Pholwat (2011), Cooksey (1997), Lin (2004), Piatek (1998). Molecular line probe assay (LPA) technology for rapid detection of multi-drug resistant tuberculosis (MDR-TB) was endorsed by the World Health Organization (WHO) in 2008. In 2009, Hain Lifescience introduced a new LPA, the Genotype MTBDR*sl* test, for the rapid determination of genetic mutations associated with resistance to fluoroquinolone, aminoglycosides (kanamycin, amikacin), cyclic peptides (capreomycin), ethambutol, and streptomycin. The assay format is similar to the Genotype MTBDR*plus* assay for the detection of mutations conferring rifampicin and isoniazid resistance, endorsed by WHO in 2008, and allows for testing and reporting results within 24 hours. Kiet (2010), Barnard (2012). Rapid microarray technique has been described to detect the resistance to FQs and second-line injectable drugs in *M. tuberculosis.* This technique detects mutations in the *gyrA* and *gyrB* genes responsible for FQ resistance and mutations in the *rrs* gene and the *eis* promoter locus that are associated with the resistance to aminoglycosides and capreomycin. Because of the accurate, unambiguous identification of a wide spectrum of relevant mutations, the uncomplicated interpretation of the results, and the high-throughput nature, reliability and reproducibility, this method can easily be implemented in any clinical laboratory that is familiar with PCR. Gryadunov D (2011), Zimenkov DV (2013). All molecular assays have certain limitations such as failure to detect mutations located outside of the targeted loci, detection of silent mutations that might not cause phenotypic drug resistance and failure to detect subpopulations with emerging resistance in a mixed population of bacteria. Therefore, confirmation with phenotypic testing is essential. Banerjee et al (2008), Lin (2004).

The Xpert MTB/RIF is a cartridge-based, semi-quantitative, nested real-time PCR *in-vitro* diagnostic test for the detection of *Mycobacterium tuberculosis* and resistance to rifampicin (RIF). Results are

available in two hours, therefore, infected patients can quickly be placed in isolation and appropriate treatment started early. Boehme (2011). *Xpert* is more sensitive than sputum smear microscopy in detecting TB, and it has similar accuracy as culture. Boehme (2010). In December 2010, the World Health Organization (WHO) endorsed *Xpert* for the rapid and accurate detection of TB, and recommends that it should be used as an initial diagnostic test in individuals suspected of having MDR-TB or HIV associated TB. WHO report (2011). Brazil, Cambodia, the Democratic Republic of Congo, India, Kenya, Tanzania, Zimbabwe and South Africa have invested heavily in procurement of Xpert MTB/RIF. http://www.who.int/tb/laboratory/mtbrifrollout/en/. In August 2012 the price of cartridges was reduced to USD 9.98 for the public sector in the 145 countries. Approximately 1.4 million Xpert MTB/RIF test cartridges and over 200 GeneXpert instruments will be made available from 2013 -2015 through the TBXpert Project to 21 countries including Bangladesh, Belarus, Cambodia, the Democratic Republic of Congo, Ethiopia, India, Indonesia, Kenya, Kyrgyzstan, Malawi, Mozambique, Myanmar, Nepal, Pakistan, Philippines, Moldova, Swaziland, Uganda, Tanzania, Uzbekistan and Vietnam. Gilpin (2012).

Thus, it is of vital importance that both public and private mycobacteriology laboratories maintain the ability to detect anti-TB drug resistance by using currently available methods which provide rapid, accurate and appropriate results of second line drug susceptibility results.

Treatment options and control of XDR-TB

XDR-TB cases are very difficult to treat because of resistance to two most potent bactericidal antitubercular drugs (INH and rifampin), therefore, associated with a high mortality rate. Yew (2011). It is estimated that about 70% of XDR-TB patients die within a month of diagnosis. **(**MDR-TB/ XDR-TB 2013). A XDR-TB outbreak occurred in South Africa, in 2006, in which 52 out of 53 people who contracted the disease died within a median of 16 days. Salim S. The situation becomes more problematic in settings of XDR-TB and HIV coinfection. Wells CD (2007), Gandhi NR (2010). Low CD4 count and high degree of drug resistance was found to be most important risk factors for mortality in a district hospital of South Africa in which 98% of XDR-TB patients with HIV coinfection died within one year of diagnosis of TB with a median CD4 count of 78 cells/mm^3. Gandhi (2012). Treatment options for XDR-TB, because of the high grade of drug resistance are severely limited. Second line drugs are not well tolerated and have high toxicity. Drug associated adverse events occur commonly during treatment of XDR-TB and often requires interruption of therapy. Study from South Africa showed that adverse effects were experienced by 58% of the XDR-TB patients who received second line drugs as therapy and 28% of the patients required discontinuation of the offending drug. Shean K (2013). Thus, treatment interruption due to any cause may potentially subvert successful outcome in patients with XDR-TB. Jager (2002), Fujita (2008), Hallbauer (2011), Mizutani (2001), Manika (2008).

