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MULTI-DRUG RESISTANT TUBERCULOSIS

STUDY GUIDE

for practical classes for students – foreign citizens of 6th course

on speciality «Phthisiology»

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The study guide is made according to the work program of phthisiology for students – foreign citizens of 6 courses for practical classes. The manual described the epidemiological situation of multi-drug resistant tuberculosis in the world, the basic concepts and terms from the topic, medicines to treat multi-drug resistant tuberculosis and patients' treatment regimens.

THE LIST OF ABBREVIATIONS

AFB – Acid fast bacilli
Am – Amikacin
Amx/Clv – Amoxicillin/Clavulanate
BTZs – 1,3-benzothiazin-4
Cfz – Clofazimine
Clr – Clarithromycin
Cm – Capreomycin
Cs – Cycloserine
DR-TB – drug resistant tuberculosis
DST – drug susceptibility test
E – Ethambutol
Et – EEthionamide
FLDs – first-line drugs
FQ – Fluoroquinolones
Gfx – Gatofloxacin
H – Isoniazid
Ipm/Cln – Imipenem/Cilastatin
Km – Kanamycin
Lfx – Levofloxacin
LPA – line probe assays
Lzd – Linezolid
MDR-TB – multi-drug resistant tuberculosis
Mfx – Moxifloxacin
MIC – minimum inhibitory concentrations
MTB – mycobacterium tuberculosis
NTP – National tuberculosis control programme
Ofx – Ofloxacin
PAS – *para*-Aminosalicylic acid
Pt – Protionamide
Rif – Rifampicinum
S – Streptomycin
SLDs – secondline anti-TB drugs
TB – tuberculosis
Thz – Thioacetazone
Trz – Terizidone
XDR – extensively drug resistant tuberculosis
Z – Pyrazinamide
WHA – World Health Assembly
2LI – second-line injectable agents

CONTENT

1. THE INTRODUCTION

Study Guide is devoted to the actual problem of modern of phthisiology – multi-drug resistant tuberculosis problem (MDR TB). Prevalence of MDR TB complicates the treatment of infectious diseases and worsens the prognosis for recovery. The first important message that must be sent to everyone tasked with managing TB patients is that, with good clinical and operational case management, all forms of drug-resistant TB (DR-TB) have the potential for cure, including those cases with a very extensive pattern of resistance. Numerous publications nonetheless show that even TB cases with extensive patterns of resistance are curable with proper clinical and operational management.

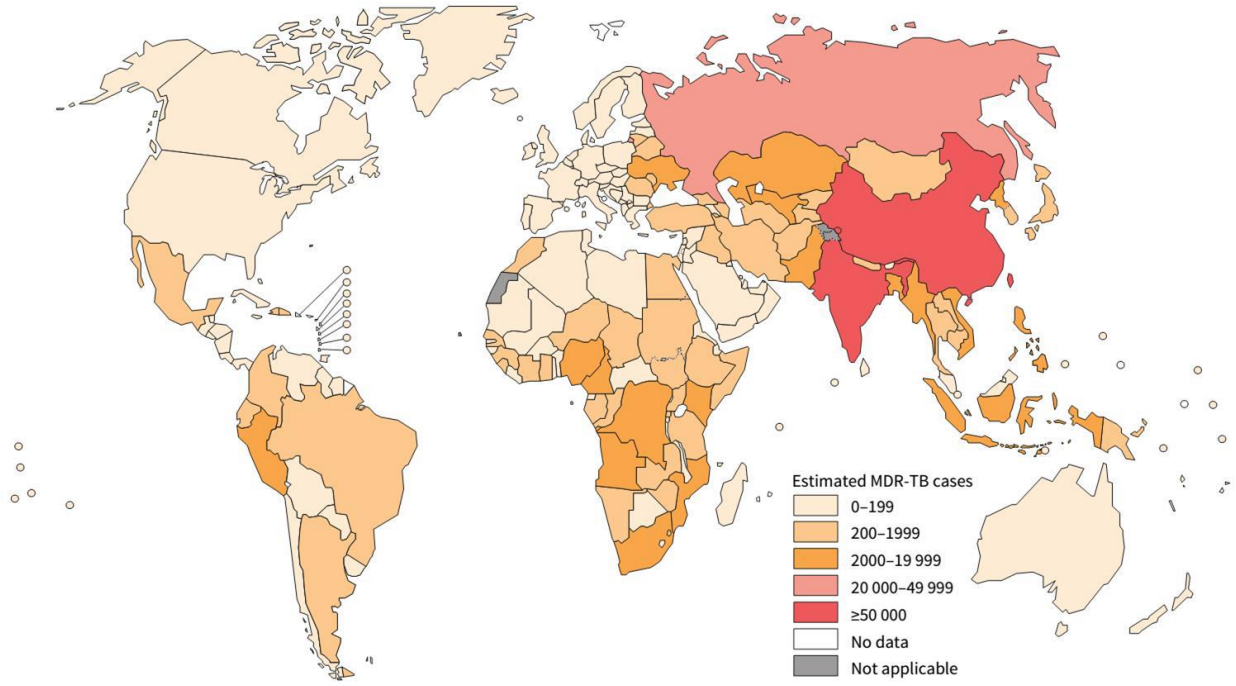
Study guide has a great practical importance because it allows students to evaluate the complexity of multi-drug resistant tuberculosis treatment`s problem and to contribute finding ways to raise treatment efficiency.

2. EPIDEMIOLOGY OF MULTI-DRUG RESISTANT TUBERCULOSIS

Globally, 5% of TB cases were estimated to have had multi-drug resistant tuberculosis (MDR-TB) in 2013 (3,5% of new and 20,5% of previously treated TB cases). Drug resistance surveillance data show that an estimated 480 000 people developed MDR-TB in 2013 and 210 000 people died.

Among TB patients reported by national TB programmes in 2013, there were an estimated 300 000 cases of MDR-TB. More than half of these cases were in India, China and the Russian Federation.

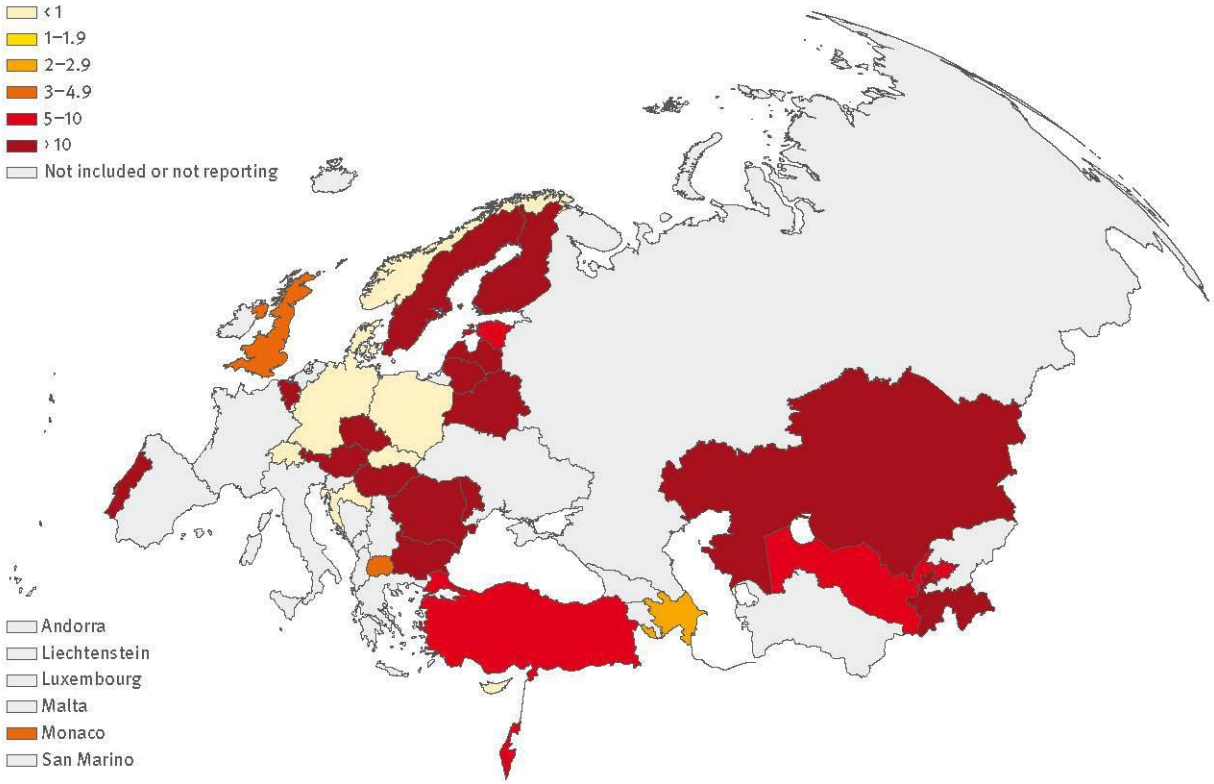
Although this appears to be relatively low, MDR-TB cases pose a disproportionate threat to the global prospects of TB control and are expensive to diagnose and treat. In several eastern European countries, at least one-third of TB cases presenting for treatment have MDR-TB. In a number of countries across the world, population rates of MDR-TB cases have increased over time.



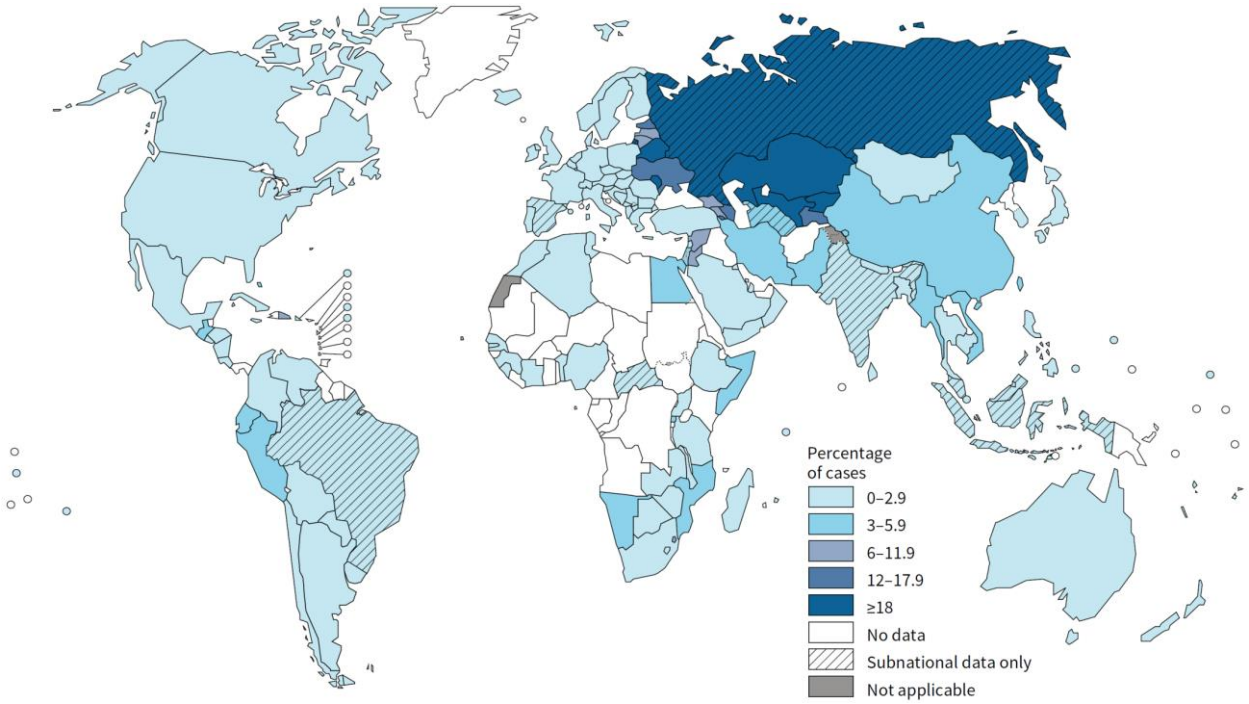
Number of MDR-TB cases estimated to occur among notified pulmonary TB cases, 2014

Of the 1,3 million TB deaths in 2012, 13% were estimated to be among people with MDR-TB. MDR-TB treatment remains arduous and unsatisfactory: whereas 87% of all newly notified TB patients reported to WHO complete their medication successfully, only about one half of MDR-TB cases do so and the likelihood of success diminishes with progressive resistance beyond MDR-TB.

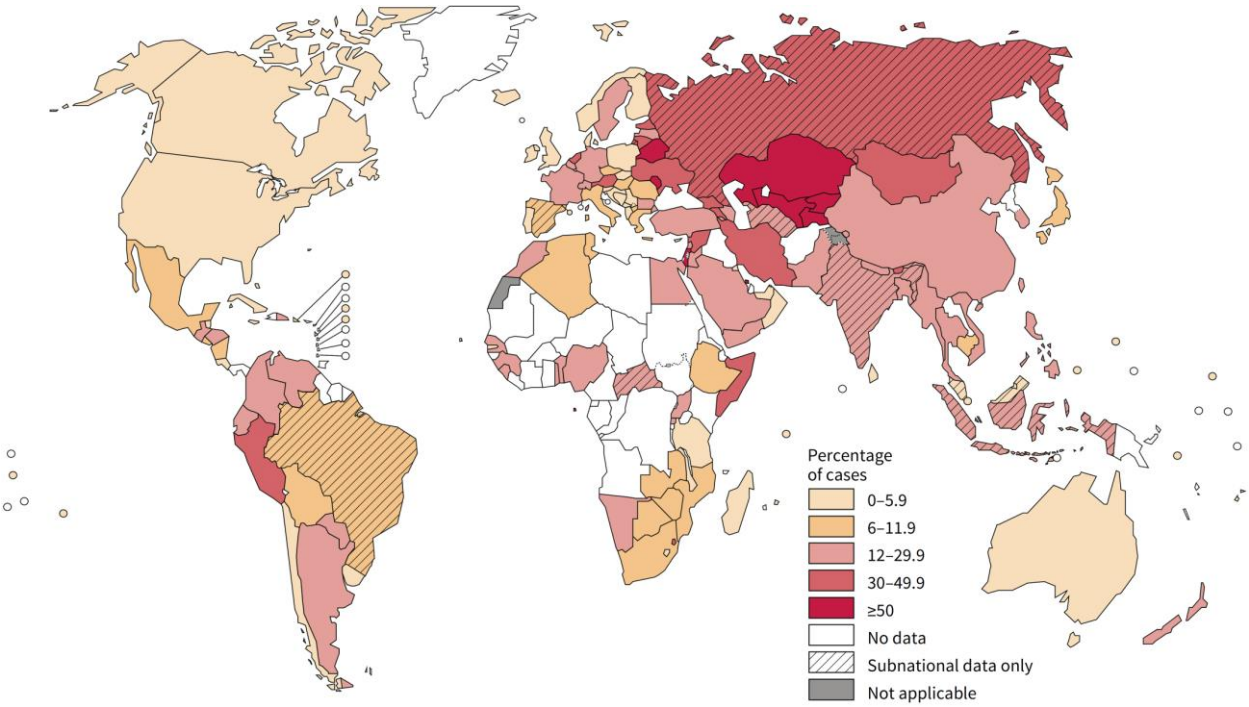
To date, 100 countries worldwide have reported at least one case of extensively drug-resistant TB (XDR-TB) (MDR-TB plus resistance to any fluoroquinolone and any second-line injectable drug). XDR-TB has been reported by 100 countries in 2013. On average, an estimated 9% of people with MDR-TB have XDR-TB.



Percentage of notified TB cases with XDR-TB among MDR-TB patients, WHO European region, 2012



Percentage of new TB cases with MDR-TB



Percentage of previously treated TB cases with MDR-TB

In 2013, the average proportion of MDR-TB cases with XDR-TB was 9,0%. The proportion of MDR-TB cases with XDR-TB was highest in Georgia (20,0%), Kazakhstan (22,7%), Latvia (21,7%), Lithuania (24,8%) and Tajikistan (Dushanbe city and Rudaki district: 21,0%).

By the end of 2013, 100 countries had notified at least one case of XDR-TB.

Only 48% of the MDR-TB patients in the 2011 cohort of detected cases were successfully treated. 16% died, 24% did not have their treatment outcome documented or interrupted treatment, and 12% were not cured despite receiving treatment. The treatment success rate for XDR-TB patients in the 2011 cohort was only 22%.

Levels of drug resistance among new cases are < 3% in 108 (75 %) of the 144 countries with drug resistance surveillance data. This includes almost all countries in the Region of the Americas, most countries in the African and South-East Asia regions, most countries in western Europe and several countries in the Western Pacific Region.

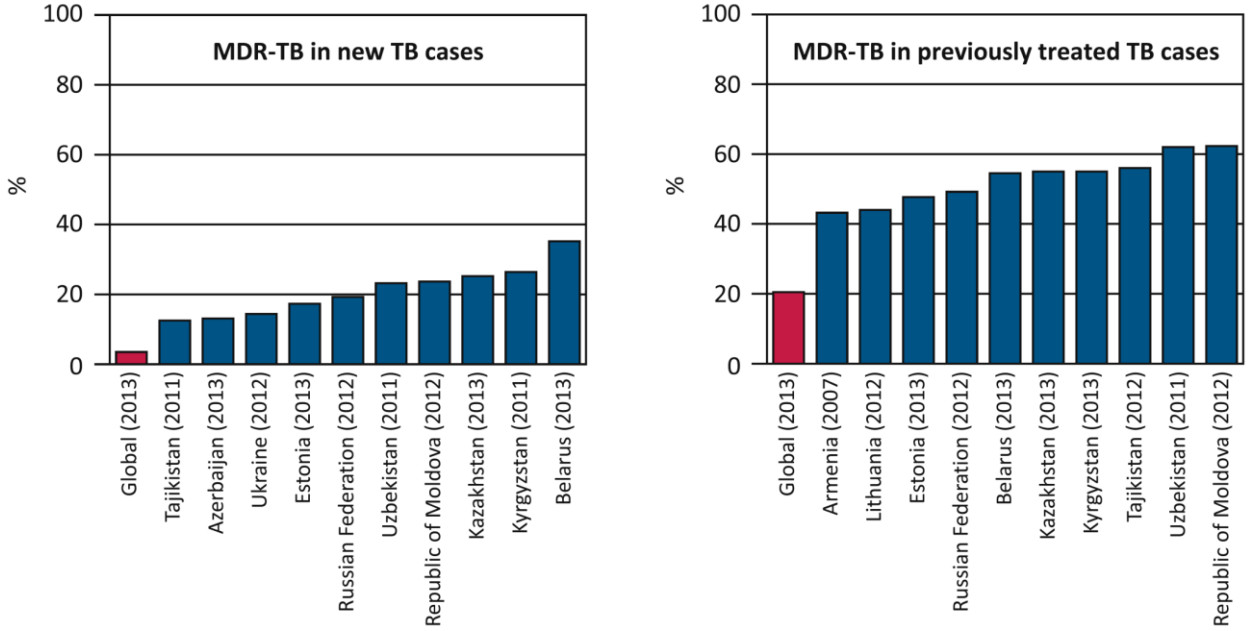
Eastern European and central Asian countries have the highest levels of MDR-TB, reaching 35% of new cases and 75% of previously treated cases in some settings.

A first time analysis of trends focussed on the period 2008−2013 suggests that globally, the estimated proportion of new cases with MDR-TB has not changed and remains at about 3,5%.

Continuous surveillance systems (i.e. systems that meet pre-defined standards for data quality and coverage, and in which TB patients are routinely tested for drug resistance) are the best way to generate robust data on trends in drug resistance.

By the end of 2013, 72 countries had continuous surveillance systems. These included 10 high MDR-TB burden countries: Belarus, Bulgaria, Estonia, Georgia, Kazakhstan, Latvia, Lithuania, Republic of Moldova, the Russian Federation and Ukraine. A further seven high MDR-TB burden countries had trend data from

special surveys of drug resistance and/or national TB prevalence surveys: China, Mozambique, Myanmar, Pakistan, the Philippines, Thailand and VietNam.



Percentage of new and previously treated TB cases with MDR-TB globally and in the top 10 countries, 2014

All countries need to build capacity for continuous surveillance. In the interim, repeat surveys should be implemented, particularly in high MDR-TB burden countries.

Rapid molecular tests such as the Xpert MTB/RIF assay are now being incorporated into drug resistance surveillance. They provide results much faster than conventional methods (culture and phenotypic drug susceptibility test (DST), do not require sophisticated laboratory infrastructure, greatly reduce and simplify laboratory work and decrease costs.

Conventional methods require timely and refrigerated transportation of sputum samples with live bacteria, which are then grown in laboratories prior to testing. Samples also need to be decontaminated to isolate TB bacteria and prevent

the growth of other organisms; in this process, there is a risk of killing TB bacilli (through too harsh decontamination) or contamination from other organisms. Molecular tests do not suffer from these limitations and as a result they may detect TB (including drug-resistant cases) that would have been missed by conventional methods.

In line with a 2009 World Health Assembly (WHA) Resolution, the Global TB Programme of World Health Organisation (WHO) monitors the efforts made by countries to expand universal access to MDR-TB care and prevention. In this study, the latest data reported to WHO by 2014 to summarise recent efforts being made to detect and cure MDR-TB patients in countries with the highest caseload of drug-resistant TB.

3. PRIORITY ACTIONS TO ADDRESS THE GLOBAL MDR-TB CRISIS

MDR-TB is a global health security risk and carries grave consequences for those affected. WHO therefore called for MDR-TB to be addressed as a public health crisis in 2013.

Five priority actions are crucial to accelerate the response against the MDR-TB epidemic:

• Prevent MDR-TB as a first priority.

• Scale up rapid testing and detection of all MDR-TB cases.

