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REVIEW ARTICLE

Extensively drug-resistant tuberculosis (XDR) and extremely drug-resistant tuberculosis (XXDR): risk factors and molecular perspectives

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INTRODUCTION

The first effective treatment for tuberculosis (TB) was developed with the discovery of streptomycin (STM) by Waksman in 1943 (1), however, immediately after its introduction many patients started showing resistance to this antibiotic (2). Basically, a single anti-TB drug should never be used in the treatment of active TB, and regimens that using more than one drug are recommended. In late 1960s, rifampicin (RMP) was introduced and with the use of combination therapy, there was a decline in drug-resistant and drug-susceptible TB (3). The principle of using combination therapy is preventing acquired drug resistance and enhancing efficacy (4). RMP resistance began to emerge in 1980s. Subsequently the emergence of HIV pandemic favor the transmission of multidrug-resistant (MDR) strains of *M. tuberculosis* (4-6). MDR-TB is defined as resistance to the two most important drugs, isoniazid (INH) and RMP, is a potential threat to TB control (7). The recently published World Health Organization (WHO) report on Global TB Control in 2009 (8) stated that there were an estimated 0.5 million cases of MDR-TB in 2007. There are 27 countries (15 in the European Region) that account for 85% of all such cases; these countries have been termed the 27 high MDR-TB burden countries. The top five countries with largest number of MDR-TB cases are India (131,000), China (112,000), the Russian Federation (43,000), South Africa (16,000) and Bangladesh (15,000). By November 2009, 57 countries and territories had reported at least one case of extensively drug-resistant TB (XDR–TB). XDR-TB is defined as TB caused by MDR strains that are also resistant to a fluoroquinolones (FQs) and, at least, one second-line injectable agent (amikacin "AMK", kanamycin "KM" and/or capreomycin "CAP"). More recently report of *M. tuberculosis* strains of totally drug-resistant (TDR) or extremely drug-resistant (XXDR) has been described (9). XXDR-TB is defined as *M. tuberculosis* isolates resistant to all first line (INH, RMP, STM, ethambutol "EMB", and pyrazinamide "PZA"), and second line drugs (ofloxacin "OFX", ciprofloxacin "CIP", cycloserine "CYC", prothionamide "PTH", AMK, KM, ethionamide "ETH", para-aminosalicylic acid "PAS", and CAP) (9). The objectives of the study were twofold: first, to highlight risk factors and current mechanisms of drug-resistant TB; and second, to recommend measures for effective control of the disease.

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Types of drug resistance TB

Primary resistance: Resistance among new cases is defined as the presence of resistant isolates of *M. tuberculosis* in patients who has not been exposed to anti-TB treatment for as much as 1 month; currently called drug resistance among new cases (10).

Acquired resistance: Acquired resistance is defined as the presence of resistant isolates of *M. tuberculosis* in patients who has been treated for TB for 1 month or more; currently called drug resistance among previously treated cases (10).

Combined proportion of drug resistance: Combined proportion of drug resistance is the proportion of resistance in the population surveyed regardless of prior treatment. This term is used when treatment history of TB is unknown (10).

Principles of anti-tuberculosis drugs selection

Successful treatment of TB requires considering three important properties on anti-TB drugs; (a) antibacterial activity (bactericidal or bacteriostatic), (b) sterilizing action for killing semi-dormant organisms, and (c) bacterial resistance inhibition activity (11). The standard regimen recommended by the WHO and International Union against Tuberculosis and Lung Disease (IUATLD) for active TB is the combination of INH, RMP, EMB, and PZA (12). All of these, except EMB, are bactericidal. INH and RMP are the most powerful bactericidal and sterilizing anti-TB drugs, respectively (11,13). PZN is also an important sterilizing drug. Therefore, both RMP and PZN are important in preventing TB relapse (14). This four drug regimen offers a rapid clinical improvement and a significant fall in the bacterial count in a few months.

It has been shown that *M. Tuberculosis* mutates to resistance against INH, STM, EMB, and RMP spontaneously and at random. The average mutation rates for the drugs, in the same order, were calculated to be 2.56×10^{-8}, 2.95×10^{-8}, 10^{-7}, and 2.25×10^{-10} mutations per bacterium per generation (15). Additionally, it has been reported that probability of resistance to three effective anti-tuberculosis drugs when used in combination would be 10^{-18} to 10^{-20} (16).