Table.2 Second line drugs and their adverse effects

Second line drugs	Adverse effects
Injectables: kanamycin and amikacin, Streptomycin, apreomycin	Ototoxicity, Nephrotoxicity
Cycloserine	Psychosis, Seizure, Depression, Gastrointestinal toxicity, Headache, insomnia, diziness
Ethionamide	Nausea, vomiting, dizziness, jaundice, allergic reactions, hypothyroidism in children
Para-aminosalicylic acid	Persistent nausea, vomiting and diarrhoea. Drug induced hypothyroidism, Hepatitis
Fluoroquinolones	Tendonitis, Photosensitivity, seizures, QT interval prolongation, Gastrointestinal and central nervous system reactions, hepatitis, renal dysfunction and hypoglycaemia

Cross-resistance between anti-TB drugs also occur which further make the treatment of XDR-TB difficult. Several previous investigations have demonstrated cross-resistance between amikacin and kanamycin, and these drugs were considered interchangeable for drug susceptibility testing. However, opposed to this, some other studies have shown discordant resistance between amikacin and kanamycin. Kruuner (2003), Jughelr L(2009), Jnawali (2013). Among fluoroquinolones, ciprofloxacin has least activity against *M. tuberculosis*. Studies have shown that addition of ciprofloxacin into a regimen resulted in a higher rate of relapse, takes a longer time to sputum-culture conversions and the emergence of resistance is far more likely when ciprofloxacin is included in the regime. Moadebi (2007), Manika (2008). The newer fluoroquinolones moxifloxacin, levofloxacin and gatifloxacin have been substantially management and control of TB disease globally. DOTS-Plus was started by WHO for the treatment and diagnosis of MDR-TB cases. Clinical shown to exert better activity and are associated with a lower probability of emergence of resistance. Nuermberger (2004), Rustomjee (2008), Zhang (2007). Therefore, it is recommended to test for levofloxacin or moxifloxacin susceptibility even if ciprofloxacin resistance has been reported. Cross-resistance between older generation fluoroquinolones and new generation agents such as moxifloxacin is unclear. Dong (1998), Zhao (1999), Somasundaram (2006).

Improvements in diagnosis and laboratory capacity are among the key elements for control of XDR-TB. Treatment must be started early with individualised regimens based on results of drug susceptibility testing. The effective implementation of rapid diagnostic tests for TB and drug resistance will increase the proportion of patients promptly placed on appropriate therapy, and therefore will improve experiences from the past few years showed that high cure rates of drug resistant TB are achieved in settings where DOTS-Plus has been established. Tupasi

(2006), Tupasi (2003), Riekstina (2007). Therefore, effective implementation of DOTS-Plus helps in reducing the emergence of XDR-TB cases.

Hence to conclude, the major challenge now is to develop innovative approaches to expand the detection and treatment of drug resistant cases globally. There is a need to develop tests for rapid detection of drug resistance for second line drugs and need to develop safe and more efficient second-line drugs and shorter second-line treatment regimens.

References

Anthony, SF., 2008. Multidrug resistant and extensively drug resistant tuberculosis: The National Institute of allergy and infectious disease research agenda and recommendations for priority research. J. Infect. Dis. 197,1493-8.

Aubry, A., Veziris, N., Cambau, E., Truffot-Pernot, C., Jarlier, V., et al., 2006. Novel gyrase mutations in quinolone-resistant and -hypersusceptible clinical isolates of *Mycobacterium tuberculosis*: functional analysis of mutant enzymes. Antimicrob. Agents. Chemother. 50,104–112.

Banerjee, R., Schecter, GF., Flood, J., Porco, TC., 2008. Extensively drug resistant tuberculosis: new strains, new challenges. Expert. Rev. Anti. Infect. Ther. 6(5),713-24.