• Ensure prompt access to appropriate MDR-TB care, including adequate supplies of quality drugs and scaled-up country capacity to deliver services.

• Prevent transmission of MDR-TB through appropriate infection control.

• Underpin and sustain the MDR-TB response through high level political commitment, strong leadership across multiple governmental sectors, ever-broadening partnerships, and financing for care and research.

1. The best prevention against MDR-TB is quality treatment of drug - susceptible TB

Adequate treatment of drugsusceptible TB remains the cornerstone of efforts to prevent the emergence and spread of drug-resistant TB.

Globally, more than 95% of people who develop TB for the first time do not have rifampicin resistance or MDR-TB and can thus be treated successfully using a standard, inexpensive, 6-month course of treatment.

Globally in 2012, the treatment success rate for drug-susceptible TB was 86%, a level that has been maintained for several years.

2. Progress in MDR-TB diagnosis essential to find and treat more people with MDR-TB

The tripling of MDR-TB detection between 2009 and 2013 follows concerted efforts to strengthen laboratories and roll out rapid tests.

EXPAND-TB, the largest global project focused on accelerating access to modern diagnostics for TB and MDR-TB, is supporting 27 low- and middle-income countries. Between the start of the project in 2009 and the end of June 2014, 89261 people with MDR-TB were detected in the 97 laboratories supported by EXPAND-TB partners.

Since 2010, when WHO approved the Xpert MTB/RIF assay (for the simultaneous detection of TB and rifampicin resistance), global roll out of the technology has been impressive. By June 2014, a total of 3269 GeneXpert machines had been procured in the public sector in 108 countries eligible for concessional pricing, and over 1 million cartridges were being procured each quarter.

3. Progress evident but health system challenges still block universal access

The Global Drug Facility (GDF) of the Stop TB Partnership increased its supplier base for second-line anti-TB drugs from 10 to 19 between 2009 and 2014, resulting in an increase in the available number of second-line drugs (12 to 23) and price reductions.

Country progress to improve delivery of MDR-TB treatment through new approaches is also evident. For example, a shift away from hospitalization to ambulatory care is happening, especially in central Asian countries; treatment of XDR-TB patients is expanding worldwide; and several countries are pioneering shorter regimens for MDR-TB under operational research conditions.

Nonetheless, health service capacity to treat patients has not kept up with the pace of diagnosis, creating growing “waiting lists” for MDR-TB treatment in several countries. In addition, lack of patient follow-up remains a major health service constraint. Patient-centred care (including enablers and social support) is important to improve treatment outcomes.

4. A vital intervention that remains neglected

TB infection control is essential to minimize the risk of disease transmission but remains one of the most neglected components of TB prevention and care.

In 2013, more than 50 % of new cases of MDR-TB were among people never before treated for TB, highlighting the importance of transmission and the lack of appropriate infection control measures, particularly at community level.

MDR-TB transmission in health care facilities and in congregate settings such as prisons is a well-known public health threat. This can be effectively addressed by appropriate infection control measures (a mix of environmental, personal protection and administrative measures), rapid identification of drug resistance, and prompt, appropriate treatment of MDR-TB patients.

5. Funding and broad collaboration are essential for implementation of current interventions and research for new tools

Without intensified political commitment, financing and coordinated action by many stakeholders, the MDR-TB crisis cannot be effectively addressed.

The WHA resolution on M/XDR-TB in 2009 called for universal access to diagnosis and care. In 2014, three further resolutions with important implications for the MDR-TB response were issued. The topics of these resolutions were (1) the post-2015 global TB strategy; (2) antimicrobial resistance; and (3) palliative care.

The increased commitment of Member States reflected in these resolutions now needs to be translated into well-defined actions. The MDR-TB response needs to be multisectoral and fully financed for current interventions and research for new tools. It needs to encompass a broad range of stakeholders from both the public and private sectors.

4. THE DEFINITIONS

Resistance

Antimicrobial agents are drugs that are used to kill or suppress the replication of microorganisms that infect human hosts. Antibiotics that are effi cacious on one organism may not be effi cacious on another, or may have reduced effi cacy due to various factors. Similarly, there are different types of TB resistance including natural resistance, primary resistance, acquired resistance, combined resistance, resistance among new patients, resistance among previously treated patients, mono-resistance, poly-resistance, multidrug resistance and extensive drug resistance.

Natural resistance in tuberculosis

M. tuberculosis has a highly hydrophobic cell wall and several potential resistance determinants, which make it naturally resistant to many antibiotics including penicillin and sulfonamides. These antibiotics cannot be used to treat TB. Although Z is an efficacious anti-TB drug, it has no effect on *M. bovis*, which is naturally resistant to it.

Wild-type mutants in tuberculosis

Whereas several bacterial species acquire resistance through mobile genetic elements (such as plasmids and transposons), resistance to anti-TB drugs is caused by spontaneous chromosomal mutation. In an untreated population, there are wild-type mutants that have spontaneous chromosomal mutations.

The average mutation rate per bacterium per generation is $2{,}56 \times 10^{-8}$ for H, $2{,}95 \times 10^{-8}$ for S, $2{,}2 \times 10^{-7}$ for E and $2{,}25 \times 10^{-10}$ for R. Alangaden and colleagues reported that fluoroquinolone (FQ)-resistant mutants appeared at frequencies of 2×10^{-6} to 1×10^{-8}. Spontaneous chromosomal mutations that confer resistance to each drug are independent; it was therefore assumed that the risk of a wild-type mutant that is resistant to two drugs is the product of the risk

related to each of the two drugs (10^{-18} to both H and R per bacterium per generation).

The prevalence of mutants is related to mutation rates and the size of the bacterial population. In larger bacterial populations, the probability that resistant mutants are present is higher. The size of the population of *M. Tuberculosis* is estimated to be of the order of 10^7–10^9 in a cavity and 10^2–10^4 in caseous foci.

In general, the bacillary population in smear-positive pulmonary TB is larger than in smear-negative pulmonary TB and extra-pulmonary TB. Typically, the prevalence of wild-type resistant mutants in an untreated *M. tuberculosis* population is very small. David estimated the prevalence of mutants at $3{,}5 \times 10^{-6}$ for H (0,2 ug/ml), $3{,}8 \times 10^{-6}$ for S (2.0 ug/ml), $3{,}1 \times 10^{-8}$ for R (1,0 ug/ml) and $0{,}5 \times 10^{-4}$ for E (2,0 ug/ml).

Acquired resistance in tuberculosis

The emergence of acquired resistance involves a process of selection in an environment of drugs that favours replication of drug-resistant mutants. An anti-TB drug kills or suppresses replication of susceptible bacilli but allows drug-resistant mutants to replicate. Selection pressure imposed by an anti-TB drug on a population of *M. tuberculosis* results in a decline of drug-susceptible bacilli and advantageous reproduction of drug-resistant mutants, which is known as the «fall and rise» phenomenon. Consequently, drug-resistant mutants may outnumber drug-susceptible bacilli and become the dominant bacilli. This is acquired resistance. As the size of the bacillary population is larger and the prevalence of mutants higher in cavitary lesions than caseous foci, the risk of selective multiplication of resistant mutants is higher in cavitary lesions; likewise, it is higher among smear-positive pulmonary TB patients than smear-negative pulmonary and extra-pulmonary TB patients.

Acquired resistance can be demonstrated if the drug susceptibility pattern of TB bacilli is determined before anti-TB treatment and repeated at a later point in treatment, and if genotyping of TB strains is available. *M. Tuberculosis* that is

susceptible to one drug prior to treatment but becomes resistant to that drug after treatment represents acquired resistance in most cases. However, reinfection with a resistant strain may result in the observation of different susceptibility patterns between pre-treatment and post-treatment strains. Acquired resistance can therefore be ascertained only if reinfection is excluded by genotyping of *M. tuberculosis* with results showing that post-treatment strains and pre-treatment strains are the same.

Primary resistance in tuberculosis

Primary resistance in TB refers to patients infected with *M. tuberculosis* that is resistant to anti-TB drugs from the outset, prior to anti-TB treatment. Patients in whom *M. tuberculosis* acquires drug resistance during anti-TB treatment may spread the drug-resistant tuberculosis in the community. Primary resistance is caused by the transmission of drug-resistant bacilli followed by the development of drug-resistant TB among those who are primarily infected with drug-resistant strains.

Drug resistance among new tuberculosis patients

Primary resistance and acquired resistance are theoretical constructs that may not be discernible if additional information is not available. In surveillance of drug-resistant TB, patients are categorised into new patients and previously treated patients. New TB patients are those who have never been treated with anti-TB drugs or who were treated briefly (for a period of less than 1 month). Patients who have been treated with a standardised anti-TB regimen for less than 1 month are at low risk for development of acquired resistance. Therefore, it is likely that drug resistance among new patients represents primary resistance due to transmission. The proportion of new patients with drug-resistant TB in a population-based survey or surveillance is used as a measure of transmission of drug-resistant TB in a community.

However, patients may not remember whether they have been previously treated with anti-TB drugs, or may not know that they were treated for TB (for instance, R and FQ can be used to treat other infectious diseases). Further, health-

care workers may not take appropriate care when obtaining histories from previous TB patients. This may lead to a misclassification of previously treated TB cases as new TB patients. As the prevalence of drug resistance among previously treated cases is commonly higher than that among new TB patients, misclassification of previously treated cases as new cases may distort drug-resistant TB surveillance results by overestimating drug resistance among new patients.

Drug resistance among previously treated tuberculosis patients

Drug resistance among previously treated TB patients refers to the presence of drug-resistant *M. tuberculosis* in patients who have been treated with anti-TB drugs for 1 month or more. Drug resistance among previously treated TB patients has three potential sources, namely primary infection with resistant bacilli, acquisition of resistance during treatment and reinfection with resistant bacilli. As susceptibility testing is not routinely performed for new TB patients, patients who are primarily infected with resistant strains may not be identified at the initiation of TB treatment but found to be infected with drug-resistant strains in retreatment. As a previous history of TB does not guarantee full protection against reinfection, TB patients may be reinfected with resistant strains during or after treatment. Therefore, drug resistance among previously treated TB patients does not necessarily indicate acquired resistance. Though sources of resistance among previously treated cases vary, in most settings, previously treated cases have a higher prevalence of drug-resistant TB than new TB cases and are the target for case finding of multidrug-resistant TB (MDR-TB). As the prevalence of drug resistance among previously treated cases is commonly higher than that of new TB patients, misclassification of new cases as previously treated may underestimate the proportion of drug-resistant TB among previously treated patients. This type of misclassification is less likely to occur than misclassifi cation of previously treated cases as new cases.

Combined resistance

Combined resistance refers to the proportion of drug resistance among all TB cases regardless of history of anti-TB treatment. The combined proportion of

drug resistance among all cases enrolled in a survey does not take previous treatment into account. In several settings where history of TB treatment cannot be reliably obtained, combined resistance is reported. Combined resistance may roughly represent the overall burden of drug resistance among all TB cases in a community.

Transient resistance

Transient resistance is a phenomenon observed in patients who have multiple sputum samples collected at several time points during treatment. Drug-resistant bacilli may be seen in sputum from patients who have adequate response to treatment in a positive culture that consists of a small number of colonies (usually less than 5^{-10}), which usually appears shortly before sputum conversion, especially when drug action is bacteriostatic. For example, in patients treated with a regimen consisting of H and p-aminosalicylic acid (PAS), H-susceptible strains are killed by H and H-resistant mutants by PAS. As H has high bactericidal activities, an H-susceptible strain will be rapidly killed. Because PAS is bacteriostatic, H-resistant mutants will die slowly and may slightly outnumber H-susceptible strains at certain points in time during treatment before sputum conversion. These resistant bacilli are transient and may not arise predominantly during treatment. Patients will eventually achieve sputum conversion without a change of the treatment regimen.

Monodrug, polydrug, multidrug and extensive drug resistance

Monodrug resistance is defined as resistance to one anti-TB drug, while polydrug resistance refers to resistance to two or more drugs. Multidrug resistance is a specifi c form of polydrug resistance defined as resistance to at least H and R. MDR-TB is difficult to manage; its treatment involves secondline anti-TB drugs (SLDs) that are more expensive and toxic than first-line drugs (FLDs). XDR-TB is a special form of MDR-TB defined as resistance to at least H and R with further resistance to an FQ and a second-line injectable agent (2LI − amikacin, kana mycin or capreomycin). In general, outcomes of XDR-TB are less favour able than for

MDR-TB cases. Recently, the term «totally drug-resistant» TB has been used by researchers to describe strains that are resistant to all TB drugs tested. As DST may not be sufficiently accurate for several of the reserved drugs, and new drugs currently undergoing clinical trials may prove effective against TDR strains, totally drug-resistant-TB remains a theoretical concept of an unwanted outcome eventually arising out of inadequate management of drug-resistant TB.

5. LABORATORY DIAGNOSIS OF DRUG-RESISTANT TUBERCULOSIS

DR-TB can occur in new as well as retreatment cases, with any type of TB (pulmonary or extra-pulmonary, smear-positive or smear-negative). However, it is rarely feasible to perform DST for each and every patient. Nor would this be advisable, given the poor predictive value of resistance test results when resistance is rare (or the tests not highly specific), as is the case for second-line drugs (SLDs) in most of the world. It should be noted that not all drug resistance is equally important. In regions with fewer resources, only the most serious types of resistance should be investigated, i.e., those carrying a poor prognosis using standard first- or second-line drug therapy. These are resistance to R (MDR-TB) and the FQ (XDR-TB). In some settings or for some patients, DST for H and 2LIs is useful, although on their own these drugs do not have a very clear impact on treatment outcome using powerful regimens. The first step will be screening and diagnosis for MDR-TB, because XDR-TB screening is most often indicated only among MDR-TB cases.

5.1. Identification of organisms

AFB in sputum are not always *Mycobacterium tuberculosis*, but in high TB prevalence countries this is nearly always the case for new patients. Among MDR suspects, confusion occurs because of the presence of other mycobacteria. This is because several species that tend to colonise old TB lesions and become opportunistic pathogens are also resistant to most first- and secondline anti-TB drugs. If not identified correctly, these patients will thus often be treated as MDR-TB, and may even be considered XDR-TB when they also fail this treatment. *M. tuberculosis* (complex) should thus be shown before DST is performed or results transmitted. This is easier to do today using the simple immune-chromatographic MPT64 antigen test from liquid or solid cultures or with the TB detection result provided simultaneously with commercial molecular rifampicin DST tests (Genotype LPA as well as Xpert MTB/RIF). Microcolony morphology (serpentine cording) has been proposed as sufficiently characteristic of *M. tuberculosis*

complex, but experience shows that errors are frequent with a higher prevalence of non-TB mycobacteriosis. Another complication arises from the fact that these other mycobacteria often grow poorly on typical, solid media but much better in liquid media, particularly the MGIT (Mycobacteria Growth Indicator Tube) system.

The other mycobacteria in question come from the environment and may be found in 10 %−20 % of MDR suspects in areas with stagnant, polluted water but may be absent in dry desert areas. It is important that these suspects not be treated as MDR-TB, although they may show temporary improvement on such treatment, and it should be recalled that DST set up for TB may yield unreliable results with non-tuberculous mycobacteria.

It is wise, in general, not to attempt any treatment, because of the usually unclear significance of their isolate, the meagre chances of success for expensive and toxic treatment, the lack of public health priority and the high risk of reinfection from the environment. Appropriate management of non-tuberculous mycobacteria disease requires expert knowledge and additional resources, so referring these patients to specialist clinicians is in the formers best interest. The TB reference laboratory can provide assistance, for instance with exact species identification using a line probe or other molecular assay specific to this purpose.

5.2. Drug susceptibility testing

Principles of drug susceptibility testing in the laboratory

During the 1950s, the establishment of laboratory methods for *M. tuberculosis* drug susceptibility testing was a tremendous challenge. At that time, when diagnostic procedures for drug susceptibility testing of bacteria were largely unexplored, sensitivity and resistance in *M. tuberculosis* were defined as follows: «sensitive» strains are those that have never been exposed to anti-TB drugs («wild» strains); «resistant» strains are those that differ from sensitive strains in their capacity to grow in the presence of higher concentrations of the drug. Fortuitously, it was found that drug- susceptible strains of *M. tuberculosis* that have not been exposed to anti-TB drugs (wild- type strains) do not exhibit much variation in minimum inhibitory concentrations (MIC) to those drugs. Depending

on which laboratory method was used for susceptibility testing, significant differences were found in the drug concentrations which discriminate most efficiently between susceptible wild- type strains and probably resistant strains. For example, with proportion testing of S a maximum discrimination was achieved with a resistance proportion of 1% on 4 mg/l dihydro-S, while with the absolute-concentration method maximum discrimination was at 16 μ g/ml.

Current procedures for drug susceptibility testing of mycobacteria are characterized by 2 peculiarities: (1) the «critical concentration» and (2) the «critical proportion» . The drug concentration which categorizes a clinical *M. tuberculosis* isolate as either susceptible or resistant is defined as the concentration that inhibits the growth of wild-type strains, without appreciably affecting the growth of strains with alterations in drug susceptibility − this concentration is termed the «critical concentration». Proportion testing in mycobacteriology is based on the observation that all strains of tuberculosis contain some bacilli that are resistant to antibacillary drugs − in resistant strains the *proportion* of such bacilli is considerably higher than in sensitive strains . The drug susceptibility of a bacterial wild- type population follows a gaussian distribution. Thus, depending on the drug concentration, a small fraction of the population will show phenotypic resistance. This observation forms the basis for combining proportion testing and «critical concentration». Standardization of the «critical concentration» has not been without controversy. For example, the recommended concentrations for E underwent adjustments over time, not the least because of low interlaboratory reproducibility of E susceptibility testing. Most likely, this is due to the very small differences between the concentration used for in vitro drug susceptibility testing and the natural drug susceptibility of wild-type isolates of *M. Tuberculosis*. Thus, minute changes in drug susceptibility will have a major impact on interpretation of in vitro test results, with only a narrow range between the MIC of susceptible isolates and resistant isolates.

Among mycobacteriologists a misperception of «critical proportion» and clinical resistance frequently prevails. While the critical proportion of cells (subpopulation) able to grow in the presence of the critical concentration is mostly defined as equal or greater than 1% of the population (1 in 100), the frequency of mutational resistance is much lower, approximately 10^{-7} (i.e. 0.00001%, or 1 in ten million).

It is, however, the mutational resistance which is responsible for treatment failure and for the emergence of resistance following inappropriate drug regimens. The critical proportion of resistance is a technical term and should not be confused with mutational resistance. In combination with the critical concentration, the critical proportion is a laboratory term used in in vitro drug susceptibility testing to define the epidemiological cut-off.

Methods and drugs to test, reliability of tests

DST methods can be divided into slow vs. rapid, conventional (or growthbased) vs. molecular (detection of resistance mutations) and direct (starting from the specimen) vs. indirect (starting from a pure subculture). All molecular techniques are rapid but indirect DST never is, and speed may be the most important criterion in classifying methods for MDR-TB management. Slow conventional methods are more reliable and perfect for drug resistance monitoring. The proportion method may be used most often, but other recognised techniques (i.e., absolute concentration and resistance ratio methods) yield equivalent results. For the most diffi cult strains, the strong inoculum and minimal inhibitory concentration technique of the absolute concentration method may provide the clearest results. With these methods, agar media such as Middlebrook 7H10 or 7H11 are easier to prepare with high consistency of drug concentration (no heating required), but they are more costly and require additives with short shelf life, and growth of diffi cult strains is decreased, even when used with CO2 enrichment as recommended. Lowenstein-Jensen egg-based medium is cheaper, ingredients are stable and easily procured, and it supports growth of all but exceptionally resistant

strains. However, inspissations by heating must be very well controlled and evenly applied to all tubes. Rapid DST techniques are needed for effi cient diagnosis and management of MDR-TB, but overall they are still less accurate than slow conventional methods.

Only molecular techniques are truly rapid, yielding results in a few hours or days. When a highly efficient, not very toxic and less expensive standard regimen is used, only diagnosis of R resistance is initially needed. If the patient is hospitalised for the initial phase of treatment, it is highly desirable to exclude XDR-TB as early as possible, but this is more difficult. In most settings where XDR-TB is still very rare, rapid tests to exclude XDR are mainly needed for patients who have previously received FQ and/or 2LIs for TB treatment. Systematic confirmation by slow conventional DST is generally recommended after the patient is already on MDR treatment.

It is true that in many settings, MDR-TB treatment can be started without proof of R resistance for Category 2 failures, a very high prevalence group. Importantly, this is not universally true, probably due to sloppy treatment observation: up to 50% of these cases have been reported to have non-MDR-TB is some settings. Infection or disease with other mycobacteria is another concern, as discussed above. On the other hand, knowledge of previous treatment regimens can give some indications regarding drugs that are likely still to work because they were never used on the patient. Resistant strains may circulate in the community, while cross-resistance with other drugs occurs as well. Constituting a regimen based on drugs previously administered will thus require good information regarding levels of primary drug resistance.

There is a risk that valuable drugs will not be included because failure or relapse can occur due to resistance to the predominantly used drugs or due to non-adherence. This is even more true for Category 1 failures, which the WHO recently added to the clinical and smear indications justifying initiation of MDR-TB treatment in the absence of DST results. Full DST covering all possible drugs may be required for treatment of XDR-TB, those previously treated with SLDs and

settings with high levels of resistance to the main SLDs. In most settings, however, the results will hardly change the management of a patient on first-time SLD treatment, and DST requirements should certainly not delay patient management.

Among first-line drugs (FLDs), only R resistance determines the choice between FLD and SLD treatment. On the other hand, the outcome of R-resistant/H-susceptible TB following treatment with FLDs is not good, peppered with relapses and, in the long term, development of MDR or death. DST for streptomycin is not useful because it is never used in the recommended regimen and there is virtually no cross-resistance with 2LIs. Pyrazinamide and ethambutol DST are difficult to perform correctly as there is not good agreement between different methods and resistance occurs less frequently with early detection. Due to its superb sterilising activity, Z is best included in any MDR regimen without the need to perform DST.