Adequate knowledge on risk factors of drug resistance development and understanding mechanisms of drug resistance is crucial for effective control measures and development of novel and effective drugs.

Risk factors influencing development of drug resistance

Previous treatment: It is an important risk factor for inducing drug resistance, particularly MDR-TB (17-23). Generally, high resistance levels are expected among previously treated cases because drug resistance is a strong risk factor for recurrent TB (17). WHO/IUATLD working group on global surveillance for anti-TB drug resistance (24) reported a prevalence of primary MDR of 1.4% and acquired resistance of 13% in previously treated patients. Therefore, prevalence of MDR-TB is 10 times higher in previously treated patients. The median combined prevalence of MDR-TB was 2.2% reportedly (24). The high rate of acquired resistance is justified with the previous inadequate treatment. There are different explanations for inadequate treatment. It may be due to inappropriate chemotherapy regimens, inadequate or irregular drug supply, unsatisfactory patients or clinicians compliance, lack of supervision of treatment, and absence of infection control measures in hospitals (17,20,25).

Immigration: It has been found as one factor leading to the elevated resistance rate of TB in some studies (18,26-28). Factors contributing to increased prevalence of drug resistance in immigrants are believed to be lack of access to health care services and inappropriate working and housing conditions. In certain studies (29,30), risk of resistance to anti-TB drugs has been reported to be 3- to 10- fold higher in immigrant than non-immigrant population. In another study, 50% of TB

cases in immigrant population had isolates that were resistant to at least one of the standard five drugs, and almost 17% were MDR-TB (31).

Age: It has been found independently associated with drug resistance and there was significantly higher proportion of MDR-TB among age group of 45-64 years (23). Faustini et al (32) found that MDR-TB was more likely in patients under 65 years, but the association was weak and more heterogeneous in patients under 45. Another study by Espinal et al. found that MDR-TB were more prevalent among age group 35–64 years old (33).

Sex: Although MDR-TB is more predominant in male (34), but there is no any influence of sex on the association between MDR-TB. It has been hypothesized that women are more compliant with treatment and therefore less likely to receive inadequate treatment (32). In contrary to MDR-TB patients, female gender has been found as a significant risk factor in XDR-TB patients; the authors attribute the reason to delayed referral female patients to hospitals because of certain social factors (35). Further studies are recommended to better understanding the role of gender in drug-resistant TB.

HIV: There is no clear association between HIV and MDR-TB cases (23,36); however, it has been found that HIV infection favors the transmission of MDR strains of *M. tuberculosis* (5,6,37).

Alcoholism: It has been found to be associated with MDR-TB, since linked with default in new TB cases and poor adherence to treatment (20,38-40).

Smoking: There are only a few reports in the literature on the association of smoking with MDR-TB (41). There was no record about this fact in almost all other literatures searched for this review.

Diabetes mellitus (DM): DM patients are prone to higher incidence of drug resistance (42,43). There is a significant association between diabetes mellitus and MDR-TB (42,40).

Socio-economic factors: There are certain socio-economic factors like drug abuse, poverty and homelessness that may induce treatment failure and subsequently emergence of drug resistance TB (20,44-46).

Mechanisms of resistance to anti-tuberculosis drugs

The mechanism of action and genes involved in mechanism of resistance to main anti-TB drugs are described in the table.

Table 1. Mechanism of action and gene involved in resistance to anti-tuberculosis drugs

Anti-TB agent	Mechanism of action	Gene involved in resistance
First line drugs		
INH	inhibition of mycolic acid biosynthesis	1-Catalase peroxidise (katG) 2-inhA (enoyl-acyl carrier protein reductase) 3-ahpC (alkyl hydroperoxide reductase) 4-kasA (β-ketoacyl-ACP synthase)
RMP	Inhibition of transcription	rpoB (β-subunit of RNA polymerase)
PZA	disrupts energy generating processes in mycobacterial membrane	pncA (pyrazinamidase)
EMB	Inhibition of arabinogalactan and lipoarabinomannan	embC, embA, embB (arabinosyl transferase)
STM	Inhibition of protein synthesis	rpsL (S12 ribosomal protein) rrs (16S rRNA)
Second line drugs		
Other aminoglycoside (AMK and KN)	Inhibition of protein synthesis	rrs (16S rRNA)
Polypeptides (CAP and viomycin)	Inhibition of protein synthesis	tlyA (rRNA methyltransferase)
FQs	Inhibition of DNA gyrase	gyrA (DNA gyrase subunit A) gyrB (DNA gyrase subunit B)
ETH	inhibition of mycolic acid biosynthesis	ethA (Flavin monooxygenase) inhA (enoyl-acyl carrier protein reductase)
CYC	Inhibit peptidoglycan synthesis	____
PAS	1-inhibit folic acid synthesis 2-Reduce iron upatake	thyA (Thymidylate synthase A)