Barry, PM., Gardner, TJ., Funk, E., Oren, E., Field, K., Shaw, T., et al., 2012. Multistate outbreak of MDR TB identified by genotype cluster investigation. Emerging. Infect. Dis. 18(1),113-6.

Basu, S., Andrews, J., Friedland, GH., 2007. The epidemic level impact of preventing nosocomial transmission of extensively drug resistant (XDR) tuberculosis in rural South African district hospitals. Lancet. 370,1500-7.

Barnard, M., Warren, R., Pittius, NGV., Helden, PV., Bosman, M., Streicher, E., et al., 2012. GenoType MTBDR*sl* line probe assay shortens time to diagnosis of extensively drug-resistant tuberculosis in a high-throughput diagnostic laboratory. American. J. Resp. Crit. Care. Medicine. 186(12),1298-1305.

Boehme, CC., Nicol, MP., Nabeta, P., Michael, JS., Gotuzzo, E., Tahirli, R., et al., 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. www.thelancet.com Published online April 19, 2011 DOI:10.1016/S0140-6736 (11) 60438-8.

Boehme, CC., Nabeta, P., Hillemann, D., Nicol, MP., Shenai, S., Krapp, F., et al.,2010. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. 363(11), 1005–1015.

Campbell, PJ., Morlock, GP., Sikes, RD., Dalton, TL., Metchock, B., Starks, AM., et al., 2011. Molecular Detection of Mutations Associated with First- and Second-Line Drug Resistance Compared with Conventional Drug Susceptibility Testing of Mycobacterium tuberculosis. *Antimicrob. Agents. Chemother*. 55(5),2032-2041.

Centers for Disease Control and Prevention. Revised definition of extensively drug resistant tuberculosis. Morb Mortal Weekly Rep 2006;*55*:1176.

Centers for Disease Control and Prevention (CDC). Emergence of

Mycobacterium tuberculosis with extensive resistance to second-line drugs-worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* 2006; 24: 55: 301-5.

Cooksey, RC., Morlock, GP., McQueen, A., Glickman, SE., Crawford, JT., 1996. Characterization of streptomycin resistance mechanisms among *Mycobacterium tuberculosis* isolates from patients in New York City. Antimicrob. Agents. Chemother. 40(5),1186-8.

Cooksey, RC., Morlock, GP., Glickman, S., Crawford, JT., 1997. Evaluation of a line probe assay kit for characterization of rpoB mutations in rifampin resistant *Mycobacterium tuberculosis* isolates from New York city. J. Clin. Microbiol. 35,1281-3.

Daniel, O., Osman, E., Oladimeji, O., Dairo, OG., 2013. Pre-Extensive Drug Resistant Tuberculosis (Pre-XDR-TB) among MDR-TB Patents in Nigeria. Global. Advanced. Research. Journal. of Microbiology. 2(2),22-25.

Datta, BS., Hassan, G., Kadri, SM., Quereshi, W., Kamili, MA., Singh, H., et al., 2010. Multidrug-Resistant and extensively drug resistant tuberculosis in Kashmir, India. J. Infect. Dev. Ctries. 4(1),019-023.

Devasia, R., Blackman, A., Eden, S., Li, H., Maruri, F., Shintani, A., et al., 2012. High proportion of fluoroquinolone-resistant *Mycobacterium tuberculosis* isolates with novel gyrase polymorphisms and a gyrA region associated with fluoroquinolone susceptibility. J. Clin. Microbiol. 50(4),1390-6.

Duong, DA., Nguyen, TH., Nguyen, TN., Dai, VH., Dang, TM., et al., 2009. Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with high-level fluoroquinolone resistance in Vietnam. Antimicrob. Agents. Chemother. 53,4835–4839.

Dong, Y., Xu, C., Zhao, X., et al., 1998. Fluoroquinolone action against mycobacteria: effects of C 8 substituents on growth, survival, and resistance. Antimicrob. Agents. Chemother. 42,2978–84.

Emergence of *Mycobacterium tuberculosis* with Extensive Resistance to Second-Line Drugs --- Worldwide, 2000—2004. MMWR weekly March 24, 2006/55(11);301-305.