Reliability of DST for p-aminosalicylic acid, ethionamide, cycloserine and thiacetazone is low and should only be performed to guide treatment of the most diffi cult cases because results may confuse more than help. DST for SLDs (or clofazimine) is of limited use in settings where resistance to these drugs is rare. This is always the case when they are not used to treat TB on a larger scale, i.e., in most low-income countries. In such circumstances, a resistant result will most often be wrong. Moreover, the short standard SLD regimen recommended here uses only the most valuable SLDs and, even with correct DST results, a switch to the remaining weak and toxic drugs may not improve outcomes. The exception is confi rmed XDR-TB or failure of the recommended MDR regimen, because this requires individualised treatment with a limited num ber of still active drugs.

In such difficult cases, the range of tests performed should cover both amikacin and capreomycin, though not necessarily the weaker kanamycin, because of varying patterns of cross-resistance. It is also useful to perform DST for the FQ and possibly H using an absolute concentration method (MIC determination). Considerably different levels of resistance exist, and MIC up to 8 μ g/ml ofloxacin or 1 μ g/ml of a fourth-generation FQ (moxifloxacin or gatifloxacin) will still be

overcome using these powerful drugs with higher dosing. This is more important than testing weak companion drugs such as PAS or Cs.

DST for R is generally highly reliable, more so than for other drugs, but some resistant strains are very difficult to diagnose using DST based on growth. Most mutations in the *rpo*B gene conferring this resistance come at a fitness cost, though it is negligible for the most common and easily tested mutations. A more important loss of fitness causing growth problems is seen with a wide range of other mutations that are each rare but may together make up 10 %−20 % of all mutated strains, particularly after fi rst TB treatment.

Routine rapid DST will regularly «miss» this resistance, and call these strains R- or even pan-susceptible whereas careful testing may show that they are resistant to all FLDs (or even drugs used in XDR-TB). Doubts have been raised regarding the clinical importance of some of these mutations (e.g., 511Pro, 516Tyr and 533Pro) because their resistance level seems very low. However, using a strong inoculum with the strain in the exponential growth phase, their MIC may prove to be several times higher than the critical concentration defining resistance. Clinically, they also cause failure of treatment and, more frequently, repeated relapse after apparent cure with final poor prognosis secondary to increasing resistance. Further, despite reduced virulence, strains with these mutations were at the origin of the MDR and XDR-TB outbreak in KwaZulu-Natal in 2006. Especially with such strains, molecular detection of R (and probably also FQ and 2LI) resistance is more reliable than conventional DST.

5.3. Rapid detection of resistance

Molecular techniques

The Gene Xpert instrument, using the Xpert MTB/RIF assay can be used to detect *M. tuberculosis* DNA from respiratory samples using real time PCR technology. The assay also detects mutations which confer rifampicin resistance. For patients likely to have acquired TB in settings where the risk of multidrug resistance (resistant to isoniazid and rifampicin) is significant, the detection of rifampicin resistance by Xpert MTB/RIF is highly predictive of MDR-TB

detection. Some extrapulmonary specimens may also be tested with the Xpert assay – such testing should be discussed with the laboratory.

The reference molecular technique (DNA sequencing) can detect DNA mutations that result in resistance for any drug. However, the molecular mechanisms of resistance are well known only for only a few drugs. Furthermore, DNA sequencing is only an option for large referral laboratories or in industrialised countries. In practice, for low- and middle-income countries, currently available methods can only reliably detect R resistance. These are line probe assays (LPA) and Xpert MTB/RIF (Xpert). Both can be applied to growth from cultures and usually work for smear-positive sputum tests as well. The most widely used LPA kit (but not Xpert) also allows detection of H resistance and results report the gene involved, but its sensitivity is too low for use in early cases. The Xpert system simplifies molecular testing by fully integrating and automating the three processes (sample preparation, amplification and detection) required for real-time PCR-based molecular testing. Xpert fails less often than LPA with smear-negative sputum. Comparing the latest generation of both tests for detection of R resistance, Xpert may be more sensitive and no longer yield more false R-resistant results. Both tests contain a positive signal confirming the presence of *M. Tuberculosis* DNA, but the differentiation between a partial test failure and presence of NTM is not reliable.

Molecular techniques are the best choice for diagnosis of MDR-TB with R resistance as its proxy. As described above regarding poorly growing strains, the gold standard technique, DNA sequencing of the *rpo*B gene, may be more accurate than phenotypic R DST in the average laboratory because it misses less than 5% of resistance. Due to non-covered mutations or resistance based on mechanisms other than *rpo*B-gene point mutations, missed resistance may be a few percent higher with commercial tests such as LPA and Xpert. With the current versions of these commercial tests, false resistance is rare and is due to cross-contamination or silent mutations.

To enhance safety, samples can be «killed» in the outlying regions prior to shipment, as described earlier, meaning that rapid or cold chain transport is not needed. The Xpert technique, in particular, is so simple that it can easily be set up and even decentralised. LPA has considerably higher requirements in terms of infrastructure, equipment and skilled staff, but its implementation has not posed major problems for start-up even in low-income countries. The main objections to molecular techniques are the relatively high cost and the temperature-sensitivity of some equipment and supplies, although so far these have not proved to be major obstacles when there is a good selection of patients and drugs to be tested and in the absence of decentralisation beyond the intermediary service level.

LPA patterns characterised by the absence of wild-type bands should be interpreted as resistance, even without the appearance of a mutation band, provided the various control bands are sufficiently developed. Presence of National treatment management can be suspected on the MTBDR*plus* LPA strips, but confirmation and species identification requires running a different LPA. Another LPA, the MTBDR*sl*, is designed to detect resistance to FQ, 2LIs and E. Reported agreement with phenotypic DST has been poor for E and not quite satisfactory for the other drugs (70 %−80 % sensitivity at most). On the other hand, there is uncertainty regarding the clinical relevance of part of the phenotypic DST resistance results for these drugs. Until this is resolved, these techniques can at least be used to rapidly confirm (but not exclude) FQ and 2LI resistance.

Conventional DST

This is performed using the BACTEC 960 MGIT liquid culture system. All *M. tuberculosis* strains receive first line DST. Drug resistant strains receive supplementary DST to the second line agents. First and second line testing each requires 10-14 days for a result. Slow growing strains may need repeat testing before a reportable result is obtained.

6. THE DRUGS FOR MDR-TB TREATMENT

6.1. Groups of drugs to treat MDR-TB

For MDR treatment, anti-TB drugs are grouped according to efficacy, experience of use and drug class. All the first-line anti-TB drugs are in Group 1, except streptomycin, which is classified with the other injectable agents in Group 2. All the drugs in Groups 2–5 (except streptomycin) are second-line, or reserve, drugs.

Group 1. Group 1 drugs are the most potent and best tolerated. If there is good laboratory evidence and clinical history that suggests that a drug from this group is effective, it should be used. If a Group 1 drug was used in a previous regimen that failed, its efficacy should be questioned even if the DST result suggests susceptibility. The newer rifamycins, such as rifabutin, have very high rates of cross-resistance to rifampicin.

Group 2. All patients should receive a Group 2 injectable agent if susceptibility is documented or suspected. Among aminoglycosides, kanamycin or amikacin is the first choice of an injectable agent, given the high rates of streptomycin resistance in drug-resistant TB. In addition, both these agents are inexpensive, cause less otoxicity than streptomycin, and have been used extensively for the treatment of drug-resistant TB. Amikacin and kanamycin are considered to be very similar and have a high frequency of cross-resistance. If an isolate is resistant to both streptomycin and kanamycin, or if DRS data show high rates of resistance to amikacin and kanamycin, capreomycin (a polypeptide) should be used.

Group 3. All patients should receive a Group 3 medication if the *M. tuberculosis* strain is susceptible or if the agent is thought to have efficacy. One of the higher generation fluoroquinolones, such as levofloxacin or moxifloxacin, is the fluoroquinolone of choice. Ciprofloxacin is no longer recommended to treat drug-susceptible or drug-resistant TB.

Table . Groups of drugs to treat MDR-TB

Groups	Drugs (abbreviations)
Group 1: First-line oral agents	• pyrazinamide (Z) • ethambutol (E) • rifabutin (Rfb)
Group 2: Injectable agents	• kanamycin (Km) • amikacin (Am) • capreomycin (Cm) • streptomycin (S)
Group 3: Fluoroquinolones	• levofloxacin (Lfx) • moxifloxacin (Mfx) • ofloxacin (Ofx)
Group 4: Oral bacteriostatic second-line agents	• *para*-aminosalicylic acid (PAS) • cycloserine (Cs) • terizidone (Trz) • ethionamide (Et) • protionamide (Pt)
Group 5: Agents with unclear role in treatment of drug resistant-TB	• clofazimine (Cfz) • linezolid (Lzd) • amoxicillin/clavulanate (Amx/Clv) • thioacetazone (Thz) • imipenem/cilastatin (Ipm/Cln) • high-dose isoniazid (high-dose H) • clarithromycin (Clr)

Group 4. Ethionamide (or protionamide) is often added to the treatment regimen because of low cost. If cost is not a constraint, *p*-aminosalicylic acid (PAS) may be added first, given that the enteric-coated formulas are relatively well tolerated and that there is no cross-resistance to other agents. When two agents are needed, cycloserine can be added. Since the combination of ethionamide (or protionamide) and PAS often causes a high incidence of gastrointestinal side-

effects and hypothyroidism, these agents are usually used together only when three Group 4 agents are needed: ethionamide (or protionamide), cycloserine and PAS. Terizidone can be used instead of cycloserine and is assumed to be equally efficacious.

Group 5. Group 5 drugs are not recommended by WHO for routine use in drug-resistant TB treatment because their contribution to the efficacy of multidrug regimens is unclear. They can be used in cases where it is impossible to design adequate regimens with the medicines from Groups 1–4, such as in patients with XDR-TB. They should be used in consultation with an expert in the treatment of drug-resistant TB.

6.2. Antituberculosis drugs characteristics

Role of first-line oral anti-tuberculosis drugs in the management of drug-resistant tuberculosis

The first group includes the four main drugs that make up the ideal regimen for initial treatment: H, R, Z and E. Clearly, the therapy focus for non-MDR-TB patients with mono- or polydrug-resistant strains is entirely different if resistance is to H or R. Patients with H mono- or poly-resistance but retained susceptibility to R are relatively common in all NTPs. As expected, R should be kept as a fundamental drug in regimen design and included among the four that make up the basic regimen. By using R, the length of treatment can be reduced to 9−12 months. A completely different situation exists if a patient with R mono- and poly-resistant strains retains susceptibility to H.

This situation is quite rare in the field, because more than 90 %−95 % of cases with R resistance are actually MDR-TB. Thus, although H should always be given in these cases, R should not be included, and the designed plan should be the same as if the patient were suffering from MDR-TB. Accordingly, the great majority of MDR-TB and XDR-TB patients have already received and failed one or several cycles of combined drugs, including Z and E. Given this, and the low reliability of the susceptibility tests for E and Z, resistance to them should often be suspected, meaning Z and E should never be considered among the four essential

drugs in regimens for these patients. In recent years, however, some evidence has been published on the value of these FLDs (including H) in the treatment of MDR-TB and XDR-TB cases.

Isoniazid (H)

It is a shame that a drug that is so good and has such potent bactericidal activity has already been lost to an important percentage of patients around the world due to proven resistance. First, H is only active against mycobacteria. Within the genus, its effect is mainly against *M. tuberculosis complex* and to a lesser extent against a few species of environmental mycobacteria, e.g., *M. kansasii*. H has the most potent early bactericidal activity of all anti-TB drugs, and adding other drugs does not increase this activity. Thus, the rapid reduction in infectiousness following initiation of chemotherapy is most likely attributable in large part to the bactericidal activity of H. It also seems logical that it will have a decisive influence on improving chances of survival in the early days/weeks of treatment and on earlier conversion to negative of sputum smear microscopies and cultures.

H appears to penetrate host cells readily and diffuses across the *M. tuberculosis* membrane. H is a pro-drug, requiring oxidative activation by the *M. tuberculosis* catalase-peroxidase enzyme KatG. Although the active metabolites of H have been reported to inhibit multiple essential cellular pathways, including synthesis of nucleic acids, phospholipids, and NAD metabolism, the primary pathway responsible for the killing activity of the drug is mycolic acid synthesis. Thus, the activated form of the drug binds tightly to the NADH-dependent enoyl acyl carrier protein (ACP) reductase InhA, a component of the fatty acid synthase II system of mycobacteria, which is essential for fatty acid elongation. H does not directly interact with InhA, as X-ray crystallographic and mass spectrometry data revealed that the activated form of H covalently attaches to the nicotinamide ring of NAD bound within the active site of InhA, causing NADH to dissociate from InhA. However, the precise mechanism by which H kills *M. tuberculosis* remains to be elucidated.

Early reports suggest that H affects cell wall integrity. Acid-fastness is lost shortly after treatment with H begins. This drug inhibits the synthesis of mycolic acids in the cell wall. When acting on the mycobacterial cell wall of continually replicating bacilli, there must be active bacilli replication for the drug to exert its potent bactericidal action. This is why its bactericidal action declines in the early weeks of treatment and nearly disappears when the sputum smears become negative, i.e., when most of the remaining bacilli are in latent or semi-latent growth phases. Interestingly, the sterilising action of H is very poor. With these important characteristics, its good tolerance and low price, it is regrettable that H is already lost to over 10 % of patients worldwide due to resistance acquired over years of misuse.

Strains resistant to low levels of H (0,2 mg/L) but susceptible to high levels of the drug (1 mg/L) are usually resistant to Eth (*inh*A) and susceptible to high doses of H (10−15 mg/kg). The latter could be true for up to 10 %−15 % of TB patients with resistance to H, in whom high doses of this drug may be useful (in spite of demonstrated in vitro resistance) to overcome the potential problem of cross-resistance to Eth. This controversial issue was recently evaluated in a randomised clinical trial after adjustment for potential confounders, subjects who received high-dose H became sputum-negative more rapidly than those who did not receive it, and were more likely to be sputum-negative at 6 months. These subjects displayed better radiological improvement without an increased risk of H toxicity. Although the results of this clinical trial are very valuable, the study was too small to control for other outcome predictors in any realistic way. We therefore conclude that adding high doses of H to the treatment of MDR-TB and XDR-TB could be a sound recommendation and should be evaluated as part of regimen design. Ideally, this recommendation should be followed only in selected patients with proven susceptibility to high-dose H or with an LPA test (GenoType) showing no mutation in the *kat*G gene. However, it often takes at least 2−3 weeks to obtain the results in many settings. Thus, in countries with a high MDR-TB burden and no facilities to provide such information, systematically adding high-

dose H to the DR-TB regimen should be considered. The use of high doses of H and Et should ensure the presence of one active drug. In these cases, vigilance for hepatotoxicity and neurotoxicity should be exercised, especially in an at-risk population.

Rifampicin and other rifamycins

Rifamycins contain an aromatic nucleus linked on both sides by an aliphatic bridge. The rifamycins easily diffuse across the *M. tuberculosis* cell membrane due to their lipophilic profile. Their bactericidal activity has been attributed to their ability to inhibit transcription by binding with high affinity to bacterial DNA-dependent RNA polymerase. Although the molecular target of rifampin has been well characterized, the precise mechanism by which this interaction leads to mycobacterial killing remains unclear.

Discovered over 40 years ago, R remains the most effective drug against *M. tuberculosis.* It has the ability to kill *M. tuberculosis* in all its growth phases. R works on mycobacterial RNA, enabling good bactericidal action (though not as good as H) and sterilising action. Its powerful sterilising action makes it the most influential drug for shortening TB treatment. A regimen with R can cure in 9 months, whereas a regimen without it needs 18 months at minimum, at least according to classical studies (although the new generations of FQ may have a similar sterilising action at high doses and thus the ability to shorten MDR-TB treatment). It appears that the other rifamycins have the same effect as R in TB treatment, though there is little solid evidence to support this. Contrary to H, R is active against a wide range of microorganisms including *Mycobacterium leprae*, *Staphylococcus aureus*, *Neisseria meningitidis* and *Legionella pneumophila.* Like all naphthalenic ansamycins (the class to which rifampicin belongs), R is a specific inhibitor of DNA-dependent RNA polymerase. R acts by interfering with the synthesis of mRNA by binding to the RNA polymerase. Three different rifamycins are currently commercially available: rifampicin, rifa butin and rifapentine. M. tuberculosis develops resistance to all of these by means of a mutation in the 81 bp

region of the RNA polymerase β-subunit (rpoB) gene. However, analysis of the diverse mutations of this gene has revealed that even if most of the isolates resistant to R are also resistant to rifapentine, about 15 %−20 % could be susceptible to rifabutin. This has also been observed in some clinical studies. Potential susceptibility to rifabutin is based on the current recommended cut-off for its DST. This cut-off has never been clinically validated, and therefore, clinical response to rifabutin should not be expected in patients whose isolates are susceptible to rifabutin but resistant to other rifamycins. Moreover, the possible use of rifabutin in MDR-TB and XDR-TB patients is limited by its high cost and the lack of availability of the drug and corresponding DST in many countries. For these reasons, the use of R or other rifamycins should not be systematically recommended in MDR-TB and XDR-TB cases. It should be considered only in isolated cases where rifabutin can be tested, and then only if results show sensitivity. It should not be among the four basic regimen drugs, but rather an addition to the core drugs.

Pyrazinamide (Z)

Z is an amide derivative of pyrazine-2-carboxylic acid and nicotinamide analog. Despite recognition of its anti-tuberculosis activity six decades ago, the mechanism of action of Z remains poorly understood. Z has been hypothesized to act against bacilli residing in acidified compartments of the lung that are present during the early inflammatory stages of infection, since the drug's sterilizing activity appears to be limited to the first 2 months of therapy. Z enters tubercle bacilli passively and via an ATP-dependent transport system. Intracellular accumulation of the drug occurs because of an inefficient efflux system unique to *M. tuberculosis*. Z, like H, is a pro-drug, requiring activation to its active form, pyrazinoic acid, by the enzyme pyrazinamidase. Uptake and intrabacillary accumulation of pyrazinoic acid is enhanced when the extracellular pH is acidic. The anti-tuberculosis activity of Z has been attributed to disruption of the proton motive force required for essential membrane transport functions by pyrazinoic

acid at acidic pH, although investigation into potential specific cellular targets is ongoing.

Z is essentially a weak drug with very limited activity only on bacilli that are intracellular and dividing in an acidic environment. Z is also only active against mycobacteria, and among the genus, mycobacteria other than *M. tuberculosis* (including *M. bovis*) are naturally resistant. It was recognised early on that Z acts only in an acid environment. The active derivative of Z is pyrazinoic acid, which preferentially accumulates in an acidic pH. Z is not active against intracellularly growing *M. tuberculosis*; only the accumulation of pyrazinoic acid through the action of the amidase pyrazinamidase by susceptible *M. tuberculosis* triggers its intracellular bactericidal action. Relatively little is known about the actual drug target, although the nicotinamide adenine dinucleotide metabolic pathway has been postulated as a potential target. Therefore, while the bactericidal ability of Z is very poor, it has powerful action on bacilli that divide very little in the presence of an acidic medium unfavourable to bacilli. This acidic environment unfavourable to bacilli is the same for most anti-TB drugs, including H and R. The difference is that Z does not lose its action in the acidic environment surrounding the bacilli when it is inside the macrophage or when there is much inflammation. On the contrary, when the acidic environment disappears, the action of Z should in theory be nil. Thus, it is recommended only during the first 2 months of initial treatment plans. This reasoning may be valid when R is kept in the regimen with its potent sterilising action, but it is very likely that if R is not present, Z may continue working after the fi rst months of treatment.

Z has very good sterilising action but poor to no bactericidal capacity. Z was commonly used between 1950 and 1970 to treat patients carrying bacilli poly-resistant to H+S (similar to todays XDR-TB patients, because R and FQ did not exist). Three interesting articles were published during that time, reporting excellent cure and/or bacteriological conversion rates for the combination of Et+Cs+Z. Presumably, Z had a major role in this regimen and remained active for the entire duration of the treatment. Moreover, a relatively frequent situation in

MDR-TB patients is continued maintenance of original susceptibility to Z. This is the case for patients with initial treatment regimen failure who develop MDR-TB, but with a strain initially resistant only to H. This could explain the results reported in two articles published recently on MDR-TB and XDR-TB patients demonstrating that adding Z, E or S to the treatment of patients who remain susceptible to these drugs improved their prognosis. Taking into account the unknown reliability of the Z susceptibility test and its low cost and moderate to low toxicity, it seems reasonable to consider adding Z to all treatment regimens for MDR-TB, although it should not be counted as one of the four basic drugs. Evaluation should be individualised and consider that the risk of hepatotoxicity may be increased for elderly and alcoholic patients. The current common practice of using Z for patients with MDR-TB (regardless of susceptibility results) needs to be critically examined to determine if there are clinical benefi ts to such treatment and whether the benefi ts justify the possible increases in toxicity.

Ethambutol (E)

The primary pathway affected by E appears to be that of arabinogalactan biosynthesis through inhibition of cell wall arabinan polymerization. E also has been reported to inhibit several other cellular pathways, including RNA metabolism, transfer of mycolic acids into the cell wall, phospholipid synthesis, and spermidine biosynthesis.