INH: It is the most widely used anti-TB drug because it is used in both standard TB chemotherapy and in the chemoprophylaxis (47). It was first discovered in 1912; however, its first use was in 1952 (47). It is active against growing tubercle bacilli, but has little activity against resting bacilli in stationary phase or under anaerobic conditions (13). The main target of INH is the inhibition of mycolic acid biosynthesis (48). It is a prodrug that needs to be converted into an active form by the catalase peroxidase enzyme (*Kat*G) encoded by the *kat*G gene. The NAD (nicotinamide adenine dinucleotide), which results from interactions of *kat*G products lead to inhibition of mycolic acid biosynthesis. The *Inh*A gene encode protein, enoyl acyl carrier protein reductase, is the primary target for INH-NAD (49,50). At least one additional enzyme *Kas*A (beta-ketoacyl ACP synthase) has been recognized as targets for INH (51).

INH has a MICs ranging from 0.01 to 0.25µg/ml (52). Resistance to INH emerges by modification of *Kat*G gene due to mutations, deletions or insertions. This is the main mechanism of INH resistance, high level, and it constitutes approximately 50% of cases (53-55). Frequent mutations occur between codons 138 and 328 with most common at codon 315 of *Kat*G gene (56). *Kat*G Ser315Thr mutation is observed most commonly, accounting for 50–95% of INH-resistant clinical isolates carrying KatG mutations (57). INH resistance also occur as a result of mutations in regulatory region of *inh*A operon, resulting in overexpression of *inh*A (58). An AGG transversion, seen in few resistant strains of INH, at position 280, is resulting in ser94Ala substitution. This mutation in the *Inh*A gene alters binding affinity of *Inh*A to NAD, resulting in INH resistance (58,59). Point mutation in *inh*A is associated with low level of resistance and it accounts for 25% of INH resistant isolates (58). Point mutations have also been demonstrated in the regulatory region of *ahp*C (alkalyl hydroperoxide reductase), which compensates for the loss of *Kat*G catalase-peroxidase activity by a second mutation, resulting in overexpression of a*hp*C. This increased expression of *ahp*C does not directly involve in INH resistance (60). Mutations in *kas*A gene, encodes β-ketoacyl-ACP synthase involved in the synthesis of mycolic acids, have been demonstrated to be a potential cause of low level of resistance (61). However, the mechanism of resistance mediated by *kas*A has not yet been clear, because similar mutations were also found in INH susceptible strains (62). Further studies in this regard are recommended.

There are other mechanisms that may induce INH resistance, but not involving mutations, the antibiotic efflux pump. Exposure of INH susceptible organisms to high level of INH can induce a high level of resistance to INH through the induction of a reserpine-sensitive efflux mechanism (63).

RMP: It is extremely effective against *M. Tuberculosis* and it is the most important drug in shortening the course of treatment and assuring good outcome (11). The MIC of RMP is 0.1 to 0.2µg/ml (64). It is active against both growing and stationary phase of TB bacilli with low metabolic activity (64). RMP inhibits DNA-dependant RNA polymerase, inhibiting transcription (65). RNA polymerase is a complex oligomer composed of four different subunits (α, ß, ß'and σ) encoded by *rpo*A, *rpo*B, *rpo*C, and *rpo*D, respectively (66). RMP resistance results from mutations in *rpo*B gene encoding the β-subunit of RNA polymerase (67). Mutations of the *rpo*B gene were found in 95% of RMP resistant *M. tuberculosis* isolates (55); most were restricted to 81 bp core region and were dominated by single nucleotide changes, resulting in single amino acid substitutions (68). These mutations mainly are results of point mutations, however, deletions and insertions also occur but at lower frequencies (55). Mutations in codon 526 and 531 of *rpo*B gene are associated with high level of resistance (MIC>32µg/ml) to