Extensively Drug-Resistant Tuberculosis - - United States, 1993-2006. Morb Mortal Weekly Rep 2007;56(11):250-3.

Edlin, BR., Tokars, JI., Grieco, MH., et al., 1992. An outbreak of multidrug resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N Engl. J. Med. 326(23),1514-21.

Espinal, MA., Laserson, K., Camacho, M., et al., 2001. Determinants of drug-resistant tuberculosis: analysis of 11 countries. Int. J. Tuberc. Lung. Dis. 5,887-93.

Elliott, AM., Berning, SE., Iseman, MD., Peloquin, CA., 1995. Failure of drug penetration and acquisition of drug resistance in chronic tuberculous empyema. Lung. Dis. 76,463-7.

Finken, M., Kirschner, P., Meier, A., Wrede, A., Böttger, EC., 1993. Molecular basis of streptomycin resistance in Mycobacterium tuberculosis: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. Mol. Microbiol. 9(6),1239-46.

Fujita, J., Sunada, K., Hayashi, H., Hayashihara, K., Saito, T., 2008. A case of multi-drug resistant tuberculosis showing psychiatric

adverse effect by cycloserine. Kekkaku. 83(1),21-5.

Fattorini, L., Iona, E., Cirillo, D., 2008. External quality control of *Mycobacterium tuberculosis* drug susceptibility testing: results of two rounds in endemic countries. Int. J. Tuberc. Lung. Dis. 12,214-7.

Gandhi, NR., Shah, NS., Andrews, JR., et al., 2010. HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. Am. J. Respir. Crit. Care. Med. 181,80–6.

Gandhi, NR., Andrews, JR., Shah, NS., 2012. Risk factors for mortality among MDR and XDR-TB patients in a high HIV prevalence setting. Int. J. Tuberc. Lung. Dis. 16(1),90-7.

Gillespie SH., 2002. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. Antimicrob. Agents. Chemother. 46(2),267-74.

Georghiou, SB., Magana, M., Garfein, RS., Catanzaro, DG., Catanzaro, A., Rodwell, TC., 2012. Evaluation of genetic mutations associated with *Mycobacterium tuberculosis* resistance to amikacin, kanamycin and capreomycin: a systematic review. Plos. One. 7(3),1-12.

Ginsburg, AS., Hooper, N., Benjamin, WH., Bishai, WR., 2003. The rapid development of fluoroquinolone resistance in M. tuberculosis. N. Engl. J. Med. 349,1977-8.

Gerdes, SR., Pfyfffer, GE., Casal, M., Chadwick, M., Siddiqi, S., 2006. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicr*obials. J. Clin. Microbiol.* 44(3),688-692.

Gryadunov, D., Dementieva, E., Mikhailovich, V., Nasedkina, T., Rubina, A., Savvateeva, E., et al., 2011. Gel-based microarrays in clinical diagnostics in Russia. *Expert. Rev. Mol. Diagn.* 11(8),839-853.

Gilpin, C., 2012. Introduction and status of the global roll-out of Xpert MTB/RIF for the diagnosis of tuberculosis.43rd Union World Conference on Lung Health, Kuala Lumpur.

Hall, L., Jude, KP., Clark, SL., Wengenack, NL., 2011. Antimicrobial Susceptibility Testing of Mycobacterium Tuberculosis Complex for First and Second Line Drugs by Broth Dilution in a Microtiter Plate Format. JoVE. 52,1-4.

Hallbauer, UM., Schaaf, HS., 2011. Ethionamide induced hypothyroidism in children. South. Afr. J. Epidemiol. Infect. 26(3),161-3.

http://www.who.int/tb/laboratory/mtbrifrollout/en/.

Iseman, MD., Madsen, LA., 1991. Chronic tuberculous empyema with bronchopleural fistula resulting in treatment failure and progressive drug resistance. Chest. 100,124-7.

Jeon, CY., Hwang, SH., Min, JH., Prevots, R., Goldfeder, LC., Lee, H., et al., 2008. Extensively drug resistant tuberculosis in South Korea: risk factors and treatment outcomes among patients at a tertiary referral hospital. Clin. Infect. Dis. 46,42-9.

Jager, DP., Altena, VR., 2002. Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. Int. J. Tuberc. Lung. Dis. 6(7),622-7.