E is a somewhat controversial drug. Based on its theoretical mode of action (on the mycobacterial cell wall), it should have significant bactericidal capacity and be very potent. Yet it seems to behave as a far weaker drug than expected; its actual important properties correspond to its excellent tolerability and ability to prevent the selection of resistance to major drugs like H and R. E is also only active against mycobacteria and, theoretically, is bactericidal on both extracellular and intracellular tubercle bacilli. Specifically, it inhibits biosynthesis of the mycobacterial cell wall and acts on the biosynthesis of arabinogalactan, the major polysaccharide of the mycobacterial cell wall. E inhibits the polymerisation of cell

wall arabinogalactan and lipoarabinomannan, indirectly inhibits mycolic acid synthesis (by limiting the availability of arabinan for the mycolic acids to attach to) and triggers a cascade of changes in lipid metabolism of mycobacteria, leading to the disaggregation of bacteria clumps into smaller clusters. The main benefits of E are its excellent tolerance and very low initial resistance rate in most countries. Furthermore, as mentioned for Z, patients with initial regimen failure who have MDR-TB but whose organisms were originally resistant only to H are likely to remain susceptible to E. This explains the benefi t of E for MDR-TB and XDR-TB patients, although there are other plausible explanations for the improved results that have been observed. Given the uncertain clinical reliability of the E susceptibility test and the low cost and toxicity of the drug, it seems reasonable to evaluate the addition of E (dose: 15 mg/kg) to the treatment of MDR-TB. However, for patients who previously received E and for whom DST shows resistance, the addition of E is not advised. Further, E should not be counted as one of the four basic drugs and its inclusion as an additional drug in an already large pill burden needs to be carefully considered in the light of its potential detrimental effect on adherence.

Injectable anti-tuberculosis drugs

Aminoglycosides inhibit protein synthesis by irreversibly binding to the 30S ribosomal subunit of M. tuberculosis, interfering with the integrity of the cell membrane. Resistance is due to mutations in the rrs gene, which encodes 16S ribosomal RNA, and in the rpsL gene, which encodes the S12 ribosomal protein gene.

Metabolization and excretion

Oral absorption of aminoglycosides is minimal, and the drugs are administered parenterally. Absorption is complete when aminoglycosides are administered i.m., and the serum levels of the drugs peak within 30-90 min after their administration; however i.m. absorption can be slower, requiring successive injections at the same site. It is recommended that i.v. administration of

aminoglycosides be carried out over a period of 15-30 min in order to reduce the risk of adverse effects, such as neuromuscular blockade. The binding of aminoglycosides to plasma proteins is low (approximately 10 %). Over a 24-h period, 80-98 % of the drug is excreted, unaltered, by the kidneys (glomerular filtration), 1 % is excreted in bile, and 1 % is excreted in feces. The half-life of streptomycin is 2-3 h, and the half-life of amikacin is 2 h, although the latter can be as long as 86 h in patients with kidney failure. The penetration of aminoglycosides into the cerebrospinal fluid is low, except in cases of meningitis.

Adverse effects

Ototoxicity. The most severe adverse reaction caused by aminoglycosides is ototoxicity due to damage to cranial nerve VIII, including vestibular damage (vertigo, ataxia, and nystagmus) and cochlear damage that can lead to hearing loss. The risk increases with age, prolonged duration of treatment, and high total accumulated dose. The risk also increases in patients who use aminoglycosides in association with diuretics (furosemide and ethacrynic acid), in dehydrated patients, and in patients with a history of hearing impairment. Vestibular damage is more common than is cochlear damage and occurs earlier. In addition, vestibular damage is more common in patients using streptomycin than in those using amikacin. Ototoxicity requires immediate discontinuation of the drug.

Neurotoxicity. Aminoglycosides can cause perioral paresthesia immediately after their administration. This adverse effect is benign.

Nephrotoxicity. Aminoglycosides produce renal toxic effects due to their accumulation in the renal tubules. Such effects are more common in elderly individuals and in patients with a history of kidney disease. Clinical and laboratory manifestations of nephrotoxicity include oliguria, urinary casts, proteinuria, and decreased creatinine clearance, as well as increased serum levels of urea and creatinine. Patients who receive more than one daily dose of the drug, patients under long-term treatment, and patients with high total accumulated dose are more likely to develop nephrotoxicity. Nephrotoxicity is more common in patients using

amikacin (occurring in 3,4-8,7 %) than in those using streptomycin (occurring in 2 %). Discontinuation of the drug is recommended.

Neuromuscular blockade. Aminoglycosides can cause neuromuscular blockade, leading to respiratory failure. Neuromuscular blockade can occur due to rapid i.v. injection of the drug in patients who concomitantly use anesthetics or neuromuscular blocking agents (curare or succinylcholine) or in those who have received massive blood transfusions in which citrate is used as an anticoagulant. Although calcium salts can reduce this effect, mechanical ventilation might be necessary.

Hypersensitivity. Hypersensitivity is rare in patients treated with aminoglycosides. However, there are hypersensitivity cross-reactions among the different aminoglycosides.

Use during pregnancy. Aminoglycosides are category D drugs. They rapidly cross the placental barrier and are contraindicated during pregnancy because they can induce ototoxicity and nephrotoxicity in neonates.

Use during breastfeeding. Aminoglycosides should be avoided during breastfeeding. Although aminoglycosides are poorly absorbed when administered orally, changes in the intestinal flora of neonates can occur.

Use in patients with liver failure. Aminoglycosides can be administered at full doses. However, patients with severe liver failure should be screened for concomitant hepatorenal syndrome.

Use in patients with kidney failure. Aminoglycosides are almost exclusively eliminated by the kidney. Therefore, in patients with a creatinine clearance < 30 mL/min, the dose of the drug should be adjusted to 12-15 mg/kg/day, administered two to three times a week. The drug is removed by peritoneal dialysis and hemodialysis. In patients on peritoneal dialysis or hemodialysis, the dose should be adjusted and administered after the procedure.

Interactions. The ototoxicity and nephrotoxicity of aminoglycosides can be potentiated by concomitant administration of amphotericin B, vancomycin, cephalosporin, cisplatin, and loop diuretics (ethacrynic acid and furosemide).

Aminoglycosides can themselves potentiate the effects of neuromuscular blocking agents. Concomitant administration of aminoglycosides and neuromuscular blocking agents can cause respiratory depression due to respiratory muscle weakness. Patients with myasthenia gravis, botulism, hypocalcemia, severe hypokalemia, or hypomagnesemia are particularly susceptible to such adverse effects. The interaction between aminoglycosides and neuromuscular blocking agents is independent of the order of their administration. Patients using aminoglycosides should be monitored for the occurrence of respiratory depression in the perioperative and postoperative periods.

Capreomycin (Cm)

Capreomycin is a polypeptide antibiotic that is obtained from Streptomyces capreolus and has been used as an antituberculosis drug since 1959. The MIC of capreomycin for M. tuberculosis is 10 μg/mL. The chemical structure of capreomycin is different from that of aminoglycosides. However, capreomycin and aminoglycosides are quite similar in terms of their antibacterial activity and adverse effects. There is no cross-reaction between capreomycin and streptomycin; however, there might be a crossreaction between capreomycin and certain strains resistant to amikacin and kanamycin.

The mechanism of action of capreomycin has yet to be fully understood. It is believed that the drug is active because it interferes with bacterial protein synthesis.

Metabolization and excretion.

Capreomycin is not absorbed when taken orally. Capreomycin is administered i.m., and absorption can be delayed in cases in which the same site of application is used repeatedly. Tissue distribution has yet to be fully understood. The serum levels of capreomycin peak within 1-2 h after the administration of the drug. The plasma half-life of capreomycin is 4-6 h in patients with normal renal function, and it can be as long as 55 h in patients with kidney failure. Most of the dose (50-60%) is excreted through glomerular filtration 12 h after administration,

and a small proportion is excreted via the biliary tract. Capreomycin reaches the cerebrospinal fluid only in patients with meningitis.

Adverse effects.

In patients treated with capreomycin, common side effects include nephrotoxicity (in 20-25% of the patients), renal tubular damage, proteinuria, electrolyte disturbances, urticaria, and maculopapular rash. Ototoxicity (especially vestibular), electrolyte changes (decreased serum levels of calcium, magnesium, and potassium), pain, edema, and abscess at the site of application occasionally occur.

Use during pregnancy. Capreomycin is a category C drug. In adults, capreomycin is less ototoxic than are aminoglycosides. However, capreomycin should be avoided during pregnancy, since it is unknown whether this can be extrapolated to the health of the fetus. If it is essential to use any given injectable antituberculosis agent during pregnancy, capreomycin should be the drug of choice.

Use during breastfeeding. The concentrations of capreomycin in breast milk are unknown. Capreomycin should therefore be avoided during pregnancy.

Use in patients with liver failure. It is not necessary to adjust the doses of capreomycin in patients with liver failure.

Use in patients with kidney failure. Capreomycin should be used with extreme caution in patients with creatinine clearance < 30 mL/min and in those on hemodialysis. In these situations, the dose should be adjusted to 12-15 mg/kg, administered twice or three times a week. The drug is removed by dialysis and should be administered after the procedure.

Interactions. Capreomycin should not be administered concomitantly with neuromuscular blocking agents, aminoglycosides, or polymyxin B due to the possibility of additive toxic effects.

Cross-resistance between all the injectables. Here again, the evidence is scarce. Isolates resistant to low concentrations of Km were susceptible to Cm (this

was not observed with the isolates resistant to high concentrations of Km, which were often resistant to Cm as well), while isolates resistant to Cm were susceptible to Km. Subsequent articles from the same author presented multiple indications of the likelihood of unidirectional cross-resistance between the injectables and therefore of the importance of the choice of injectable. Analysis of more recent publications studying the MIC of each 2LI and genetic mutations determining their resistance has led us to conclude that:

1 Isolates acquiring resistance to S are usually susceptible to Km, Am and Cm. However, rare strains with apparently single-step mutations conferring resistance to both S and Km have been observed.

2 Isolates acquiring resistance to Cm can be susceptible to Km and Am. However, a diverse proportion (dependent on setting) may be resistant to Km and even to Am.

3 Isolates acquiring resistance to Am almost always acquire resistance to Km and Cm.

4 Isolates acquiring resistance to Km show different levels of crossr esistance to Am and Cm. Hence, while available evidence seems to demonstrate that Cm causes less cross-resistance than the others, this is not the case in all *M. tuberculosis* cultures, and results seem to vary according to setting. Moreover, the susceptibility test for all these 2LIs is not very reliable. Whenever one is used and shows resistance, possible cross-resistance to the other two must be suspected, afactor that must be kept in mind when designing treatment regimens.

The best sequence of use. To avoid cross-resistance that may interfere with the activity of other injectables in subsequent treatment regimens, the most reasonable sequence would be the following: S, Cm, Km and finally Am. However, S should never be used in the treatment of MDR-TB or XDR-TB, even if DST indicates a sensitive isolate (because DST is not reliable for these), because the rate of primary resistance is extraordinarily high and increases significantly in scenarios with resistance to H as in MDR-TB and XDR-TB patients. Moreover, there are other injectables available to ensure the effi cacy of this mainstay group

of drugs. The injectable of choice would hypothetically be Cm, except that it lacks large-scale availability at global level, has a short shelf life (24 months) and is more expensive than Km. In many countries, of necessity and practicality, Km is the first option in the field, as it is much more readily available and cheaper. In recent years, though, there have also been Km supply problems, meaning that many countries have no other option but to use Am. Indeed, Am is the most widely available injectable drug in all hospitals because of its excellent activity against other bacteria. Given the good efficacy and low/moderate toxicity of these 2LIs, the treatment of MDR-TB and XDR-TB should always include one of them, with the choice dependent on the history of previous use for each one and the likelihood of resistance, particularly in XDR-TB patients. In the case of MDR-TB, the 2LI must be counted as one of the four basic drugs, but not in the case of XDR-TB due to the risk of cross-resistance. Again, the question of which 2LI to use (and at what dose) needs further clinical study.

Fluoroquinolones (FQ)

Fluoroquinolones have been used as salvage drugs in the treatment of tuberculosis since 1985. However, recent studies involving third-generation and fourth-generation fluoroquinolones (levofloxacin, moxifloxacin, and gatifloxacin) have demonstrated the enormous potential of these drugs and, consequently, the great interest of the scientific community in using these drugs for the treatment of tuberculosis.

After their administration, fluoroquinolones are widely distributed to the body and have the remarkable property of reaching the interior of the cells, including macrophages, which explains the strong effect of these drugs on intracellular mycobacteria. There is no cross-resistance between fluoroquinolones and other antituberculosis drugs, and, although in vitro studies have reported cross-reactions among the different fluoroquinolones, levofloxacin and moxifloxacin have been used even in cases of previous M. Tuberculosis resistance to ofloxacin. Fluoroquinolones are bactericidal and show different degrees of effectiveness

against M. tuberculosis. The most effective fluoroquinolones are moxifloxacin and gatifloxacin, followed by levofloxacin, ofloxacin, and ciprofloxacin. In vitro, the MIC of ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin for M. tuberculosis are 0,5-4,0 μg/mL, 1,0-2,0 μg/mL, 1,0 μg/mL, 0,20-0,25 μg/mL, and 0,12‑0,50 μg/mL, respectively. Recent studies have shown that ciprofloxacin should not be included in the antituberculosis regimen. In regions where the prevalence of tuberculosis is high, fluoroquinolones have been administered as monotherapy for the empirical treatment of patients under clinical and radiological suspicion of having bacterial pneumonia; however, the respiratory symptoms can temporarily improve in patients with unsuspected or undiagnosed pulmonary tuberculosis, thus delaying the diagnosis of tuberculosis and selecting fluoroquinoloneresistant bacilli.

Fluoroquinolones inhibit M. Tuberculosis DNA gyrase activity or topoisomerase II activity, which regulates DNA topology and is essential to the survival of M. tuberculosis. The DNA molecule of M. tuberculosis is compacted by DNA gyrase and becomes biologically active. When fluoroquinolones inhibit this enzyme, the DNA molecule stops supercoiling in order to occupy a small cellular space for its expression, recombination, and replication. Free DNA ends induce uncontrolled mRNA synthesis, protein synthesis, exonuclease production, and chromosome degradation. These factors lead to cell death. In vitro, fluoroquinolones also inhibit topoisomerase IV; however, this does not contribute to the bactericidal effect on M. tuberculosis, since this enzyme is absent in the bacillus. Bacterial resistance occurs rapidly when a fluoroquinolone is used as monotherapy or when it is included in regimens that failed.

Metabolization and excretion

Fluoroquinolones are rapidly absorbed after oral administration, and their serum levels peak within 1-3 h. The bioavailability of fluoroquinolones ranges from 90 % for moxifloxacin to 99 % for levofloxacin. Only a small proportion is metabolized in the liver into d-ofloxacin, which has a limited bactericidal effect. Fluoroquinolones are excreted principally by the kidney through tubular secretion

or glomerular filtration, and 65-80 % of the drug is eliminated unaltered. A small proportion (4-8 %) is excreted in bile and feces. Moxifloxacin, however, is metabolized in the liver, and 60 % of the drug is excreted in bile, 45 % being excreted unaltered (25 % in urine and 20 % in feces). The half-life of fluoroquinolones ranges from 4 h for ciprofloxacin to 10-13 h for moxifloxacin.

The penetration of fluoroquinolones into the cerebrospinal fluid is poor. However, in cases of meningitis, the cerebrospinal fluid levels of ciprofloxacin and ofloxacin can be as high as 40-90 % of the plasma levels of these drugs.

Adverse effects

Gastrointestinal effects. The most common side effects of fluoroquinolones are gastrointestinal. Patients can present with nausea, vomiting, aerophagy, anorexia, abdominal discomfort, and diarrhea. Gastrointestinal effects occur in 3-17 % of the patients. Pseudomembranous colitis is rare.

Central nervous system effects. Dizziness, headache, insomnia, tremors, and mood disorders occur in 0,9-11 % of patients treated with fluoroquinolones. Hallucinations, delusions, and convulsions are rare. Greater attention should be paid to these effects in elderly patients and in those using theophylline or nonsteroidal anti-inflammatory drugs (NSAIDS).

Skin reactions and allergies. Erythema, pruritus, and skin rash occur in 0,4-2,2 % of patients treated with fluoroquinolones. Phototoxicity can occur when patients are exposed to ultraviolet light. Urticaria, angioedema, anaphylactic reactions, and vasculitis are uncommon.

Musculoskeletal effects. Arthropathy and cartilage erosion have been observed in young animals treated withfluoroquinolones (especially for prolonged periods or at high doses), and the use of fluoroquinolones in children is therefore restricted. However, the use of fluoroquinolones in special situations (e.g., in children with cystic fibrosis) has increased. Arthralgia has been observed in only 2 % of such cases and has always been reported to be reversible. Achilles tendinopathy and Achilles tendon rupture have been reported to occur. These are rare and bilateral, occurring in 50 % of the cases. These are often associated with

predisposing factors such as previous corticosteroid use, rheumatoid arthritis, kidney failure, and hemodialysis.

Cardiovascular effects. In patients treated with fluoroquinolones, prolongation of the electrocardiographic QT interval can occur, leading to ventricular tachycardia, including polymorphic ventricular tachycardia (torsades de pointes). This is a rare event. Electrocardiographic QT interval prolongation is dose-dependent. Patients with kidney failure, liver failure, cardiomyopathy, hypomagnesemia, or hypokalemia, as well as those using class IA antiarrhythmic drugs (procainamide and quinidine) or class III antiarrhythmic drugs (amiodarone and sotalol), together with those using terfenadine, erythromycin, cisapride, or tricyclic antidepressants, should receive special attention.

Urinary tract effects. Interstitial nephritis, characterized by the presence of eosinophils and crystals in urine, can occur in patients treated with fluoroquinolones. These are rare events. However, patients using fluoroquinolones who present with dehydration, diarrhea, and vomiting should receive appropriate fluid supplementation.

Endocrine effects. Changes in glycemia levels, including symptomatic hypoglycemia and, less commonly, hyperglycemia, have been reported to occur in patients with diabetes who use fluoroquinolones in conjunction with oral hypoglycemic agents or insulin.

Biochemical effects. Leukopenia and eosinophilia occur in less than 1 % of the cases, and increased transaminase levels occur in 1-3 % of the patients. Therapy is rarely discontinued due to these changes.

Use during pregnancy. Fluoroquinolones are category C drugs. In principle, fluoroquinolones should not be used during pregnancy. There is no evidence that the incidence of abnormalities is higher in children treated with fluoroquinolones. However, studies involving animals and ciprofloxacin have suggested that there are risks of damage to the articular cartilages of the fetus and, consequently, of juvenile arthritis and joint lesions. Fluoroquinolones should be used during pregnancy only when the benefits of the treatment outweigh the

potential risks. The decision to use a fluoroquinolone can be made only after clinicians who have considerable experience in managing tuberculosis have been consulted.

Use during breastfeeding. Ofloxacin is excreted in breast milk. There are no data regarding breastfeeding and the use of levofloxacin or moxifloxacin. Considering the potential adverse effects of the drugs on infants, the WHO suggests that fluoroquinolones be used only in cases in which they are vital to the health of the mother.

Use in patients with liver failure. Fluoroquinolones can be used without restrictions in patients with mild or moderate liver failure (Child-Pugh classes A and B). However, in cases of severe liver disease (Child-Pugh class C), as occurs with any other drug, patients should be closely monitored, through clinical evaluation and laboratory testing.

Use in patients with kidney failure. The dose of any given fluoroquinolone should be adjusted in patients with kidney failure. The WHO suggests that, in patients with kidney failure and creatinine clearance < 30 mL/min, the dose of ofloxacin be adjusted to 600-800 mg, administered three times a week, and the dose of levofloxacin be adjusted to 750-1,000 mg, administered three times a week. It is not necessary to adjust the dose of moxifloxacin. Fluoroquinolones are not removed by peritoneal dialysis or hemodialysis.

Interactions. Foods, with the exception of dairy products with a high concentration of calcium, do not interfere with the absorption of ofloxacin, levofloxacin, or moxifloxacin, as they do with the absorption of other fluoroquinolones. Patients using ciprofloxacin should be instructed to avoid excessive use of foods with high caffeine content, since ciprofloxacin inhibits the cytochrome P450 system, thereby reducing caffeine clearance.

Antacids containing calcium, aluminum, or magnesium interfere with the absorption and concentration of fluoroquinolones. Sucralfate inhibits the absorption of the drugs. Fluoroquinolones should not be administered until 2 h

after the use of antacids. The administration of H2 receptor blockers does not interfere with the absorption of fluoroquinolones.

Vitamin supplements containing zinc or iron interfere with the gastrointestinal absorption of fluoroquinolones. Fluoroquinolones can inhibit numerous cytochrome P450 subfamilies, which increases the plasma concentrations of drugs that are metabolized via the cytochrome P450 system. Fluoroquinolones increase the serum levels of theophylline, glibenclamide, and cyclosporine, as well as increasing the effect of oral anticoagulants. Third-generation and fourth-generation fluoroquinolones (levofloxacin and moxifloxacin) do not inhibit the cytochrome P450 enzyme system and therefore do not interact with the aforementioned drugs. However, when a fluoroquinolone is concomitantly administered with oral anticoagulants, the international normalized ratio should be closely monitored. Probenecid and cimetidine can increase the serum levels of fluoroquinolones. Concomitant administration of fluoroquinolones and NSAIDS can increase central nervous system stimulation and the possibility of convulsions.

Fluoroquinolones effectiveness.