RMP and associated with cross resistance to other rifampicin, whereas mutations in codon 511, 516, 518, 522, 529 and 533 result in lower level RMP resistance and associated with susceptibility rifabutin and the new rifampicin KRM1648 (69-71). Resistance to RMP in *M. tuberculosis* occurs at a frequency of 1 in 10 (67). Interestingly, monoresistance to RMP is rare, whereas to INH is common. Therefore, resistance to RMP can be used as surrogate marker for MDR-TB (72). There has been report (57) about strains that grow better in the presence of RMP, a potentially worrying finding, RMP dependent strains *M. tuberculosis*. These are not true RMP resistant, as they can very poorly grow in the absence of RMP. The mechanism for development of these strains is not clear, however, they may develop upon repeated treatment with rifampicin in re-treatment patients.

PZN: It was shown to be active against TB in 1952, but it became a first line anti-TB drug of short-course chemotherapy in the mid-1980s. When used in combination with INH and RMP, it shortens the duration of treatment from 9 to 12 months to 6 months (72). It is active against bacilli in semi-dormant state residing in acidic environment (64). PZN is a nicotinamide analogue prodrug, needs to be converted to its active form, pyrazinoic acid (POA) by the pyrazinamidase (PZase) encoded by *pnc*A (74). The POA disrupts energy generating processes in the *M. tuberculosis* membrane (75). The major mechanism for PZN resistance is due to defective PZase activity resulting from mutations in *pnc*A gene (76,77); this occurs in 72-97% of cases (57). Some PZN resistance strains do not show *pnc*A mutations. One type of such strains is PZase negative, with a high level of resistance, which may be due to mutations in an undefined *pnc*A regulatory gene. Another type of such strains has low level resistance (MICs=200–300 µg/ml, with a resistance cut-off of 100µg/ml PZA) and positive PZase activity without *pnc*A mutations; their mechanism of resistance remains to be determined (57). The drug is highly specific for *M. tuberculosis*, with little or no activity against other mycobacteria. The reason behind is that PZN needs to be activated by the PZase enzyme. Hence, many mycobacterial species are resistant to PZN because they lack efficient PZase (78). *Mycobacterium bovis* is naturally resistant to PZA due to a unique C-G point mutation in codon 169 of the *pnc*A gene (76). This is an important criterion for differentiating *M. bovis* from *M. tuberculosis.* Some PZN-resistant mycobacterial species like *M. Avium* and *M. smegmatis* have an active PZase, its resistance to PZA is probably due to effective pyrazinoic acid efflux. Therefore, the susceptibility of a mycobacterium to PZA under acidic conditions thus appears to be determined by the relative contributions of its PZase and pyrazinoic acid efflux activities (79).

EMB: It is a first line anti-TB drug, which is used in combination with other drugs. EMB inhibits an arabinosyl transferase (*emb*B) involved in the biosynthesis of mycobacterial cell wall components arabinogalactan and lipoarabinomannan (80). Three *emb* genes have been recognized in *M. tuberculosis*, namely *emb*C, *emb*A, and *emb*B. These genes encode mycobacterial arabinosyl transferases, which are involved in EMB resistance (81). The most frequent mutations for EMB resistance are substitutions of codon 306 in the *M. tuberculosis* (82,83). In this codon, five mutations have been recognized resulting in substitution of Met with Val, Leu and Ile in EMB-resistant organisms (83). These five mutations constitute 70–90% of all EMB resistant strains (55). *M. tuberculosis* isolates with Met306Leu and Met306Val replacements demonstrated a higher MIC for EMB (40µg/ml) than those for organisms with Met306Ile substitutions (20µg/ml). Some EMB-resistant isolates demonstrated mutations in the region of *emb*C and *emb*A and did not show mutations at the *emb*B locus (84). Ramaswamy et al reported that in 24% of EMB resistant isolates, no mutation of

emb gene could be detected (84). This postulates that there may be another mechanism of EMB resistance like permeability and efflux pumps. Therefore, further studies to understand exact mechanism of EMB are required.