Jughelr, L., Bzekalava, N., Rigouts, L., 2009. High Level of Cross-Resistance between Kanamycin, Amikacin, and Capreomycin among *Mycobacterium tuberculosis* Isolates from Georgia and

a Close Relation with Mutations in the *rrs* Gene. Antimicrob. Agents. Chemother. 53(12),5064-8.

Jnawali, HN., Yoo, H., Ryoo, S., Lee, KJ., Kim, BJ., Koh, WJ., et al., 2013. Molecular genetics of *Mycobacterium tuberculosis* resistant to aminoglycosides and cyclic peptide capreomycin antibiotics in Korea. World. J. Microbiol. Biotechnol. 29(6),975-82.

Kim, H., Seo, M., Park, YK., Yoo, JI., Lee, YS., Chung, GT., et al., 2013. Evaluation of MGIT 960 System for the Second-Line Drugs Susceptibility Testing of *Mycobacterium tuberculosis*. Tuberculosis. Research. and Treatment. 2013,1-6.

Kiet, VS., Lan, NTN., An, DD., Dung, NH., Hoa, DV., Chau, NV., et al., 2010. Evaluation of the MTBDRsl test for detection of second-line-drug resistance in *Mycobacterium tuberculosis.* J. Clin. Microbiol. 48(8),2934–2939.

Kruuner, A., Jureen, P., Levina, K., Ghebremichael, S., Hoffner, S., 2003. Discordant resistance to kanamycin and amikacin in drug-resistant *Mycobacterium tuberculosis*. Antimicrob. Agents. Chemother. 47**,**2971-3.

Lomtadze, N., Aspindzelashvili, R., Janjgava, M., et al., 2009. Prevalence and risk factors for multidrug-resistant tuberculosis in the Republic of Georgia: a population-based study. Int. J. Tuberc. Lung. Dis. 13,68-73.

Laszlo, A., Rahman, M., Espinal, M., Raviglione, M., 2002. Quality assurance programme for drug susceptibility testing of *Mycobacterium tuberculosis* in the WHO/IUATLD supranational reference laboratory network: five rounds of proficiency testing, 1994-1998. Int. J. Tuberc. Lung. Dis. 6,748-56.

Lin, SYG., Probert, W., Lo, M., Desmond, E., 2004. Rapid detection of isoniazid and rifampin resistance mutations in *Mycobacterium tuberculosis* complex form cultures or smear positive sputa by use of molecular beacons. J. Clin. Microbiol. 42,4204-8.

Michael, JS., John, TJ., 2012. Extensively drug resistant tuberculosis in India: A review. Indian. J. Med. Res. 136(4),599-604.

Marrakchi, H., Laneelle, G., Quemard, A., 2000. InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II. Microbiology. 146,289-96.

Maus, CE., Plikaytis, BB., Shinnick, TM., 2005. Mutation of tlyA confers capreomycin resistance in *Mycobacterium tuberculosis*. Antimicrob. Agents. Chemother. 49(2),571-7.

Malik, S., Willby, M., Sikes, D., Tsodikov, OV., Posey, JE., 2012. New Insights into Fluoroquinolone Resistance in *Mycobacterium tuberculosis*: Functional Genetic Analysis of *gyrA* and *gyrB* Mutations. PLoS. One. 7(6),e39754.

MDR-TB/ XDR-TB. 2013 global alliance for TB drug development.

Mizutani, H., Horiba, M., Shindoh, J., Kimura, T., Son, M., Wakahara, K., 2001. Two cases of multi-drug-resistant pulmonary tuberculosis with para-aminosalicylic acid (PAS)-induced hypothyroidism. Kekkaku. 76(10),667-72.

Manika, K., Kioumis, L., 2008. The role of fluoroquinolones in the treatment of tuberculosis. Pneumon. 21(4),396-401.

Moadebi, S., Harder, CK., Fitzgerald, MJ.,

Elwood, KR., Marra, F., 2007. Fluoroquinolones for the treatment of pulmonary tuberculosis. Drugs. 67(14),2077-99.

Nuermberger, EL., Yoshimatsu, T., Tyagi, S., Williams, K., Rosenthal, I., O'Brien, RJ., et al., 2004. Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis. Am. J. Respir. Crit. Care. Med. 170,1131-4.

Narvskaya, O., Otten, T., Limeschenko, E., et al., 2002. Nosocomial outbreak of multidrug resistant tuberculosis caused by a strain of Mycobacterium tuberculosis W-Beijing family in St. Petersburg, Russia. Eur. J. Clin. Microbiol. Infect. Dis. 21(8),596-602.