Evidence is limited it this regard. It seems that Cfx is somewhat less effective and therefore should not be recommended. There is only one clinical study comparing Ofx and Lfx. Lfx has been clearly proved to be more effective in patients whose *M. tuberculosis* shows confi rmed susceptibility to Ofx, as well as in patients with *M. tuberculosis* resistant to this drug. The latter finding suggests that there is not complete cross-resistance among FQ. Despite the lack of clinical studies, the pharmacodynamic data showed that Cfx is the least effective and that the effectiveness of FQ has increased with the new-generation agents. Lfx was superior to Ofx, but Mfx and gatifl oxacin (Gfx) were even better than Lfx. The early studies used Lfx at doses of 500 mg/day. More recent studies using Lfx at 1000 mg/day demonstrated the best early bactericidal activity among the FQ (even a little better than Mfx and Gfx), but with the highest area under the concentration-time curve (from 0 to 24 h)/minimum inhibitory concentration (AUC24/MIC),

even a little higher than Mfx. Other studies have shown that Mfx and Gfx are better than Ofx, but without comparison against Lfx. To date, there have been no studies comparing high-dose Lfx with high-dose Mfx or Gfx. High doses of Gfx were used in the successful Bangladesh trial (short MDRTB regimen), where nearly 90 % of MDR-TB patients who had never before received SLDs had successful outcomes with a treatment regimen of just 9 months. With practically no evidence of relapses in this 9-month regimen, one may postulate that these high doses of Gfx have potent sterilising activity, probably very similar to R.

Cross-resistance between all the fluoroquinolones.

This was initially thought to be the case, as they all act upon the same *gyr*A gene. However, subsequent analyses of these gene mutations have demonstrated that approximately half of the isolates resistant to Ofx could remain susceptible to Mfx and to high-dose Lfx. These findings may account for the reported effi cacy of Lfx in patients with resistance to Ofx. Furthermore, an interesting recent meta-analysis by Jacobson et al. analysed the outcomes of XDR-TB patients treated in 13 different settings. Although the favourable outcome rate was low (43,7 %, while 20, 8% died), favourable outcomes totalled 59 % in settings where a new-generation FQ was systematically used for verifi ed XDR-TB cases. In settings where this new generation of FQ was not used, the favourable outcome rate was just 31 %. The most pertinent fact here is that studies in which a higher proportion of patients received a latergeneration FQ reported a higher proportion of favourable treatment outcomes, presenting new evidence that cross-resistance among the FQ is not absolute, especially with the new-generation drugs.

The best fluoroquinolone to recommend in the treatment of multidrug-resistant and extensively drug-resistant tuberculosis.

In the light of this discussion and the cost of Lfx, the ideal doses of FQ may be 750−1000 mg/day. The slightly better profi le for Lfx (at the higher dose) compared with Mfx and Gfx is not likely to be clinically signifi cant, and has not been studied clinically. Therefore, Mfx and Gfx can be good options as well. Given the low toxicity of these new FQ generations and the available evidence, it

is justifi able to always include one in the treatment of MDR-TB and XDR-TB. In the case of MDR-TB, the FQ is counted as one of the four basic drugs, but not in the case of XDR-TB due to the risk of cross-resistance to Ofx. Again, the question of which FQ to use (and at what dose) needs further clinical study, including an evaluation of possible long-term side effects.

Thioamides (Ethionamide (Et) and Prothionamide (Pt)

Ethionamide has been used as a secondline drug in the treatment of tuberculosis since 1956. It is an inactive prodrug, the structure of which is analogous to that of isoniazid. However, there is no cross-resistance to ethionamide and isoniazid. Ethionamide needs to be activated by the bacterial enzyme EthA (a monooxygenase containing flavin adenine dinucleotide, and it is NADPH-specific. Ethionamide acts on intracellular and extracellular bacilli. The MIC of ethionamide for M. tuberculosis is 0,6-2,5 μg/mL. At the usual doses, ethionamide is bacteriostatic.

Although ethionamide is similar to isoniazid, the former inhibits the activity of the inhA gene of M. tuberculosis. Although the mechanisms of action are different, the result is the same: the two drugs inhibit protein synthesis, preventing mycolic acid biosynthesis and affecting the bacterial cell membrane. Resistance to ethionamide is due to genetic alterations in EthA. M. tuberculosis strains that are resistant to isoniazid due to alterations in the katG gene (catalase/peroxidase enzyme) remain sensitive to ethionamide, which indicates that the enzymes that are responsible for the activation of isoniazid and ethionamide are different.

Metabolization and excretion.

Ethionamide is rapidly and completely absorbed when administered orally, and serum levels of the drug peak within approximately 1h after its administration. Approximately 30 % of the drug binds to plasma proteins, and the bioavailability of the drug is 80 %. Ethionamide is metabolized in the liver and excreted in urine, 1-5 % being excreted as active drug (unaltered) and the remainder being excreted

as metabolites. The half-life of ethionamide is 2 h. The CSF and plasma levels of ethionamide are similar.

Adverse effects.

*Gastrointestinal effects.*Ethionamide produces intense gastrointestinal effects, including a metallic taste in the mouth, excessive salivation, nausea, vomiting (commonly severe), loss of appetite, and abdominal pain. The symptoms improve if the drug is taken at mealtime or at bedtime. In some cases, it might be necessary to increase the doses progressively until the full dose is reached or to use antiemetics (or to do both).

Hepatotoxicity. Ethionamide and isoniazid have a similar structure. Therefore, the two drugs can cause similar side effects. Hepatotoxicity (toxic hepatitis) occurs in approximately 4,3% of the patients, especially in those with a history of liver disease or alcoholism. Liver changes can occur up to five months after the initiation of treatment with the drug, and it remains unclear whether these changes are due to direct toxicity or to hypersensitivity. Hepatotoxicity habitually resolves when the drug is discontinued.

Neurotoxicity. Peripheral neuritis, optic neuritis, diplopia, irritability, anxiety, depression, hallucinations, convulsions, and psychosis have been reported to occur in 1-2 % of the patients. In patients with a history of mental instability, ethionamide should be administered with caution. The neurological effects can be minimized by administering 50-100 mg/day of pyridoxine.

Cardiovascular effects. Postural hypotension can occur in patients treated with ethionamide.

Endocrine effects. Patients receiving ethionamide can develop gynecomastia, alopecia, hypothyroidism, impotence, or menorrhagia. Ethionamide makes glycemic control difficult in patients with diabetes mellitus.

Skin reactions. Acne, photosensitivity, and exanthema can occur in patients treated with ethionamide. Thrombocytopenia and purpura have been reported to occur sporadically.

Use during pregnancy. Ethionamide is a category C drug. It crosses the placental barrier. The use of ethionamide during pregnancy is controversial. In rodents, high doses of ethionamide have been associated with teratogenic effects. In humans, two studies reviewing 47 cases showed no deleterious effects; however, one study reported malformations in 7 of 23 neonates born to mothers who used the drug. Ethionamide should be used during pregnancy only when the benefits of the treatment outweigh the potential risks. The decision to use the drug can be made only after clinicians who have considerable experience in managing tuberculosis have been consulted.

Use during breastfeeding. There are no data regarding the concentration of ethionamide in breast milk. It is advisable to avoid the use of ethionamide during breastfeeding.

Use in patients with liver failure. Ethionamide should be used with caution and under continuous monitoring in patients with liver disease.

Use in patients with kidney failure. In patients with creatinine clearance lower than 30 mL/min or in those who are on hemodialysis, the dose of ethionamide should be reduced to 250-500 mg/day. The drug is not removed by hemodialysis.

Interactions. The effects of foods on the bioavailability of ethionamide are minimal. Antacids do not interfere with the absorption of ethionamide. Concomitant use of ethionamide and terizidone or isoniazid can potentiate the neurotoxic effects (hallucinations, irritability, tremors, depression, convulsions, psychosis, and peripheral neuropathy). Concomitant use of ethionamide and para-aminosalicylic acid can increase hepatotoxicity and the possibility of hypothyroidism. Concomitant use of ethionamide and alcohol can produce psychotic reactions.

Thioamides are by far the best of the Group 4 drugs, as documented by numerous studies showing their effi cacy and ability to cure, even when only associated with weak drugs such as Z and Cs. Due to their mechanism of action, they are somewhat like a slightly weaker H, but when all is said and done their

action is similar, meaning there obviously may be cross-resistance with H. Following the discovery of the pyridine-containing H, numerous pyridine derivatives were tested, and the activity of thio-isonicotinamide against *M. tuberculosis* was noted by several groups. Et was one of these thioamides. Thioamides are active against *M. tuberculosis* and, to a lesser extent, against other mycobacteria. Although the mechanism of action of thioamide drugs has not been fully elucidated, like H, they appear to inhibit mycolic acid biosynthesis. Pt is rapidly absorbed and excreted. Both thioamides show excellent penetration into cerebrospinal fluid. Resistance develops rapidly if used alone and cross-resistance is complete between Et and Pt. Thioamides are generally good drugs except for low gastric tolerance and, as mentioned before, the risk of cross-resistance to H. As such, they often become a basic anti-TB drug in MDR-TB and XDR-TB treatment regimens and are in fact included in the great majority of standardised MDR-TB regimens. Reliability of DST for Eth is very poor, so it is advisable to use it empirically and not rely on DST. Results for Et DST must be interpreted with caution, always considering previous use of the drug. Modern LPA (GenoType Plus) techniques are able to test for the *inh*A gene, a target of thioamides (and of H, as noted above), and if the mutation is present, a possible resistance to thioamides must be suspected.

Cycloserine (Cs)/Terizidone (Trz)

Cycloserine/terizidone, synthesized in 1952, is a structural analogue of D-alanine amino acid, a component that is important to the formation of the bacterial cell wall. Terizidone results from the combination of two cycloserine molecules. There is no cross-reaction between cycloserine/terizidone and other antituberculosis drugs. The MIC of cycloserine/terizidone for M. Tuberculosis is 5-20 mg/mL. At the usual doses, cycloserine/terizidone has a bacteriostatic effect.

Cycloserine/terizidone acts by competition, inhibiting the enzymes D-alanyl-D-alanine synthetase, alanine racemase, and alanine permease, which are indispensable for the synthesis of the peptidoglycan that confers rigidity and

stability to the M. Tuberculosis cell membrane. Although the mechanisms of M. tuberculosis resistance to cycloserine/terizidone have yet to be fully clarified, it is presumably due to genetic mutations in the aforementioned enzymes.

Metabolization and excretion.

Cycloserine/terizidone is rapidly absorbed after oral administration, and the bioavailability of the drug ranges from 70 % to 90 %. Plasma levels of the drug peak within 3-4 after ingestion. The half-life of cycloserine/terizidone is 10 h. The drug does not bind to plasma proteins. Only a small proportion of cycloserine/terizidone is metabolized in the liver. Most of the dose (70 %) is excreted by the kidney, unaltered, within 72 h. A small proportion of the drug is excreted in feces. The cerebrospinal fluid and serum concentrations of cycloserine/terizidone are similar.

Adverse effects.

Central nervous system effects. Cycloserine/terizidone has neurological adverse effects (headache, vertigo, dysarthria, somnolence, convulsion, mental confusion, and memory deficit) and psychiatric adverse effects (psychotic states with catatonic, paranoid, and depressive reactions, with a risk of suicide) that occur especially when the daily dose is higher than 500 mg or when cycloserine/terizidone is concomitantly administered with other neurotoxic drugs, such as isoniazid and ethionamide. Cases of peripheral neuropathy and changes in the pressure and quantity of proteins in the cerebrospinal fluid have been described. The administration of pyridoxine can aid in preventing the neurotoxic effects, the recommended dose being 100-200 mg/day.

Patients with a history of epilepsy, severe mental disturbances, suicidal tendencies, or alcoholism should be closely monitored when receiving cycloserine/terizidone. In cases of convulsive seizures caused by cycloserine/terizidone, the drug should be temporarily discontinued, anticonvulsant therapy should be initiated, and cycloserine/terizidone should be reinitiated at lower doses. In cases of psychotic symptoms, cycloserine/terizidone should be discontinued for one to four weeks, and antipsychotic drugs should be given until

the symptoms have been controlled. If necessary, the dose of cycloserine/terizidone can be reduced, provided that this does not interfere with the therapeutic regimen. Occasionally, it is necessary to use antipsychotic drugs concomitantly with the therapeutic regimen for the duration of the treatment. Family members and health care workers should be on the alert to detect, in a timely manner, personality changes or signs and symptoms of depression.

Skin effects. Although rare, skin rash and Stevens-Johnson syndrome can occur in patients treated with cycloserine/terizidone.

Use during pregnancy. Cycloserine/terizidone is a category C drug. It has no teratogenic effects in laboratory animals. Although it is known that cycloserine/terizidone crosses the placental barrier, there are no data as to whether the drug is safe for the fetus. Cycloserine/terizidone should be used only when there is no therapeutic alternative.

Use during breastfeeding. The American Academy of Pediatrics considers cycloserine/terizidone to be compatible with breastfeeding. Infants exposed to cycloserine/terizidone must receive supplemental doses of pyridoxine.

Use in patients with liver failure. In patients with liver failure, cycloserine/terizidone can be used without precautions, except in patients with hepatitis due to alcoholism or in those at a high risk for convulsions.

Use in patients with kidney failure. The dose of cycloserine/terizidone should be adjusted in patients with kidney failure. In patients with creatinine clearance < 30 mL/min, the recommended dose is 250 mg/day or 500 mg three times a week. These doses are not well-established. Patients should be closely monitored for signs of neurotoxicity. Hemodialysis removes 56 % of the drug, and patients on hemodialysis should receive cycloserine/terizidone after the procedure, at a dose of 500 mg administered three times a week.

Interactions. Foods increase the time required for cycloserine/terizidone to be absorbed by 3,5 times, and there can be a 35 % reduction in the maximum concentration of the drug. Orange juice (and probably other acidic beverages)

reduces the maximum concentration of the drug by 15 %. Whenever possible, the drug should be ingested with water, well before or after meals.

Antacids do not significantly interfere with the absorption and concentration of cycloserine/terizidone.

There is evidence that combining cycloserine/terizidone with ethionamide and isoniazid can potentiate the neurotoxic effects. Cycloserine/terizidone can increase the serum levels of phenytoin and oral anticoagulants, as well as decreasing those of pyridoxine. In patients using anticonvulsants and neuroleptics, the dose of cycloserine/terizidone should be adjusted.

However, due to the potential effect that cycloserine/terizidone has on the central nervous system, patients should be closely monitored for side effects of this drug combination. Concomitant use of cycloserine/terizidone and fluoroquinolones can worsen the effects on the central nervous system. Concomitant use of cycloserine/terizidone and alcohol increases the risk of convulsions.

There are similarities between Cs and Et. It is surprising that Cs is such a weak drug, because its mechanism of action involves the mycobacterial cell wall and it is derived from a streptomycete. Cs is only bacteriostatic and competitively blocks the enzyme that incorporates alanine into an alanyla lanine dipeptide, an essential component of the mycobacterial cell wall. Cs is active against *M. tuberculosis* and several species of gram-positive bacteria. Among the advantages of Cs are its high gastric tolerance (compared with the other two drugs in this group) and lack of cross-resistance to other agents. The two main drawbacks of Cs are adverse psychiatric reactions (psychotic reactions with suicidal tendencies), which necessitate a psychiatric interview prior to treatment initiation, and a short shelf life (24 months). Terizidone is a combination of two molecules of Cs, potentially causing fewer a dverse events, although reports concerning this drug are scarce and not always relevant. Cs has become a basic drug in MDR-TB and XDR-TB treatment regimens in spite of its lower activity and adverse effects. The reality is that Cs is used extensively worldwide only because there are no better drugs to include in MDR-TB regimens and because at least four drugs are needed

to ensure the highest probability of therapeutic success. Its only contribution may be that it protects the core pharmaceuticals in these treatment plans (FQs and 2LIs) from resistance selection. Replacing Cs with Cf as the fourth drug seems to have been a good choice in the Bangladesh (short MDR-TB) regimen.

p-Aminosalicylate (PAS)

Para-aminosalicylic acid has been used as an antituberculosis drug since 1946. Beginning in 1955 and for nearly 15 years, paraaminosalicylic acid was considered a first-line drug in a combination regimen with isoniazid and streptomycin. Para-aminosalicylic acid is used as an acid or as a sodium salt. Para-aminosalicylic acid is bacteriostatic, and the MIC of the drug for M. tuberculosis is 1 μg/mL. Para-aminosalicylic acid acts preferentially on extracellular bacilli. The drug can currently be administered in granules stored in 4-mg envelopes, replacing the former 500-mg capsules.

The mechanism of action of paraaminosalicylic acid has yet to be elucidated, and it is believed that the mechanism is related to interference with bacterial folic acid synthesis and inhibition of iron uptake.

Metabolization and excretion.

Para-aminosalicylic acid is administered orally. Para-aminosalicylic acid granules are better tolerated than are para-aminosalicylic acid capsules. The ingestion of 4 g of paraaminosalicylic acid granules leads to a maximum serum concentration of 20-60 μg/mL after 4-6 h. The serum levels of para-aminosalicylic acid peak within 90-120 min after the ingestion of para-aminosalicylic acid capsules. The halflife of para-aminosalicylic acid is 1 h, and the plasma concentrations of the drug after 4-5 h are minimal, which justifies the need for doses of 10-12 g in order to maintain the bacteriostatic activity. Para-aminosalicylic acid is metabolized in the intestines and liver, via acetylation, into N-acetyl-para-aminosalicylic acid. More than 80 % of the drug is excreted by the kidney through glomerular filtration and tubular secretion.

In the presence of meningitis, the cerebrospinal fluid concentration of para-aminosalicylic acid is 10-50 % of the plasma concentration of the drug.

Adverse effects.

In patients treated with para-aminosalicylic acid, gastrointestinal effects (anorexia, diarrhea, nausea, and vomiting) and hypothyroidism, the latter occurring especially when paraaminosalicylic acid is administered concomitantly with ethionamide, are common. Thyroid function returns to normal when the drug is discontinued. Hepatitis (in 0,3-0,5 % of the cases), allergic reactions (fever, rash, and pruritus), hemolytic anemia, agranulocytosis, leukopenia, thrombocytopenia, malabsorption syndrome, and increased thyroid volume are rare, as are cardiovascular adverse effects (pericarditis), neurological adverse effects (encephalopathy), respiratory adverse effects (eosinophilic pneumonia), and ocular adverse effects (optic neuritis). Para-aminosalicylic acid should be used with caution in patients with glucose-6-phosphate dehydrogenase deficiency and in those who are allergic to aspirin.

Use during pregnancy. Para-aminosalicylic acid is a category C drug. There have been reports of congenital anomalies associated with the administration of the drug in the first trimester of pregnancy. Therefore, the drug should be used in pregnant women only when there is no therapeutic alternative.

Use during breastfeeding. Para-aminosalicylic acid is secreted in breast milk (at 1,4 % of the maternal plasma concentration of the drug). Para-aminosalicylic acid can be used during breastfeeding.

Use in patients with liver failure. Para-aminosalicylic acid should be used with caution in patients with liver failure. Hepatic enzyme levels should be monitored.

Use in patients with kidney failure. It is not necessary to adjust the doses of para-aminosalicylic acid in patients with kidney failure. However, the drug can exacerbate acidosis and crystalluria in patients with severe kidney failure. Sodium para-aminosalicylate can also increase blood volume in this situation.

Interactions. Foods increase the absorption of paraaminosalicylic acid. The drug can be administered with water, orange juice, or fatty foods. Antacids do not interfere with the absorption of para-aminosalicylic acid. Digoxin can reduce the

absorption of paraaminosalicylic acid. Ethionamide can increase hepatotoxicity and hypothyroidism in patients treated with para-aminosalicylic acid. Isoniazid increases acetylation, which results in an increase in the serum levels of para-aminosalicylic acid. Concomitant use of angiotensin-converting enzyme inhibitors and para-aminosalicylic acid can reduce the antihypertensive effect of the latter, and the use of calcium channel blockers can increase the anticoagulant effect of para-aminosalicylic acid. Concomitant use of para-aminosalicylic acid and carbonic anhydrase inhibitors potentiate the adverse effects of both drugs, and concomitant use of paraaminosalicylic acid and systemic corticosteroids can also increase the number and severity of adverse effects, especially gastrointestinal effects. Para-aminosalicylic acid can reduce the effect of loop diuretics, and, conversely, loop diuretics can increase the serum levels of para-aminosalicylic acid. With the exception of diclofenac, nonselective NSAIDS can increase the adverse effects of para-aminosalicylic acid. Para-aminosalicylic acid can increase the hypoglycemic effects of sulfonylurea, as well as increasing the risk of bleeding when administered in conjunction with oral anticoagulants, thrombolytics, or salicylates.

There are very few arguments for using PAS. It is quite weak, has scant activity (just bacteriostatic), is very poorly tolerated (particularly gastric adverse effects) and is very expensive. It is therefore relegated to the last level for drug selection for DR-TB treatment plans. Analogous to the observation that benzoic acid inhibits respiration of tubercle bacilli, PAS might be built into coenzyme F of the bacterium instead of para-aminobenzoic acid and thereby inhibit growth. The fi rst PAS compound used in various studies was the acid salt. The use of p-aminosalicylate sodium (PAS sodium), requiring doses 30 % higher than the PAS acid, became progressively widespread in the 1950s and 1960s. From the 1970s until nearly 2000, PAS sodium was used in most countries, despite its well known gastric intolerance. However, over the last 10 years, thanks to global demand for MDR-TB and XDR-TB treatment, PAS was re-introduced, particularly in the form of enteric-coated PAS granules, and is now gradually replacing PAS sodium.

Nonetheless, many countries still use the sodium formulation, because experience around the world has demonstrated its effi cacy. Signifi cant current demand for this agent has led to the use of both formulations of PAS. The main advantage of entericcoated PAS acid seems to be better gastric tolerance and lower dose requirement, although it needs to be kept refrigerated, therefore requiring cold chain transport that is not always available in developing countries. In contrast, the major advantage of PAS sodium is its simple storage requirements with no need for a cold chain. In any case, PAS displays very low effectiveness and poor tolerance with high costs, which relegate it to the last place in Group 4.