STM, other aminoglycosides, and polypeptides: STM is an aminoglycoside antibiotic that was indeed the first effective anti-TB drug. Other aminoglycosides such as AMK, KM and paromomycin; and basic peptides like CAP and viomycin are used as second line anti-TB drugs. Aminoglycoside antibiotics act at the ribosomal level of *M. tuberculosis*, which inhibits mRNA translation, thus prevents protein synthesis (85). The mechanism of resistance to STM is attributed to mutations in S12 ribosomal protein, encoded by *rps*L gene and mutations in the *rrs* operon encoding the 16S rRNA (86). The most common point mutations occur at codon 43 of the *rps*L gene encoding the S12 protein, which results in substitution of (AAG→AGG; Lys→Arg) and less frequently substitution of (AAG→ACG; Lys→Thr) (87). This type of STM resistance accounts for 53% of cases and results in high level of resistance (55). Mutations in *rrs* gene are the second most common mechanism of STM resistance in *M. tuberculosis*, which constitutes 20% of cases and resulting in intermediate level of STM resistance. Cooksey et al (88) demonstrated that mutations in *rps*L and *rrs* were not seen with low-level STM resistance isolates. This indicates that other mechanisms of resistance are existing. Point mutations in the 1400 position of the *rrs* gene are associated with high level resistance to both AMK and KM (89). KAN resistance has been specifically associated with mutations at positions 1400, 1401 and 1483 of the *rrs* gene (90). Cross resistance within kanamycin and amikacin may be seen but not to STM and thus these antibiotics are alternatives in cases of STM resistance (90,91).

Viomycin and CAP, act by binding to the 50S and 30S ribosomal subunits and inhibit the translocation reaction, thus inhibiting protein synthesis (92). There is cross resistance between viomycin and CAP (93,94) because of structural similarity between the 2 basic peptides (95). It has been shown that mutations in the 30S or 50S ribosomal subunits are associated with resistance to viomycin in *M. smegmatis* (96,97). Maus et al demonstrated that mutation of the *tly*A gene, encoding a putative rRNA methyltransferase, confers resistance to CAP and to viomycin in both *M. tuberculosis* and *M. smegmatis* (98). Some CAP resistant clinical isolates did not show *tly*A mutations but did have an A1401G change in their *rrs* genes (98).

FQs: The FQs comprise a group of antimicrobials, such as ciprofloxacin, ofloxacin, levofloxacin, and moxifloxacin that have marked antimycobacterial activity (99,100). The mechanisms of action of fluoroquinolones is to inhibit DNA gyrase, encoded respectively A and B subunits of *gyr*A and *gyr*B, a member of the type II DNA topoisomerases, which is essential for the replication, transcription, and repair of bacterial DNA (101). The FQs have been increasingly used in the treatment of MDR-TB. It is also frequently used in the hospital and community acquired infections. Together, these features lead to increased emergence of FQs-resistant TB (102-104). Acquired FQs resistance TB has been shown to be mainly due to mutations in the quinolone resistance-determining regions (QRDRs) of the *gyr*A and *gyr*B genes (105-107). Mutations frequently occur at conserved 320-bp and 375-bp regions of the *gyr*A and *gyr*B genes, respectively (101). This type of mutations is usually associated with high level of resistance. Mutations in *gyr*A are most frequent cause of FQs resistance (105,106). Resistance mutations to FQs occur at a frequency of 2×10^{-6} to 2×10^{-8} (108). Point mutations in DNA gyrase confer cross resistance within the group FQ agents (109,110). Mutation codons that confer resistance to FQs have been reported in codons 88-94 (101,111). In contrary, mutations at codon 95 have not been associated with acquiring FQs

resistance (112). Some *M. tuberculosis* resistant strains do not show *gyr*A or *gyr*B mutations. Here, the mechanism of FQs resistance appears to be due to mutations elsewhere in the target genes or via other mechanisms (104,105). The mechanism of such resistance is not clear and further studies are recommended. An active efflux pump, *Lfr*A, has been reported to confer low level resistance in a quinolone-resistant isolate of *M. smegmatis*; however, this mechanism of resistance has not been demonstrated in *M. tuberculosis* (57). Recently, Hedge et al (113) demonstrated a new mechanism of FQs resistance in *M. tuberculosis*. A family of proteins from *M. tuberculosis*, *MtMfp*A, appears to confer FQs resistance via a novel mechanism based on DNA mimicry. This explains both the inhibitory effect on DNA gyrase and FQs resistance. In *M. smegmatis* a chromosomal gene, *mfp*A, which encodes a 192 amino acid PRP has been identified. It is an intrinsic quinolone resistant determinant and has 67% similarity to *MtMfpA* of *M. tuberculosis* (114).