Pang, Y., Lu, J., Wang, Y., Song, Y., Wang, S., Zhao, Y., 2013. Study of the rifampin monoresistance mechanism in *Mycobacterium tuberculosis*. Antimicrob. Agents. Chemother. 57(2),893-900.

Pitaksajjakul, P., Wongwit, W., Punprasit, W., Eampokalap, B., Peacock, S., et al., 2005. Mutations in the *gyrA* and *gyrB* genes of fluoroquinolone-resistant *Mycobacterium tuberculosis* from TB patients in Thailand. Southeast. Asian. J. Trop. Med. Public. Health. 36,228–237.

Pholwat, S., Heysell, S., Stroup, S., 2011. Rapid first and second line drug susceptibility assay for *Mycobacterium tuberculosis* isolates by use of quantitative PCR. *J. Clin. Microbiol.* 49,169-75.

Piatek, AS., Tyagi, S., Pol, AC., 1998. Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis.* Nat. Biotechnol. 16,359-63.

Richter, E., Gerdes, SR., Hillemann, D., 2009. Drug-susceptibility Testing in TB: Current Status and Future Prospects. Expert. Rev. Resp. Med. 3(5),497-510.

Rastogi, N., David, HL., 1993. Mode of action of anti-tuberculous drugs and mechanisms of resistance in *Mycobacterium tuberculosis*. Res. Microbial. 144,133-43.

Riekstina, V., Leimane, V., Holtz, TH., Leimans, J., Wells, CD., 2007. Treatment outcome cohort analysis in an integrated DOTS and DOTS-Plus TB program in Latvia. Int. J. Tuberc. Lung. Dis. 11(5),585-587.

Rustomjee, R., Lienhardt, C., Kanyok, T., Davies, GR., Levin, J., Mthiyane, T., et al., 2008. *A phase II study of the sterilizing activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis.* Int. J. Tuberc. Lung. Dis. 12,128-*138.*

Salim, S., Karim, A. High mortality in a South African XDR-TB outbreak. AIDS Clinical Care.

Shah, NS., Wright, A., Drobniewski, F., et al., 2005. Extreme drug resistance in tuberculosis ("XDR-TB"): global survey of supranational reference laboratories for *Mycobacterium tuberculosis* with resistance to second-line drugs. Int. J. Tuberc. Lung. Dis. 9(Suppl 1),S77.

Shean, KP., Willcox, PA., Siwendu, SN., Laserson, KF., Gross, L., Kammerer, S., et al., 2008. Treatment outcome and follow-up of multidrug-resistant tuberculosis patients, West Coast/Winelands, South Africa, 1992–2002. Int. J. Tuberc. Lung. Dis. 12,1182–9.

Shean, K., Streicher, E., Pieterson, E., Symons, G., Smit, RVZ., Theron, G., et al., 2013. Drug associated adverse events and their relationship with outcomes in patients receiving treatment for extensively drug resistant tuberculosis in South Africa. Plos.

One. 8(5),e63057.

Shah, NS., Wright, A., Bai, GH., Barrera, L., Boulahbal, F., Casabona, NM., et al., 2007. Worldwide emergence of extensively drug resistant tuberculosis. Emerging. Infect. Dis. 13(3),380-7.

Shah, NS., Richardson, J., Moodley, P., Moodley, S., Babaria, P., et al., 2011. Increasing drug resistance in extensively drug-resistant tuberculosis, South Africa. Emerg. Infect. Dis. 17,510–3.

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

Somasundaram, S., Paramasivan, NC., 2006. Susceptibility of Mycobacterium tuberculosis strains to gatifloxacin and moxifloxacin by different methods. Chemotherapy. 52,190–5.

Tupasi, TE., Gupta, R., Quelapio, MI., Orillaza, RB., Mira, NR., Mangubat, NV., et al., 2006. Feasibility and cost-effectiveness of treating multidrug-resistant tuberculosis: a cohort study in the Philippines. PLoS. Med. 3(9),e352.

Tupasi, TE., Quelapio, MI., Orillaza, RB., Alcantara, C., Mira, NR., Abeleda, MR., et al., 2003. DOTS-Plus for multidrug-resistant tuberculosis in the Philippines: global assistance urgently needed. Tuberculosis (Edinb). 83(1-3),52-58.