Most effective drugs in Group 5 and recommended sequence of use

This is a very heterogeneous group that includes drugs for which experience is very limited in human TB treatment and which display very low efficacy or a high toxicity profile. As a consequence, the drugs in Group 5 are considered minor or adjuvant drugs and each should be counted only as 0,5 within the total of four core drugs for treatment of MDR-TB and XDR-TB. When it is necessary to resort to this group, at least two compounds should thus be chosen. The mechanism of action of the great majority of these drugs has not been clearly defi ned. Based on effectiveness, potential adverse reactions and cost, the sequence of introduction of drugs in this group should be as follows.

Clofazimine (Cfz)

Although experience with Cfz in TB treatment is limited, it may turn out to be a much better drug than believed to date, with potential intracellular and extracellular activity. Adequate dose management facilitates control of adverse reactions, in particular photosensitivity and gastric intolerance. Low cost is another advantage, but current availability in the market is not assured as this drug has been almost exclusively restricted to treatment for leprosy. Some countries where Cfz is available include it in standardised regimens because of its benefi ts and low cost. This is the case for the shortened MDR-TB Bangladesh regimen, in which Cfz is used for just 9 months with a success rate approaching 90 % and included in

the plan throughout treatment. In addition, one of the most promising lines of new drug studies and new plans associates Cfz with other drugs like Mfx and/or Z. It appears that regimens with Cfz clearly work better than those not using this drug, so it is possible that it acts as a facilitator for other drugs. Given these qualities, an initiative should be undertaken to facilitate global availability of Cfz.

Adverse effects of Cfz are generally dose-related and mainly affect the skin, eye and gastrointestinal tract. Pink to brownish-black discolouration of skin, cornea, retina and urine occur in a high proportion of patients within a few weeks of treatment. Other skin problems include i chthyosis, dryness, rash and pruritus. Gastrointestinal disturbances include abdominal pain, nausea, vomiting and diarrhoea.

Amoxicillin/clavulanate (Amv/Clv)

Beta-lactams antibiotics have not been regarded as useful drugs for TB treatment because *M. tuberculosis* is naturally resistant to most of them in vitro. Resistance is thought to be mediated by a class A β-lactamase which hydrolyses penicillins and cephalosporins. Resistance may be overcome by 1) inhibition of the β-lactamase or 2) use of an antibiotic that is not a substrate for it. An example of the former strategy is the use of a combination of a β-lactam and a β-lactamase inhibitor like Amx/Clv, which is active in vitro and has early bactericidal activity in patients with pulmonary TB. Anecdotally, Amx/Clv combined with other SLD has been successfully used in selected patients infected with MDR strains. This approach has been met with considerable scepticism and the role, if any, of Amx/Clv remains unclear. In any case, the lack of effective drugs for the treatment of MDR-TB and XDRTB, the good tolerance and the low toxicity profi le of this drug have made Amx/Clv a drug of choice from Group 5.

Linezolid (Lzd)

Linezolid is the first compound belonging to the oxazolidinone class approved for clinical use. Due to its ability to penetrate macrophages, linezolid is active against intracellular bacilli, exerting its activity by binding to the ribosomal

50S subunit and thus inhibiting an early step in protein synthesis. Linezolid is most commonly used to treat drugresistant TB, but its use has been limited by toxicity concerns, particularly hematological disturbances such as leukopenia and thrombocytopenia, as well as peripheral neuropathy, which may be irreversible. While resistance to linezolid in *M. tuberculosis* clinical isolates is rarely reported, *in vitro*-selected mutants with high-level resistance to linezolid (MIC = 16–32 mg/L) have been found to contain mutations at G2061T and G2576T in the 23S rRNA gene (Hillemann, Rusch-Gerdes et al. 2008). On the other hand, mutants with lower level linezolid resistance (MIC = 4–8 mg/L) lack mutations in the 23S rRNA gene, implicating other possible mechanisms of resistance.

More than 10 years ago, studies on a mouse model demonstrated the effectiveness of Lzd and other oxazolidinones against *M. tuberculosis* despite its possible low bactericidal activity. This activity had been confi rmed in a number of reports concerning patients with MDR-TB and XDR-TB, though most of them included a limited number of cases. Lzd and the other oxazolidinones researched to date (see below) are new orally administered antibiotics that act by interfering with early protein synthesis. They have a very broad spectrum of activity on aerobic and anaerobic gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermitis* and *enterococcus.* Lzd would be a drug of choice in the management of MDR-TB and XDR-TB (not only from Group 5, to which it is currently assigned) were it not for the fact that it displays a high toxicity profi le in the long term (25 %−45 % rate of severe anaemia and/or thrombocytopenia and peripheral and optic neuropathy) and is expensive. Costs and toxicity would clearly decrease, without decreasing effi cacy, by reducing the initial dose (600 mg/12 h) by 50 % (600 mg/day). There are some studies using 300 mg/day. Although the ideal dosage to use in TB treatment has not been clearly defi ned, there is near unanimous agreement on recommending 600 mg/day. Some recent publications also demonstrate that the rate of adverse side effects is not so important or serious if they are addressed early on and aggressively, and that cost problems are very much linked to manufacture and distribution, because in

countries like India the drug may be even cheaper than Km or Cm. Two recent meta-analyses found a therapy success rate near 70% in complicated DR-TB cases in which Lzd was systematically included in the treatment regimen. In any case, countries that can afford the drug and control the adverse reactions end up using Lzd (600 mg/day) as a basic drug against XDR-TB and probably in many cases against MDR-TB.

Diarrhoea and nausea are common in patients treated with Lzd. Administering Lzd for a prolonged period of time is associated with haematological and neurological toxicities. Haematological adverse effects are mostly reversible and include myelosuppression manifesting as anaemia, leucopenia, pancytopenia or thrombocytopenia. Peripheral and optic neuropathies have been reported. The adverse drug effects of Lzd are generally dose-related.

Carbapenems

Following the rationale invoked for Amx/Clv, the carbapenems offer a second approach to overcoming the β-lactam resistance of *M. tuberculosis.* They are poor substrates for both class A and class C β-lactamases, and two carbapenems, meropenem and imipenem, are active in vitro against *M. tuberculosis.* Effectiveness has been demonstrated in some reports on MDR-TB and XDR-TB patients treated with imipenem and meropenem combined with clavulanic acid. Though experience with the drug is very limited to date and involves very isolated XDR-TB patients who have an even more extensive pattern of resistance, outcomes appear to be rather successful. Still, limited experience, unknown long-term toxicity and high costs make carbapenems a group to be used only in extreme situations.

Macrolides

The macrolides are broad-spectrum antibiotics, which exert their antibacterial effect by binding to the bacterial 50S ribosomal subunit and inhibiting RNA-dependent protein synthesis. However, these drugs have limited activity against wild-type *M. tuberculosis*. The possible role of macrolides in TB treatment

is an area of active investigation, as recent studies have reported synergy of macrolides in combination with other antibiotics.

The effectiveness of clarithromycin against *M. tuberculosis* is very weak and no role has been demonstrated for it in the treatment of TB. Based on the isolated reports that have been published with a restricted number of patients receiving multiple other drugs, only a minor anti-TB role can be assigned to clarithromycin. The sole advantage of this agent is relatively good tolerance and a low toxicity profi le. It is of doubtful and low effectiveness against *M. tuberculosis.* Clarithromycin is only used when no other drug is left, and then with more scepticism than hope.

Thiacetazone (Thz)

This is one of the oldest and most widely used drugs in the treatment of TB, even though its action has always been considered very weak and is only bacteriostatic. Due to its high toxicity, particularly in patients infected with human immunodefi ciency virus (HIV), entailing an even higher mortality rate, it has been almost eliminated from the anti-TB therapeutic arsenal. Moreover, Th exhibits partial cross-resistance to Eth. The use of this agent should be restricted to cases with an extensive profi le of drug resistance, with close follow-up for adverse reactions and exclusion of patients coinfected with HIV because of documented incidents of Stevens Johnson syndrome and toxic epidermal necrolysis. Given all of its weaknesses, this agent is hardly ever used in practice.

7. NEW DRUGS FOR DRUG-RESISTANT TUBERCULOSIS TREATMENT

In recent years, considerable research has been conducted in the hunt for new medicines/derivatives of existing compounds and new forms of therapy that will improve TB treatment and accelerate disease control. Four principal avenues are under study:

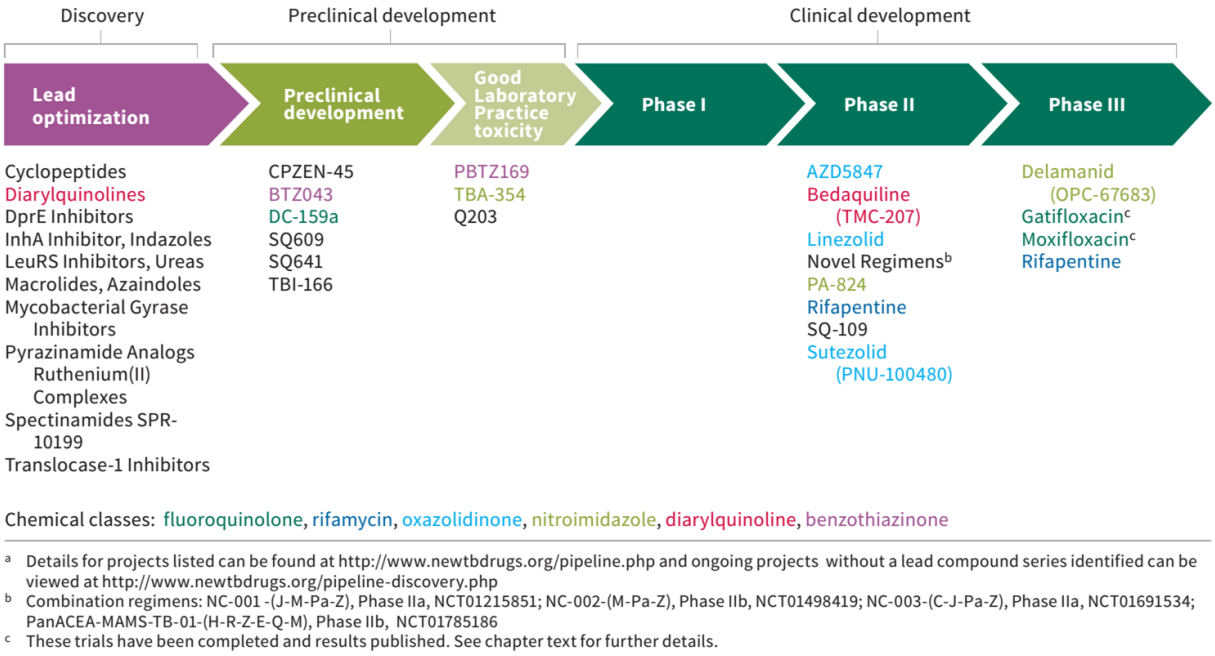
1) new anti-TB drugs,

2) new uses of existing antimicrobials,

3) immunomodulators, and

4) new routes of drug administration.

Unfortunately, as with most pharmacotherapeutic development, the discovery process of a new anti-TB drug takes 10 to 15 years. Some 10 000 substances must customarily be analysed, at a cost of many millions of dollars, to fi nd a single promising compound for clinical use. To be accepted as a new medication, it must go through complex stages of validation in experiments on animals and humans, which usually entails 10−15 years of research.



[a] Details for projects listed can be found at http://www.newtbdrugs.org/pipeline.php and ongoing projects without a lead compound series identified can be viewed at http://www.newtbdrugs.org/pipeline-discovery.php

[b] Combination regimens: NC-001 -(J-M-Pa-Z), Phase IIa, NCT01215851; NC-002-(M-Pa-Z), Phase IIb, NCT01498419; NC-003-(C-J-Pa-Z), Phase IIa, NCT01691534; PanACEA-MAMS-TB-01-(H-R-Z-E-Q-M), Phase IIb, NCT01785186

[c] These trials have been completed and results published. See chapter text for further details.

New drugs for drug-resistant tuberculosis treatment

Despite these obstacles, several dozen new chemical compounds are currently in varying stages of development. We analyse below those for which full development appears to be the most promising. Of all the drugs under study for DR-TB treatment, only TMC207 (Bedaquiline) and OPC-67683 (Delamanid) are in Phase III trial. We may therefore be able to turn to these two compounds in the near future. For many of the compounds below, it is too early to discern the potential role they might play in the initial treatment of drug-sensitive TB.

Diarylquinolines (TMC207 [Bedaquiline])

TMC207 (also named R207910 or "J compound") is a first-in-class anti-TB diarylquinoline with bactericidal and sterilizing activities against drug-susceptible and drug-resistant *M.tuberculosis in vitro* and in animal models, including in a murine model of latent TB infection. Mouse model studies suggest a synergistic relationship between TMC207 and PZA. TMC207 inhibits ATP synthase, a critical enzyme in the synthesis of ATP for *M. tuberculosis*. The addition of TMC207 to standard therapy for MDR-TB significantly reduced the time to conversion to a negative sputum culture and increased the proportion of patients with conversion of sputum culture as compared with placebo. Resistance to TMC207 is mediated by mutations in the *atpE* gene encoding the transmembrane and oligomeric C subunit of ATP synthase, typically at positions 63 or 66. However, more recent studies have shown that a majority of *in vitro*-generated mutants resistant to TMC207 lacked mutations in *atpE*, indicating alternative mechanisms of drug resistance.

There are currently high expectations here due to publications showing that a derivative of the diarylquinolines, R-207910 (TMC207), acting on both sensitive and resistant bacilli and in active growth and latent phases, could cut TB treatment time in half. It is more bactericidal than H (its initial early bactericidal activity is less than H and R but equals it at 14 days), and when combined with R or Z, it enhances the sterilising power of these drugs. In rats, the combination of TMC207, rifapentine and Z administered once a week was much more effective than the

standard regimen of H+R+Z five times a week. It appears to be synergistic with Z. The appeal of TMC207 is that it is the first anti-TB drug in the last 40 years with a totally new mechanism of action: it acts by inhibiting the *M. tuberculosis* ATP synthase. This drug is currently in Phase III trials for the treatment of MDR-TB patients and has generated high expectations. There are already publications on its use in MDR/XDR-TB patients showing very promising results. In a Phase IIb study in MDR-TB, the addition of TMC207 to a treatment regimen with SLDs versus placebo plus SLDs administered over 8 weeks showed sterilised sputum in 48 % of the patients versus 9 % for the placebo group. After 2 years of treatment, 81 % of patients who received TMC207 + the standard regimen were cured 57 % of those who received only the standard regimen. However it may have unfavourable interactions with R, although it appears to lose no bactericidal activity.

Nitroimidazopyrans (PA-824 and OPC-67683 [Delamanid])

Reduced oxygen tension may be an important microenvironmental condition encountered by persistent bacilli within necrotic lung granulomas in the human host. Interestingly, although *in vitro* exposure to microaerophilic conditions renders *M.tuberculosis* less susceptible to killing by H and rifampin, the bacilli become susceptible to metronidazole a nitroimidazole drug used to treat anaerobic infections. Metronidazole, which becomes reductively activated by the pyruvate: ferredoxin oxidereductase system under anoxic conditions lacks antituberculous activity in mouse models and in guinea pigs, but displays activity in *M. tuberculosis*-infected rabbits. Clinical studies evaluating the activity of metronidazole against MDR-TB are ongoing.

A series of nitroimidazopyrans originally investigated as radiosensitisers for use in cancer chemotherapy were shown to have in vitro and in vivo activity against *M. tuberculosis.* Newer derivatives showed substantial activity against *M. tuberculosis* and lacked mutagenicity shown previously with bicyclic nitroimidazoles. There is considerable in vivo activity (in mouse studies) against

M. tuberculosis, comparable to that of H. Their action involves inhibition of fatty acid and mycolic acid synthesis. Similar to the nitroimidazoles (to which metronidazole belongs), these drugs show substantial in vitro bactericidal activity against bacilli held in a hypoxic stationary phase.

PA-824

PA-824, a small molecule nitroimidazopyran related to metronidazole, exhibits bactericidal activity against actively multiplying and stationary-phase cultures of *M. tuberculosis*, as well as in murine and guinea pig models of TB infection. In addition, PA-824 is highly active against multidrug-resistant clinical isolates of *M. tuberculosis* (MIC < 1 μ g/mL), suggesting no cross-resistance with current antituberculosis drugs. Like metronidazole, PA-824 is a pro-drug requiring reductive activation of an aromatic nitro group, which involves an F420-dependent glucose-6-phosphate dehydrogenase encoded by Rv0407 (*fgd1*) and deazaflavin-dependent nitroreductase encoded by Rv3547, in order to exert its antitubercular effect. The activity of PA-824 is at least partially mediated through inhibition of the oxidation of hydroxymycolates to ketomycolates, a terminal step in mycolic acid synthesis. Recently, formation of the des-nitroimidazole metabolite of PA-824 was shown to generate reactive nitrogen species, including nitric oxide, which appears to contribute to the killing activity of PA-824 and may explain the activity of the drug against non-replicating bacilli. Similar to H, resistance to PA-824 is most commonly mediated by mutations that lead to loss of pro-drug activation, including those in the genes Rv0407 and Rv3547 encoding the activating enzymes.

A series of nitroimidazoles, related to metronidazole, have shown to be bactericidal against *M. tuberculosis* both in vitro and in vivo. Experimental studies with a nitroimidazopyran called PA-824, which proved to be the most active metronidazole, showed action similar to H, with a spectrum of action very specifi c to TB. Like H, PA-824 acts on the biosynthesis of cell wall lipids but in different metabolic states, and also inhibits protein synthesis. Also like H, PA-824 acts on the bacilli in the exponential multiplication phase, although in an anaerobic culture model it also appears to act on latent bacilli. It has shown effectiveness against

strains of *M. tuberculosis* that are resistant to the usual drugs. More study is needed, but it may become a good alternative first-line drug.

OPC-67683[Delamanid]

OPC-67683 is a nitro-dihydro-imidazooxazole derivative with potent activity against drugsusceptible *M. tuberculosis* and MDR-TB. The drug exerts its killing activity by inhibiting the synthesis of methoxy- and keto-mycolic acids. E in the standard regimen alongside rifampin and Z led to more rapid sterilization of *M.tuberculosis*-infected mouse lungs. Like the other nitroimidazoles, OPC-67683 is a pro-drug requiring reductive activation by *M. tuberculosis*. As in the case of PA-824, mutations in the Rv3547 gene have been identified in strains resistant to OPC-67683, indicating defective drug activation.

The substitution of OPC-67683 for H and Otsuka Pharmaceutical is testing a new compound from the nitroimidazoles series, a dihydroimidaze-oxazol (OPC-67683 (Delamanid)), on patients that appear to have great anti-mycobacterial activity. It is already in Phase III testing and showing very promising results.

Derivatives of the oxazolidinones (linezolid, PNU-100480 and AZD5847)

Oxazolidinones are a new chemical class of synthetic antibiotics related to cycloserine with broad-spectrum activity against gram-positive pathogens through inhibition of protein synthesis.

A role for Lzd in TB treatment has been extensively analysed, as this is the only drug marketed from this group of new orally administered antibiotics. Recent studies show that PNU-100480 is more potent than Lzd and signifi cantly improves the bactericidal activity of several anti-TB combinations, including Mfx, suggesting that it may be a new candidate to shorten TB treatment. Tests still in Phase I-II suggest that AZD5847 may be even more potent.

PNU-100480

PNU-100480, another oxazolidinone, has been shown to have more potent activity against *M. tuberculosis* than linezolid, as the MIC of PNU100480 is half

that of linezolid, and is as bactericidal as isoniazid in an acute model of TB infection in mice. Recent studies in the mouse model have shown that the addition of PNU-100480 to the standard first-line regimen of rifampin, H, and Z can shorten the duration of treatment necessary to prevent relapse, suggesting that this drug may have sterilizing activity against drug-susceptible and drug-resistant *M. tuberculosis*. Recent Phase I studies have shown that PNU-100480 is safe and well tolerated at all tested doses, and exhibits synergy with PZA in an ex vivo whole-blood culture assay. Resistance mechanisms are expected to be similar to those of linezolid.

AZD5847

AZD5847 was originally designed for treatment of gram-positive infections, but was later repositioned for TB treatment with the goal of improving the toxicity profile associated with linezolid, including inhibition of mitochondrial protein synthesis, thrombocytopenia, and myelosuppression. Like linezolid, AZD5847 has bactericidal activity against *M. tuberculosis* in macrophages, as well as in murine models of acute and chronic TB infection. Recent Phase I trials revealed that oral administration of the drug up to 800 mg twice daily for 14 days was satisfactorily tolerated in healthy volunteers. Although bioavailability decreases with increasing dose, this effect may be largely compensated if taken within 2 hours of meals, and the exposures achieved in man correspond to efficacious exposures in the mouse model of TB infection. Phase 2 studies to be conducted in South Africa are in the planning stage.

Ethylendiamines (SQ109)

SQ109 was identified by screening a large synthesized combinatorial library based on the 1,2-ethylenediamine structure of E, and was found to have limited toxicity and potent activity against intracellular bacilli as well as in a murine model of chronic TB infection. Early clinical data reveal the drug's potential to enhance the treatment of TB during the first 2 months of intensive therapy and also to treat MDR-TB. Whether upregulation of *ahpC* expression, observed in strains resistant

to H, E, and SQ109, plays a role in resistance to SQ109 or merely reflects a compensatory metabolic mechanism remains to be determined.

SQ109, the most potent compound from among 2,796 similar preparations, demonstrates anti-TB activity. Although it is a diamine that began study as an analogue to E, its chemical structure and mechanism of action are not the same, and it in fact appears to have no cross-resistance with E. SQ109 acts by inhibiting synthesis of the mycobacterial cell wall and enhances the action of H and R, shortening the treatment time in a mouse model of TB. It appears to be synergistic in the murine TB model with H, R and TMC207.