ETH: It is one of the most frequently used second line anti-TB drugs (115). Its structure and mechanism of action is similar to INH (58). ETH is a prodrug that is activated by *Eth*A (favin monooxygenase) (116). It exerts a toxic effect on the mycolic acid, thus inhibiting cell wall biosynthesis (58). Mutations in the mycobacterial *inh*A gene can confer co-resistance to INH and ETH (58). It has been observed that low level INH resistant strains is commonly associated with low level of ETH resistance, whereas high level of INH resistance is usually ETH susceptible (117). It has been demonstrated that mutations in the *Eth*A gene is associated with ETH resistance (116,118) and it has no detectable association with INH resistance (118).

PAS: It is a bacteriostatic second line anti-TB drug active against extracellular TB. The mechanism of action of PAS is to inhibit folic acid synthesis or it may inhibit synthesis of cell wall component (mycobactin), resulting in decrease iron uptake by the *M. tuberculosis*. Rengarajan et al demonstrated that resistance in PAS is linked to mutations of *thy*A gene, encoding thymidylate synthase A, which is required for thymine biosynthesis in the folic acid pathway (119). It has been shown that only 37% of *thy*A mutations were involved in PAS resistance (119). Totally, 63% of resistant PAS isolates did not show mutations in any gene (*thy*A, *dfr*A, *fol*C, *fol*P1, *fol*P2, *thy*X, nhoA, *aac*1, and *aac*2 genes), thus other mechanisms of resistance to PAS are postulated (120).

CS: It is a broad spectrum antibiotic but because of its toxicity; it is not commonly used for bacterial infections. CS is bacteriostatic and only used against *M. tuberculosis* resistant to main anti-TB drugs. The mechanism of action is the inhibition of peptidoglycan synthesis, competing with D-Alanine ligase (*Ddl*) and D-alanine racemase (*Alr*), both enzymes are necessary for peptidoglycan biosynthesis (92,121). It has been demonstrated that spontaneous CS mutants strains of *M. smegmatis* exhibited a promoter-up mutation in the D-alanine racemase enzyme, encoded by *alr*A gene (122). A single transversion (G→T) in the *alr* promoter may lead to the overexpression of *alr* (122). Feng (123) documented that overexpression of either the *M. smegmatis* or the *M. tuberculosis ddl* gene in *M. smegmatis* confers resistance to CS, but at lower levels than the overexpression of the *alr* gene. Furthermore, a strain overexpressing both the *alr* and *ddl* genes displayed an eightfold-higher level of resistance. Further studies are needed to underline the genetic basis of CS resistant in *M. tuberculosis*.

XDR-TB and XXDR-TB: Magnitude and trends of the problem

Anti-TB drug resistance is present everywhere in the world and it is certain that MDR-TB, i.e. *M. tuberculosis* strains resistant to at least INH and RMP, is extensively widespread. A high prevalence of drug resistance have been noticed in certain

regions of the world, like Latvia, Estonia and Russia in the former USSR, the Dominican Republic and Argentina in the Americas, Ivory Coast in Africa, and Asia (7). Recently more worrying strains, XDR-TB, i.e. MDR-TB strains with resistance to at least three of the six classes of second-line drugs, have been found in all regions of the world (124). And more recently the emergence of XXDR-TB, i.e. *M. tuberculosis* strains resistant to all first-line drugs and to the six second-line classes, has added to the complexity of TB care and treatment (9,125). According to Centres for Disease Control (CDC) and the WHO, a survey was conducted based on an international network of TB laboratories for year 2000–2004. The result showed that 20% and 2% of *M. tuberculosis* isolates were MDR and XDR, respectively. Additionally it was reported that the total number and proportion of XDR-TB isolates observed worldwide (excluding South Korea) increased from 14 (5% of MDR-TB isolates) in 2000 to 34 (7% of MDR-TB isolates) in 2004 (126).