Udwadia, ZF., Amala, RA., Ajbani, KK., Rodrigues, C., 2012. Totally Drug-Resistant Tuberculosis in India. *Clin. Infect. Dis.* 54(4),579-581.

Udwadia, ZF., Pinto, LM., Uplekar, MW., 2010. Tuberculosis control by private practitioners in Mumbai, India: has anything changed in two decades? PloS. One. 5,e1203.

Vareldzis, BP., Grosset, J., de Kantor, I., et al., 1994. Drug resistant tuberculosis: laboratory issues. World Health Organization recommendations. Tubercle. Lung. Dis. 75,1-7.

Vilcheze, C., Morbidoni, HR., William, R., 2000. Inactivation of the inhA encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the FASI end products and cell lysis of *Mycobacterium smegmatis*. J. Bacteriol. 182(14),4059-67.

Weyer, K., Mirzayev, F., Migliori, G., et al., 2012. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert(R) MTB/RIF. *Eur. Respir. J.* published online Nov 22.DOI:10.1183/09031936.00157212.

World Health Organization. XDR TB-extensively drug-resistant TB, November 2006: outcomes of the WHO Global Task Force on XDR TB, October 9–10. Available at: http:// www.who.int/tb/xdr/xdr_nov06_en.pdf . Accessed 28 March 2008.

World Health Organization. Anti-tuberculosis drug resistance in the world. Report no. 4. WHO/HTM/TB/2008.394. Geneva, Switzerland: WHO, 2008. http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf Accessed July 2009.

World Health Organization. Global tuberculosis report 2012. Geneva, Switzerland (2012).

World Health Organization. Global consultation of the WHO-GLI TB Supranational Reference Laboratory Network. Meeting Report. Geneva, Switzerland: WHO, 2010.

World Health Organization. TB diagnostics and laboratory strengthening. Geneva, Switzerland (2013).

World Health Organization (WHO). Rapid

implementation of the Xpert MTB/RIF diagnostic test. Geneva*:*WHO*;*2011.

Wengenack, N., Hall, L., Labombardi, V., Parrish, N., 2010. Evaluation of the New Sensititre (MYCOTB) MIC Plate for the susceptibility testing of Mycobacterium tuberculosis (Mtb) to First and Second Line Drugs. Sept. 12, 2010. Abstract 2450. Presented at 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, Boston, MA.

Wells, CD., Cegielski, JP., Nelson, LJ., et al., 2007. HIV infection and multidrug-resistant tuberculosis: the perfect storm. J. Infect. Dis. 196 (Suppl 1),S86–107.

Young, DB., Perkins, MD., Duncan, K., Barry, CE III., 2008. Confronting the scientific obstacles to global control of tuberculosis. J. Clin. Invest. 118,1255-65.

Yew, WW., 2011. Management of multi drug resistant tuberculosis and extensively drug resistant tuberculosis: current status and future prospects. Kekkaku. 86(1),9-16.

Zignol, M., Van Gemert, W., Falzon, D., et al., 2012. Surveillance of antituberculosis drug resistance in the world: an updated analysis, 2007–2010. *Bull. World. Health. Organ.* 90,111–19.

Zhao, Y., Xu, S., Wang, L., Chin, DP., Wang, S., Jiang, G., et al., 2012. National survey of drug resistant tuberculosis in China. N. Engl. J. Med. 7,2161-70.

Zhang, Y., Yew, WW., 2009. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. Int. J. Tuberc. Lung. Dis. 13(11),1320-30.

Zimenkov, DV., Antonova, OV., Kuzmin, AV., Isaeva, YD., Krylova, LY., Popov, SA., et al., 2013. Detection of second-line drug resistance in *Mycobacterium tuberculosis* using oligonucleotide microarrays. *BMC. Infectious. Diseases.* 13,240-7.

Zhang, Y., 2007. Advances in the treatment of tuberculosis. Clin. Pharmacol. Ther. 82,595-600.

Zhao, BY., Pine, R., Domagala, J., et al., 1999. Fluoroquinolone action against clinical isolates of Mycobacterium tuberculosis: effects of a C-8 methoxyl group on survival in liquid media and in human macrophages. Antimicrob. Agents. Chemother. 43,661–6.