Pyrroles (LL3858)

Various pyrroles have shown notable action against specifi c sensitive and resistant strains of *M. tuberculosis.* Their mechanism of action is unknown. The new LL3858 compound sterilises the lung and spleen of infected rats in a shorter time than conventional pharmacotherapy. It also appears to act intermittently, so a LL3858+rifapentine+Z regimen administered once a week has the same efficacy as H+R+Z administered fi ve times a week in rats. It is currently in Phase II trials for evaluation in human TB.

Phenothiazines

The antipsychotic phenothiazine drug thioridazine has been reported to be active against drug-susceptible and drug-resistant *M. tuberculosis*, both in macrophages as well as in murine models. Although serum concentrations above the MIC for *M. tuberculosis* (8-16 mg/L range) cannot be safely attained in humans, thioridazine still has potential as an antimycobacterial drug because of intracellular accumulation, such that concentrations inside macrophages are at least 10-fold higher than in serum. Despite the favorable toxicity profile of thioridazine relative to chlorpromazine and other phenothiazines, cardiac arrhythmia associated with prolongation of the QT interval remains a risk. Thioridazine has been used successfully to cure patients with XDR-TB in Argentina and as salvage therapy in

similar patients in India. The mechanism of action of thioridazine is likely multifactorial, as the drug appears to act on enzymes involved in fatty acid metabolism and membrane proteins, particularly efflux pumps, in addition to inhibiting type II NADH:menaquinone oxidoreductase as a phenothiazine. Mechanisms of *M. Tuberculosis* resistance to the phenothiazines remain to be elucidated.

Benzothiazinones

The 1,3-benzothiazin-4-ones (BTZs) represent a new class of drugs, which have activity against *M. tuberculosis in vitro, ex vivo*, and in murine TB models. BTZs are activated in *M. tuberculosis* by reduction of an essential nitro group to a nitroso derivative, which then specifically reacts with a cysteine residue in the active site of the enzyme decaprenylphosphoryl-D-ribose 2'-epimerase. Inhibition of this enzymatic activity abolishes the formation of decaprenylphosphoryl arabinose, a key precursor that is required for the synthesis of the cell-wall arabinans, thus causing bacterial lysis and death.

Although spontaneous BTZ-resistant laboratory mutants were found to have a Ser or Gly substitution at codon Cys387 of *dprE1*, resistance to BTZs has not been reported in clinical *M. tuberculosis* isolates. Recently, a novel resistance mechanism to BTZ was described in *M. smegmatis* involving the overexpression of the nitroreductase, which leads to the inactivation of the drug by reduction of a critical nitro-group to an amino-group. However, *M. Tuberculosis* seems to lack nitroreductases able to inactivate BTZs.

New drugs from already known families

These include new rifamycins (rifabutin, rifapentine and rifalazil) and the new FQ (Lfx, Mfx and Gfx), the role of which in the treatment of DR-TB has already been largely reviewed and discussed above. Conversely, other macrolides like clarithromycin have shown a very reduced effect in DR-TB treatment. While clarithromycin and other macrolides, such as roxithromycin and azithromycin, have demonstrated anti-mycobacterial activity with good MIC in vitro, in reality,

this does not happen in all cases and there is insuffi cient clinical experience to recommend its use. On the other hand, both clarithromycin and azithromycin are very active against mycobacteria other than tuberculosis, and are thus the basis for treatment of many of these mycobacterioses.

New drug combinations

There is a growing conviction that, in addition to the properties of each individual drug, an evaluation must be made of their most effective combinations, because therapeutic success depends more on the treatment regimen than the activity of each component taken separately. Combined treatments were initially proposed to prevent the development of bacterial resistance. Today, we are also seeking to augment effi cacy through the benefi ts of varying drug combinations. For example, many combinations under study show that Cfz may have great value when combined with other drugs, possibly facilitating their mechanism of action. Of the various associations currently known to be under study, a combination of Cfz with Z and Mfx looks like it may have excellent bactericidal and sterilising activity.

8. THE ANTI-TUBERCULOSIS DRUGS RESISTANCE MECHANISMS

Drug-resistant TB is not a recent phenomenon. *M. Tuberculosis* strains that were resistant to streptomycin appeared soon after the introduction of the drug for treatment of TB in 1944. Genetic resistance to an anti-tuberculosis drug is due to spontaneous chromosomal mutations at a frequency of 10^{-6} to 10^{-8} mycobacterial replications. Mobile genetic elements such as plasmids and transposons, which are known to mediate drug resistance in various bacterial species, do not do so in *M. tuberculosis.* Because such mutations resulting in drug resistance are unlinked, the probability of developing bacillary resistance to three drugs used simultaneously becomes 10^{-18} to 10^{-20}. In theory, the chance of drug resistance is thus virtually non-existent when three effective drugs are used in combination for TB treatment.

Amplification of the afore-mentioned genetic mutation through human error results in clinically drug-resistant TB. These include «monotherapy» due to irregular drug supply, inappropriate doctor prescription and, most importantly, poor patient adherence to treatment. Subsequent transmission of resistant *M. Tuberculosis* strains from the index patient to others aggravates the problem. The MDR/XDR phenotype is caused by sequential accumulation of mutations in different genes involved in individual drug resistance.

Although the defi nitions of «acquired» and «primary» drug resistance are conceptually relatively clear, in reality they are often subject to misclassification when previous treatment cannot be readily ascertained. The term «initial» drug resistance is thus often preferred to ‘primary’ drug resistance to include «unknown» or «undisclosed» acquired drug resistance. The matter is currently further simplifi ed by categorizing drug resistance in new cases and previously treated cases of TB. The latter refers to cases with treatment lasting for at least 1 month.

To date, there has been no single chromosomal mutation found to cause r esistance to two or more anti-TB drugs. Polydrug-resistant TB (including MDR-TB) is caused by sequential mutations in different genes. Susceptible TB bacilli

develop resistance fi rst to one drug (acquired resistance) and subsequently to another drug (amplifi cation of resistance). This evolution involves multiple cycles of «fall» (susceptible strains) and «rise» (resistant strains) in tubercle bacilli. The first cycle includes a decline in susceptible bacilli and predominant multiplication of a strain resistant to one drug, and results in monodrug resistance. The second cycle occurs in the background of monodrug resistance and results in acquisition of resistance to another drug (amplifi cation of resistance), while the third cycle in the background of resistance to two drugs leads to acquisition of resistance to the third drug.

Therefore, resistance to multiple drugs takes time to develop and is the cumulative result of human errors. It is worth noting that Colijin and colleagues recently reported that the rate of spontaneous occurrence of MDR-TB may be much higher than previously expected. Because *M. Tuberculosis* bacilli in an immunocompetent host are killed by immune response, a bacillary population observed in vivo likely has experienced more replication events than the same size of bacillary population in vitro without death. They estimated that the probability of the emergence of resistance to both H and R before anti-TB therapy ranges from 10^{-5} to 10^{-4}.

The size of the bacillary population is the largest and the probability of chromosomal mutations the highest in the subset of bacilli that multiply the fastest. In the current standard regimen of H, R, Z and E, H has the highest early bactericidal activity of the drugs and kills the majority of the subpopulation of rapidly replicating bacilli. Selection pressure imposed by H on a population of susceptible *M. tuberculosis* usually exceeds other fi rst-line anti-TB drugs. When patients are administered a regimen of H, R, Z and E, the fi rst drug to which *M. tuberculosis* becomes resistant is usually H. H has the highest ability to prevent resistance to companion drugs, followed by R. It is essential to pay attention to H-resistant TB because it is the precursor of MDR-TB. In the background of H resistance, resistance to R may emerge, resulting in MDR-TB. Certain conditions may promote the emergence of R resistance prior to H resistance, resulting in R

mono-resistance; examples include monotherapy with R, infection with HIV, use of rifapentine and inadequate dosage or poor quality of H.

8.1. Common mechanisms associated with the emergence of drug resistance in individuals

An inappropriate drug regimen, use of a lower-than-recommended dosage, inferior drug quality and poor adherence to treatment are commonly associated with the emergence of drug resistance in individual patients. Inappropriate regimens include exposure to (functional) monotherapy, continued administration of a failing regimen and inadequate modifi cation of a failing regimen. Examples of monotherapy include the use of an FQ in the treatment of TB patients who are misdiagnosed with pneumonia and the administration of H preventive therapy in individuals with undiagnosed TB.

Examples of functional monotherapy include the use of H and R in the treatment of patients with H-resistant TB. Continued administration of a failing regimen for a prolonged period may result in the emergence of resistance to one drug followed by amplifi cation of resistance to another drug. For example, in a patient who has poor response to a regimen consisting of H, R and E, H resistance may emerge; if the patient continues H, R and E after the emergence of H resistance without proper modifi cation of the regimen, resistance to R may develop. Inadequate modifi cation of a failing regimen, such as adding a single drug to a failing regimen, may result in amplification of resistance to the newly added drug. Use of a lower-thanrecommended dosage may result in inadequate serum concentration of drugs, and use of poor quality drugs may have the same effect as using a lower-than-recommended dosage. Poor adherence to treatment includes 1) selective intake of drugs of a treatment regimen and 2) irregular intake of a treatment regimen. Selective intake of one drug or another may result in functional monotherapy. With irregular intake of a treatment regimen, even if non-selective (such as in a fi xed-dose combination formulation), drug r esistance may still emerge.

Mitchison proposed four theoretical mechanisms that may result in selective multiplication of drug-resistant mutants due to irregular intake of anti-TB drugs:

1) differences in bactericidal activity during initial killing,

2) monotherapy resulting in sterilisation of specifi c populations,

3) sub-inhibitory drug concentrations during regrowth,

4) differences in post-antibiotic effects during regrowth.

These mechanisms may change the ratio of the population size of susceptible and resistant bacilli in each cycle of i rregular intake of drugs.

Risk factors for MDR-TB

Resistance always begins as a man-made problem, as it is the result of inadequate treatment somewhere along the chain of transmission: prescription error, shortages of specifi c anti-TB drugs at the health centre level or incomplete and/or irregular intake of the drugs by the patient. In new patients, resistance occurs when a patient develops TB after being infected by another patient with resistant microorganisms. In previously treated patients, resistance may have developed during the previous course of treatment, for example, treatment with a single drug in patients with smear-positive pulmonary TB (sometimes referred to as monotherapy), or administration of powerful drugs to a patient harbouring TB microorganisms that are resistant to all but one of the drugs administered. For some patients, initial resistance is present from the start, but as systematic drug susceptibility testing (DST) is neither recommended nor possible in the majority of settings, the initial susceptibility of the patient strain is usually unknown: resistance is discovered when a patient fails treatment or returns for retreatment. This classification is interesting as it easily identifi es high-risk groups. Because the regimen for MDR-TB patients must be different from those for non-MDR-TB patients, it is important that they be identifi ed as soon as possible and offered the treatment most likely to rapidly sterilise their sputum (to avoid dissemination) and ensure defi nitive cure. Identifi cation of high-risk populations for drug-resistant

tuberculosis, especially MDR-TB, is a key issue for guiding investigations in resource-constrained environments.

Patients who have been ***treated previously for TB*** according to the criteria outlined above can be divided into four subcategories:

1 *Initial treatment failure* refers to a patient who, while on treatment for the first time with an R-containing regimen (Category 1), is smearpositive at 5 months or later during the course of treatment.

2 *Retreatment failure* refers to a patient who, while on the retreatment regimen with fi rst-line drugs (FLDs, Category 2), is still smear-positive at the end of the retreatment regimen.

3 *A relapse case* is one where a previously treated patient who was declared cured or completed treatment becomes sputum smear-positive again.

4 *Treatment after default* refers to a case where a patient who had been on treatment for 1 month or longer returns to the health service sputum smear-positive after having interrupted treatment for 2 or more months.

The highest risk group for MDR is *retreatment failures*, with MDR rates frequently exceeding 85 %. When the regularity of patient drug intake is monitored during the retreatment regimen, MDR treatment can sometimes be initiated before DST results are available. The second highest risk group is usually *initial treatment failures.* However, rates vary widely from one country to the next, ranging from 0% in Malawi to 22 % in Benin, and as high as 88% in Peru. These variations are related to many factors: quality of directly observed treatment, short-course (DOTS), initial MDR-TB rates, initial H resistance rates, whether the regimen is intermittent or daily, extent of the disease, etc. Typically, when the initial MDR-TB rate is higher, there is a higher rate of MDR-TB after failure. Nevertheless, there are frequently circumstances in the fi eld that result in operational failure (smear-positive at 5 months or later) in fully susceptible patients.

The prevalence of MDR among *relapse* and *treatment after default* cases also varies greatly according to setting and rates are usually fairly similar, but not always: 32 % for the two categories of patients from aggregate data from 10

countries, with respective rates of 13 % and 19 % in Taiwan and 4 % and 12 % in Benin. Because the risk in each category of patients varies widely from one setting to another, it is important to have a good surveillance system capable of measuring the level of risk in each subpopulation.

Except in Russia, the former Soviet Union republics and some parts of China, the MDR rate among ***new cases*** is low, usually less than 2 %−3 %. However, the household contacts of MDR-TB cases deserve particular attention. Active TB cases among contacts are not so common, but these individuals are at high risk of having MDR bacilli themselves, with risk rates often exceeding 80%. For a new TB patient, failure is declared after 5 months of treatment. In some countries, however, doctors are more comfortable looking for resistance before the fi fth month. The problem is the cost-effectiveness of such strategies, because among patients who are still smear-positive at 2−3 months, much more sputum must be analysed than at month 5 to identify one MDR case.

Other risk factors

Human immunodeficiency virus

Limited information is available about the link between human immunodeficiency virus (HIV) and drug-r esistant TB at the population level. In the United States and Europe, an association between HIV and MDR-TB has been reported in several studies, often related to nosocomial transmission. In sub-Saharan Africa, where HIV prevalence is very high, this association has not been documented. On the other hand, dramatic nosocomial microepidemics can sometimes occur in health institutions, and rigorous infection control is key to preventing such epidemics. Resistance to R alone (and not to H) is very uncommon; nevertheless, it seems that in high HIV settings, mono-resistance to R occurs more frequently.

Intermittent regimens

While there is no clear proof, intermittent treatment (twice or thrice weekly) is suspected in the development of both R resistance and MDR bacilli, probably related to irregular intake of drugs in regimens where each dose is important. This

is likely to occur when an intermittent regimen is prescribed for the duration of treatment, even in the intensive phase.

Country of origin

In low-TB prevalence countries, country of birth is regularly collected as an indicator for TB surveillance. In countries with low TB prevalence, TB rates are much higher for non-native individuals than natives. The same holds true for MDR-TB in low-TB prevalence countries in that rates are much higher among foreign-born patients than the native-born, as seen, for example, in Western Europe and the United States.

Others

Some publications report an increased risk of MDR-TB in other circumstances, such as in patients treated in the private sector, patients from countries with a history of drug stock-outs or poor-quality drugs, patients with other co-morbidities facilitating malabsorption, etc. If resources are available, culture and DST against FLDs should be performed.

Risk factors for extensively drug-resistant tuberculosis

Very few studies have been published on the risk factors for extensively drug-resistant TB (XDR-TB) because the number of cases is so limited to date. Insuffi cient case management of MDR-TB clearly plays a major role in XDR-TB development, and the cumulative duration of previous treatment with second-line drugs (SLDs) is identifi ed as the main risk factor. XDR-TB occurs more frequently in settings where SLDs are widely available, especially fl uoroquinolones and second-line injectables. Mortality rates are quite high in HIV-infected patients with concomitant nosocomial TB infection.

8.2. Genetic markers of resistance to anti-tuberculosis drugs

Isoniazid

Isoniazid was introduced in 1952 as an anti-TB agent and it remains, together with rifampicin, as the basis for the treatment of the disease. Unlike

rifampicin, isoniazid is only active against metabolically-active replicating bacilli. Also known as isonicotinic acid hydrazide, isoniazid is a pro-drug that requires activation by the catalase/peroxidase enzyme KatG, encoded by the *katG* gene, to exert its effect. Isoniazid acts by inhibiting the synthesis of mycolic acids through the NADH-dependent enoyl-acyl carrier protein (ACP)-reductase, encoded by *inhA.* Although simple in its structure, resistance to this drug has been associated with mutations in several genes, such as *katG*, *inhA, ahpC, kasA* and NDH.

The two main molecular mechanisms of isoniazid resistance are associated with gene mutations in *katG* and *inhA* or its promoter region. Indeed, numerous studies have found mutations in these two genes as the most commonly associated with isoniazid resistance. Among these, the most prevalent gene mutation has been identified as S315T in *katG* resulting in an isoniazid product deficient in forming the isoniazid-NAD adduct needed to exert its antimicrobial activity. This mutation has been consistently associated with high-level resistance (MIC > 1 μg/mL) to isoniazid and occurs more frequently in MDR strains. The second most common mutation occurs in the promoter region of *inhA* causing an overexpression of InhA or less frequently, a mutation in its active site, which decreases its affinity for the isoniazid-NAD adduct. The most prevalent mutation found is at position −15C/T and is more commonly associated with low level resistance to isoniazid (MIC < 1 μg/mL). Mutations in *inhA* not only cause resistance to isoniazid but also to the structurally related drug ethionamide, which shares the same target. A recent study found that a mutation in the *inhA* regulatory region together with a mutation in the *inhA* coding region produced high-level isoniazid resistance and also cross-resistance to ethionamide.

One recent interesting finding showed that the 4R isomer of the isoniazid-NADP adduct causes inhibition of the dihydrofolate reductase (DfrA) in *M. tuberculosis,* suggesting that mutations in *dfrA* could possibly play a role in resistance to isoniazid. Moreover, an analysis of the proteome of isoniazid targets in *M. tuberculosis* identified 16 other proteins, in addition to InhA and DfrA, that were bound by these adducts with high affinity, which could signal other not yet

clearly defined actions of isoniazid on the bacteria [34]. Two recent studies, however, have failed to identify any mutation in *dfrA* associated with resistance to isoniazid.

In *M. tuberculosis*, *ahpC* encodes an alkyl hydroperoxidase reductase that is implicated in resistance to reactive oxygen intermediates and it was initially proposed that mutations in the promoter of *ahpC* could be used as proxy markers for isoniazid resistance. It is now better understood that mutations in the promoter of *ahpC* are compensatory mutations for the loss of catalase/peroxidase activity rather than the cause for isoniazid resistance. Moreover, overexpression of AhpC does not confer resistance to isoniazid.

Several studies have found single nucleotide polymorphisms in other genes in isoniazid resistant clinical isolates of *M. tuberculosis*, including *kasA* and the *oxyR-ahpC* and *furA-katG* intergenic regions. However, their direct role as a cause of isoniazid resistance has not been fully demonstrated. On the other hand, co-resistance to isoniazid and ethionamide has been clearly demonstrated to be caused by mutations in ndh in M. smegmatis and M. bovis BCG, by altering the NADH/NAD ratios inside the cell, leading to a competitive inhibition of the INH-NAD adduct. A recent study has also found that a silent mutation in mabA conferred isoniazid resistance through upregulation of *inhA* in *M. tuberculosis*.

Rifampicin

Rifampicin is a rifamycin derivative introduced in 1972 as an antituberculosis agent. It is one of the most effective anti-TB antibiotics and together with isoniazid constitutes the basis of the multidrug treatment regimen for TB. Rifampicin is active against growing and non-growing (slow metabolizing) bacilli. The mode of action of rifampicin in *M. tuberculosis* is by binding to the β-subunit of the RNA polymerase, inhibiting the elongation of messenger RNA. The majority of rifampicin-resistant clinical isolates of *M. tuberculosis* harbor mutations in the *rpoB* gene that codes for the β-subunit of the RNA polymerase.

As a result of this, conformational changes occur that decrease the affinity for the drug and results in the development of resistance.

In about 96 % of *M. tuberculosis* isolates resistant to rifampicin, there are mutations in the so-called ―hot-spot region of 81-bp spanning codons 507–533 of the *rpoB* gene. This region is also known as the rifampicin resistance-determining region. Mutations in codons 516, 526 and 531 are the most commonly associated mutations with rifampicin resistance in the majority of studies. Although less frequent, some reports have also noted the occurrence of mutations outside of the hot-spot region of *rpoB*. Cross-resistance with other rifamycins can occur. Mutations in some codons (e.g., 518 or 529) have been associated with low-level resistance to rifampicin but still susceptible to other rifamycins, such as rifabutin or rifalazil. This is important for TB patients that need to receive antiretroviral therapy since rifabutin is a less effective inducer of the cytochrome P450 CYP3A oxidative enzyme. On the other hand, monoresistance to rifampicin is quite rare and almost all rifampicin-resistant strains are also resistant to other drugs, especially to isoniazid. This is the reason why rifampicin resistance is considered as a surrogate marker for MDR-TB.

Recent genome sequencing studies have uncovered the acquisition of compensatory mutations in *rpoA* and *rpoC*, encoding the α and β' subunits of RNA polymerase, in rifampicin-resistant strains with mutations in *rpoB*. These compensatory mutations would be responsible for restoring the fitness of these strains *in vivo* and have also been associated with a higher transmissibility in some settings.

Ethambutol

Ethambutol was first introduced in the treatment of TB in 1966 and is part of the current first-line regimen to treat the disease. Ethambutol is bacteriostatic against multiplying bacilli interfering with the biosynthesis of arabinogalactan in the cell wall. In *M. tuberculosis*, the genes *embCAB*, organized as an operon, code

for arabinosyl transferase, which is involved in the synthesis of arabinogalactan, producing the accumulation of the intermediate D-arabinofuranosyl-P-decaprenol.