In order to reverse the increasing trend in drug-resistant TB, effective treatment, prevention and control of emergence and transmission of drug-resistant TB is required from all countries. The WHO recommended that the best way to prevent emergence of drug-resistant TB is to encourage adoption of DOTs programme. The programme involves giving effective and regular anti-TB drug supply, government security and financing commitment, case detection and diagnosis by smear microscopy, and monitoring the performance and outcome (127,128). Nevertheless, failure of treatment may occur due to many factors as discussed above, resulting in emergence of MDR-TB. In the case of drug-resistant TB in general and MDR-TB in particular, the WHO established DOTS-Plus within the context of basic DOTS programme. The programme relies on quality-assured and internationally recommended treatment regimens administered under strict supervision must be scaled up and strengthened to prevent spread of drug-resistant strains i.e. MDR-TB and XDR-TB. Strictly speaking the goal of DOTS-Plus is to prevent further development and spread of MDR-TB (129). The emergence of MDR-TB strains is of great concern, because it requires the use of second line drugs that are difficult to cure, and much more toxic and expensive than the first line regimen (130). It is noteworthy that the lengthy treatment course of drug-resistant TB results in complexity and problematic treatment outcome. Because diagnosis takes too long time, difficult adherence to treatment and some default from treatment. For these reasons, more aggressive form of drug resistant-TB emerges i.e. XDR-TB, TDR-TB, and may be even beyond in the future. XDR-TB treatment is much more difficult and costly than MDR-TB. Furthermore, the treatment outcome is found to be significantly worse than that of other MDR-TB cases (35, 130,131).

Although drug-resistant TB (MDR-TB and XDR-TB) is a critical alarm to patient life, yet treatment is feasible and cost effective if WHO guidelines are followed, with cure rates of up to 80% among MDR cases and up to 60% among XDR cases in low-resource settings. Inappropriate treatment that is not in line with the recommended guidelines runs the risk of raising mortality; increasing resistance and spreading resistance even further (132).

The newly emerging form of drug-resistant TB strains (XXDR-TB) is potentially untreatable since them are resistant to all first-line drugs and to the six second-line classes. XXDR-TB constitutes a deadly threat to the affected patients because we do not know how to treat these patients and what kind of combination should we use (9). The current ineffective anti-TB drugs for such patients increase the complexity of the situation, and perplexing TB treatment 60 years back to the era before antibiotics.

Overall, XDR-TB (133,134) and XXDR-TB (9) constitute an emerging threat for the TB control

and the further spread of drug resistance. It has been stated that drug-resistant TB is equally infectious as drug-susceptible TB (135). One of the important factors that facilitate transmission of drug-resistant TB is HIV infected patients; such patients have a rapid progressive course to fatal disease (36). Therefore, drug-resistant TB patients co-infected with HIV should be diagnosed quickly and prompt combination treatment commenced (136,137). This is important to prevent further transmission of drug-resistant strains. Finally, rapid detection of drug resistance to both first- and second line anti-TB drugs is a key component of TB control programs.

In conclusion, It important to know that we are in real fight with TB as a result of current global resurgence of the disease and progress in emergence of drug-resistant TB i.e. MDR-TB, and especially XDR-TB and XXDR-TB. Additionally, the drug-resistant TB and HIV association has increasing the complexity of the situation. There is pressing need of urgent new and more effective drugs for treatment of XDR-TB and XXDR-TB. Nevertheless, although a number of anti-TB drugs are in the pipelines, it would be unwise not to protect the currently available agents. Therefore, in depth understanding of the mechanisms of action and resistance at the molecular basis of anti-TB drugs is essential; this provides an insight into the pathogenicity of resistant strains and prevents its further spread. It is noteworthy that fluoroquinolones remain significant antimycobacterial antibiotics and an international recommendation for optimal use of these agents is essential. It is highly recommended to strictly follow the appropriate WHO treatment guidelines, to ensure adequate success rate of treatment in drug-susceptible and drug-resistant strains; this will limit emergence of resistant strains and prevent spread of the disease. The emergence of aggressive new forms of drug-resistant TB is worrying that requires reinforcement of control measures. This demands special attention to case detection and prompt treatment of MDR-TB, XDR-TB, and XXDR-TB to prevent transmission of the disease and further development of drug-resistant strains beyond this stage. A prospective population-based surveillance encompassing all regions of the world is warranted with the implementation of standardized protocols to further understand trends of drug resistance.

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