The recognized mechanism of resistance to ethambutol has been linked to mutations in the gene *embB* with mutations at position *embB*306 as the most prevalent in most of the studies performed. Some studies, however, have also found mutations in *embB*306 in ethambutol susceptible isolates. Moreover, a study with a large number of *M. tuberculosis* isolates found that mutations in *embB*306 were not necessarily associated with resistance to ethambutol but with a predisposition to develop resistance to increasing number of drugs and to be transmitted. In fact, allelic exchange studies have shown that individual mutations causing certain amino acid substitutions produced ethambutol resistance, while other amino acid substitutions had little or no effect on ethambutol resistance. The same authors have more recently reported that mutations in the decaprenylphosphoryl-B-D-arabinose (DPA) biosynthetic and utilization pathway genes, Rv3806c and Rv3792, together with mutations in *embB* and *embC* accumulate, giving rise to a range of MICs of ethambutol depending on mutation type and number. These findings could have influence on the correct detection of ethambutol resistance by current molecular methods. Mutations in *embB*306 then, cause variable degrees of ethambutol resistance and are required but are not enough to cause high-level resistance to ethambutol. There remain about 30% ethambutol resistant strains that do not present any mutation in embB stressing the need to identify other possible mechanisms of drug resistance to this drug.

Pyrazinamide

Pyrazinamide was introduced into TB treatment in the early 1950s and constitutes now part of the standard first-line regimen to treat the disease. Pyrazinamide is an analog of nicotinamide and its introduction allowed reducing the length of treatment to six months. It has the characteristic of inhibiting semi-dormant bacilli residing in acidic environments such as found in the TB lesions. Pyrazinamide is also a pro-drug that needs to be converted to its active form,

pyrazinoic acid, by the enzyme pyrazinamidase/nicotinamidase coded by the *pncA* gene. The proposed mechanism of action of pyrazinamide involves conversion of pyrazinamide to pyrazinoic acid, which disrupts the bacterial membrane energetics inhibiting membrane transport. Pyrazinamide would enter the bacterial cell by passive diffusion and after conversion to pyrazinoic acid it is excreted by a weak efflux pump. Under acid conditions, the protonated pyrazinoic acid would be reabsorbed into the cell and accumulated inside, due to an inefficient efflux pump, resulting in cellular damage. One study has also found that pyrazinoic acid and its n-propyl ester can inhibit the fatty acid synthase type I in replicating *M. tuberculosis* bacilli.

A recent study, however, has challenged the previous model by proposing that pyrazinoic acid inhibits trans-translation, a process of ribosome-sparing in *M. tuberculosis*. The study was performed in pyrazinamide-resistant strains lacking mutations in pncA but that had mutations in *rpsA* identifying the ribosomal protein 1 (RpsA) as the proposed target. Overexpression of RpsA conferred increased resistance to pyrazinamide and pyrazinoic acid was confirmed to be bound to RpsA. While a very intriguing hypothesis as a target for pyrazinamide, the failure to perform allelic transfers in this study makes it difficult to conclude that in fact mutations in rpsA are the target of pyrazinamide.

Mutations in the gene pncA remain as the most common finding in pyrazinamide resistant strains. These mutations, however, are scattered throughout the gene but most occur in a 561-bp region in the open reading frame or in an 82-bp region of its putative promoter. Some few studies have reported the occurrence of pyrazinamide resistant strains without any mutation in pncA stating that the resistance could be due to mutations in another not yet identified regulatory gene. Based on the current evidence, the contribution of mutations in rpsA to pyrazinamide resistance remains limited.

Streptomycin

Originally isolated from the soil microorganism Streptomyces griseus, streptomycin was the first antibiotic to be successfully used against TB.

Unfortunately, as soon as it was prescribed, resistance to it emerged, a result of being administered as monotherapy. Streptomycin is an aminocyclitol glycoside active against actively growing bacilli and its mode of action is by inhibiting the initiation of the translation in the protein synthesis. More specifically, streptomycin acts at the level of the 30S subunit of the ribosome at the ribosomal protein S12 and the 16S rRNA coded by the genes *rpsL* and *rrs*, respectively.

Consequently, mutations in *rpsL* and rrs are the major mechanisms of resistance to streptomycin but account for 60%–70% of the resistance found. Among the mutations reported in *rpsL*, a substitution in codon 43 from lysine to arginine has been the most commonly reported. This mutation produces high-level resistance to streptomycin. In *rrs* the most common mutations occur around nucleotides 530 and 915. There remain an important percentage of strains resistant to streptomycin that lack mutations in either of these two genes, suggesting additional mechanisms of resistance.

In the last years, it has also been reported that mutations in *gidB*, a gene encoding a conserved 7-methylguanosine methyltransferase specific for the 16S rRNA, confers low-level resistance to streptomycin.

Fluoroquinolones

Fluoroquinolones are currently in use as second-line drugs in the treatment of MDR-TB. Both ciprofloxacin and ofloxacin are synthetic derivatives of the parent compound nalidixic acid, discovered as a by-product of the antimalarial chloroquine. Newer-generation quinolones such as moxifloxacin and gatifloxacin are being evaluated in clinical trials and proposed as first-line antibiotics with the purpose of shortening the length of treatment in TB.

The mode of action of fluoroquinolones is by inhibiting the topoisomerase II (DNA gyrase) and topoisomerase IV, two critical enzymes for bacterial viability. These proteins are encoded by the genes *gyrA*, *gyrB*, *parC* and *parE*, respectively. In *M. tuberculosis*, only type II topoisomerase (DNA gyrase) is present and, thus, is the only target of fluoroquinolone activity. Type II topoisomerase is a tetramer

formed by two α and β subunits, coded by *gyrA* and *gyrB*, respectively, which catalyzes the supercoiling of DNA. The main mechanism of development of fluoroquinolone resistance in *M. tuberculosis* is by chromosomal mutations in the quinolone resistance-determining region of *gyrA* or *gyrB*. The most frequent mutations found are at position 90 and 94 of *gyrA* but mutations at position 74, 88 and 91 have also been reported. A recent systematic review of fluoroquinolone-resistance-associated gyrase mutations in *M. tuberculosis* has been published.

One interesting finding in *M. tuberculosis* is the presence of a natural polymorphism at position 95 in *gyrA* that is not related to fluoroquinolone resistance since it is also found in fluoroquinolone-susceptible strains. Another interesting finding has been the report that the simultaneous occurrence of mutations T80A and A90G in *gyrA* led to hypersusceptibility to several quinolones. This finding could point out that the problem of fluoroquinolone resistance in *M. tuberculosis* might be more complex than was thought initially.

Cross-resistance is assumed to occur between fluoroquinolones although isolated reports have acknowledged the presence of strains resistant to gatifloxacin and moxifloxacin that were still susceptible to ofloxacin. Also, the involvement of efflux mechanisms has been suggested as a possible cause for fluoroquinolone resistance in *M. tuberculosis*.

Kanamycin, Capreomycin, Amikacin, Viomycin

These four antibiotics have the same mechanism of action by inhibiting the protein synthesis but, while kanamycin and amikacin are aminoglycosides, capreomycin and viomycin are cyclic peptide antibiotics. All four are second-line drugs used in the management of MDR-TB.

Kanamycin and amikacin inhibit protein synthesis by alteration at the level of 16S rRNA. The most common mutations found in kanamycin-resistant strains are at position 1400 and 1401 of the *rrs* gene, conferring high-level resistance to kanamycin and amikacin. However, mutations at position 1483 have also been reported . Full cross-resistance between kanamycin and amikacin is not complete,

as previously thought. Some studies have shown variable levels and patterns of resistance suggesting that other mechanisms of resistance might be possible. In concordance with this, a low-level resistance to kanamycin has been associated with mutations in the promoter region of the *eis* gene, encoding an aminoglycoside acetyltransferase. Mutations at position −10 and −35 of the *eis* promoter led to an overexpression of the protein and low-level resistance to kanamycin but not to amikacin. These mutations were found in up to 80% of clinical isolates showing low-level resistance to kanamycin.

Capreomycin and viomycin, on the other hand, have a similar structure and bind at the same site in the ribosome, at the interface of the small and large subunits. They show full cross-resistance as reported in previous studies. Mutations in the *tlyA* gene have also been associated with resistance to capreomycin and viomycin. TlyA is an rRNA methyltransferase specific for 2'-O-methylation of ribose in rRNA. Mutations in t*lyA* determine the absence of methylation activity. Although some studies did not find this association, a recent meta-analysis, evaluating the association of genetic mutations and resistance to second-line drugs, has confirmed the presence of *tlyA* mutations in addition to mutations in *rrs* and *eis*.

Ethionamide

Et (2-ethylisonicotinamide) is a derivative of isonicotinic acid, and is bactericidal only against *M. tuberculosis*,*M. avium-intracellulare* and *M. leprae*. Like INH, Et is also a prodrug that is activated by EtaA/EthA (a monooxygenase) and inhibits the same target as INH, the InhA of the mycolic acid synthesis pathway. Prothionamide (Pt, 2-ethyl-4-pyridinecarbothioamide) shares structure and activity almost identical to that of Et. Ethionamide is structurally similar to isoniazid. It is also a pro-drug requiring activation by a monooxygenase encoded by the ethA gene. It interferes with the mycolic acid synthesis by forming an adduct with NAD that inhibits the enoyl-ACP reductase enzyme. EthA is regulated by the transcriptional repressor EthR. Resistance to ethionamide occurs by

mutations in *etaA*/*ethA*, *ethR* and also mutations in *inhA*, which cause resistance to both isoniazid and ethionamide. Moreover, studies with spontaneous isoniazid- and ethionamide-resistant mutants of *M. tuberculosis* found that they map to mshA, encoding an enzyme essential for mycothiol biosynthesis.

Para-aminosalicylic acid

Although it was one of the first anti-tuberculosis drugs used in the treatment of the disease, together with isoniazid and streptomycin, para-amino salicylic acid or PAS is now considered as a second-line drug part of the treatment regimen for MDR-TB. Until recently, its mechanism of action was not completely defined. It has been proposed that being an analog of para-amino benzoic acid, it must compete with it for dihydropteroate synthase, interfering in the process of folate synthesis. A study using transposon mutagenesis identified mutations in the thyA gene associated with resistance to PAS that were also present in clinical isolates resistant to PAS. A recent study has also identified various missense mutations in folC encoding dihydrofolate synthase that conferred resistance to PAS in laboratory isolates of *M. tuberculosis*. In a panel of 85 clinical MDR-TB isolates, mutations in *folC* were identified in five isolates resistant to PAS. Nevertheless, just less than 40% of PAS-resistant strains had mutations in thyA indicating that still other mechanisms of resistance to the drug might exist.

Cycloserine

Cycloserine is an oral bacteriostatic second-line anti-tuberculosis drug used in MDR-TB treatment regimens. It is an analog of D-alanine that by blocking the activity of D-alanine: D-alanine ligase inhibits the synthesis of peptidoglycan. It can also inhibit D-alanine racemase needed for the conversion of L-alanine to D-alanine. Although the actual target of cycloserine in *M. tuberculosis* is not completely elucidated, in previous studies in *M. smegmatis* it was shown that overexpression of *alrA* led to resistance to cylcoserine in recombinant mutants. More recently, it has also been shown that a point mutation in cycA, which

encodes a D-alanine transporter, was partially responsible for resistance to cycloserine in *M. bovis* BCG.

Thioacetazone

Thioacetazone is an old drug that was used in the treatment of TB due to its favourable *in vitro* activity against *M. tuberculosis* and its very low cost. It has toxicity problems, however, especially in patients co-infected with HIV. It belongs to the group 5 drugs of the WHO and acts by inhibiting mycolic acid synthesis.

Clofazimine

Clofazimine is a riminophenazine compound reported long ago as having anti-TB activity. Due to the availability of other effective anti-TB drugs at the time and some undesirable side-effects, such as pigmentation of the skin, its use was more limited to the treatment of leprosy. It is now considered in the group 5 drugs of the WHO for the management of MDR-TB. Until recently, the exact mode of action of this antibiotic was not completely understood. Recent studies, however, have signalled the outer membrane as the possible target of clofazimine. Another study found that in *M. tuberculosis* clofazimine is reduced by NADH dehydrogenase and subsequently after spontaneous reoxidation liberates bactericidal levels of reactive oxygen species.

Resistance to clofazimine has not yet been fully characterized; however, a recent study has found that in spontaneous mutants of the reference strain H37Rv, mutations in the transcriptional regulator Rv0678 caused an upregulation of MmpL5, a multisubstrate efflux pump, which not only caused resistance to clofazimine but also to bedaquiline.

Macrolides

Macrolides are more frequently recommended for the treatment of other mycobacterial infections due to their limited activity against *M. tuberculosis*. Among them, clarithromycin is considered as part of the group 5 drugs of the

WHO. Intrinsic resistance to macrolides has been attributed to low cell wall permeability and the expression of *emr37*, a gene that codifies for a methylase at a specific site in the 23S rRNA, blocking the binding of the antibiotic. In studies performed with *M. tuberculosis* and Mycobacterium microti it was found that this intrinsic resistance was inducible with sub-inhibitory concentrations of clarithromycin, leading to four- to eight-fold increase in MIC values. Moreover, in studies performed with clinical isolates of *M. tuberculosis*, sub-inhibitory concentrations of ethambutol reversed resistance to clarithromycin, signalling a permeability barrier as the cause of the intrinsic resistance to the macrolide.

Linezolid

Also part of the category 5 drugs of second-line anti-TB drugs, linezolid is an oxazolidinone originally approved for clinical use in the treatment of skin infections and nosocomial pneumonia caused by Gram-positive bacteria. The mode of action of linezolid is by inhibition of an early step in the synthesis of proteins, binding to the 50S ribosomal subunit. Resistance to linezolid in *M. tuberculosis* is still unusual, but a study analyzing 210 MDR strains found 1.9% of strains being resistant to linezolid. Further analysis of *in vitro* selected linezolid-resistant mutants found that strains with mutations in the 23S rRNA had MICs of 16–32 μg/mL, while strains with MICs of 4–8 μg/mL or susceptible strains showed no mutations. A more recent study using next-generation sequencing has also found the mutation T460C in *rplC*, encoding the 50S ribosomal L3 protein, in *in vitro*-selected mutants and clinical isolates of *M. tuberculosis* resistant to linezolid. Previous studies have also found evidence of the possible involvement of efflux pumps in the resistance of *M. tuberculosis* to linezolid.

Bedaquiline

Formerly known as TMC207 or R207910, bedaquiline is a new antibiotic belonging to the class of diarylquinolines with specific activity against *M. tuberculosis*, which has also shown *in vitro* activity against other non-tuberculous

mycobacteria. Bedaquiline was discovered after a high-throughput evaluation of thousands of compounds using *Mycobacterium smegmatis* in a whole-cell assay. The drug showed *in vitro* and *in vivo* activity against *M. tuberculosis* and then entered into clinical evaluation for drug susceptible and MDR-TB. Based on the results of two phase II clinical trials, bedaquiline has recently received conditional approval for the treatment of MDR-TB under the trade name Sirturo. A ―black box‖ warning is, however, accompanying this authorization due to the reported unexplained deaths and QT interval prolongation. Recent reviews and evaluation of this new drug have been published. A phase III clinical trial was scheduled to begin in 2013 but has not yet started. Bedaquiline is also being evaluated in new combination regimens with the purpose of reducing the length of treatment.

The mode of action of bedaquiline is by inhibiting the ATP synthase of *M. tuberculosis*, which was a completely new target of action for an antimycobacterial drug. This mode of action was discovered by analyzing *M. tuberculosis* and *M. smegmatis* mutants resistant to bedaquiline. By sequencing the genome of these mutants and comparing to that of the susceptible strains, the only mutation found was in the *atpE* gene, which encodes the c part of the F0 subunit of the ATP synthase. This is a complex structure that generates the ATP needed by the mycobacterial cell for which bedaquiline has a favored specificity compared to mitochondrial ATP synthase. The most prevalent mutation in the *atpE* gene found in bedaquiline resistant mutants is A63P but also I66M has been found. The latter introduces a modification that interferes the proper binding of bedaquiline to its target. Nevertheless, in a study to further assess the mechanisms of resistance to bedaquiline in *M. tuberculosis*, it was found that only 15 out of 53 resistant mutants had mutations in *atpE*. The other 38 strains lacked mutations in *atpE* or even in the F0 or F1 operons, which suggests that other mechanisms of resistance are still possible.

Delamanid

Delamanid, previously known as OPC-67683, is a derivative of nitro-dihydro-imidazooxazole with activity against *M. tuberculosis* that acts by

inhibiting the synthesis of mycolic acid and is undergoing clinical evaluation in a phase III trial. Delamanid was previously shown to have a very good *in vitro* and *in vivo* activity against drug-susceptible and drug-resistant *M. tuberculosis*, as well as good early bactericidal activity comparable to that of rifampicin. Delamanid has more recently shown its safety and efficacy in a clinical evaluation for MDR-TB. The specific mode of action of delamanid is by inhibition of the mycolic acid synthesis but it differs from isoniazid in that, it only inhibits methoxy- and keto-mycolic acid while isoniazid also inhibits α-mycolic acid. Delamanid also requires reductive activation by *M. tuberculosis* to exert its activity. In experimentally generated delamanid-resistant mycobacteria, a mutation was found in the Rv3547 gene, suggesting its role in the activation of the drug.

PA-824

PA-824 is a bicyclic derivative of nitroimidazole that showed specific activity against *M. tuberculosis*. The structure of PA-824 is shown in Figure 3. This small-molecule compound showed a very good *in vitro* and *in vivo* activity in animal models and it also showed to be safe and well tolerated. PA-824 is currently undergoing further clinical evaluations. PA-824 needs to be activated by a nitroreductase to exert its activity and it inhibits the synthesis of protein and cell wall lipids. The mechanism of resistance to PA-824 has been shown to be most commonly associated with loss of a specific glucose-6-phosphate dehydrogenase (FGD1) or the dezaflavin cofactor F420. More recently, a nitroimidazo-oxazine-specific protein causing minor structural changes in the drug has also been identified.

SQ-109

Compound SQ-109 is a synthetic analogue of ethambutol that has shown *in vitro* and *in vivo* activity against drug-susceptible and drug-resistant *M. tuberculosis*. The structure of SQ-109 is shown in Figure 4. It has also been shown to possess synergistic *in vitro* activity when combined with first-line drugs, and

more interestingly, when combined with bedaquiline and the oxazolidinone PNU-10048. SQ-109 is currently being evaluated in a phase II clinical trial.

The mode of action of SQ-109 is by interfering with the assembly of mycolic acids into the bacterial cell wall core, resulting in accumulation of trehalose monomycolate, a precursor of the trehalose dimycolate. Transcriptional studies have shown that, similar to other cell wall inhibitors such as isoniazid and ethambutol, SQ-109 induces the transcription of the iniBAC operon required for efflux pump functioning. Moreover, by producing spontaneously generated resistant mutants to SQ-109 analogs and performing whole-genome sequencing, mutations in the *mmpL3* gene were identified, suggesting MmpL3 as the target of SQ-109 and signaling MmpL3 as transporter of trehalose monomycolate.

Benzothiazinones

A new class of drug with antimycobacterial activity, 1,3-benzothiazin-4-one or benzothiazinone (BTZ), was recently described. The lead compound, 2-[2-S-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl]-8-nitro-6-(trifluoromethyl)-4H-1,3-benzothiazin-4-one (BTZ043) was found to have *in vitro*, *ex vivo* and *in vivo* activity against *M. tuberculosis*. It was also found to be active against drug-susceptible and MDR clinical isolates of *M. tuberculosis*.

By transcriptome analysis, the mode of action of BTZ043 was initially spotted at the cell wall biogenesis level. By further genetic analysis, using *in vitro* generated mutants, the target of the drug was identified at the level of the gene rv3790, which together with rv3791 encode proteins that catalyze the epimerization of decaprenylphosphoryl ribose to decaprenylphosphoryl arabinose, a precursor for arabinan synthesis needed for the bacterial cell wall. DprE1 and DprE2 were proposed as names for these two key enzymes. More recent studies have characterized more precisely the mechanism of action of BTZ043 by showing that the drug is activated in the bacteria through reduction of an essential nitro group to a nitroso derivative, which can react with a cysteine residue in DprE1. In studies with *M. smegmatis*, an alternative mechanism of resistance has been

suggested. The overexpression of a nitroreductase NfnB led to the inactivation of the drug by reducing an essential nitro group to an amino group. Although *M. Tuberculosis* apparently lacks nitroreductases able to reduce the drug, this finding could be important for development of new BTZ analogues with improved activity.

Just recently a series of piperazine-containing BTZ has been reported. The lead compound PBTZ169 has improved activity, safety and efficacy in animal models and has shown *in vitro* synergy with bedaquiline signaling it as an attractive new candidate for further clinical development.

9. GENERAL PRINCIPLES IN DESIGNING AN MDR-TB TREATMENT REGIMEN

The general principles apply whether an NTP manager is selecting an empirical or standard MDR-TB regimen for the country or a clinician is constructing a regimen for an individual patient. These principles also apply to XDR-TB cases.

Treatment regimens should consist of at least four drugs with either certain, or almost certain, effectiveness. Where evidence about the effectiveness of a certain drug is unclear, the drug can be part of the regimen but it should not be depended upon for success. Often, more than four drugs may be started if the susceptibility pattern is unknown or the effectiveness of one or more agents is questionable.

Susceptibility testing for isoniazid, rifampicin, the fluoroquinolones, and the injectable agents is fairly reliable. For other agents it is less reliable, and basing individualized treatments on DST for these agents should be avoided. The clinical effectiveness or ineffectiveness of a drug cannot be predicted by DST with 100 % certainty.

Each dose in an MDR regimen is given as DOT throughout the treatment.

Programmatic strategies for treatment of MDR-TB

Programmatic approaches to MDR-TB treatment depend in part on the type of laboratory method used to confirm MDR. Once MDR-TB is confirmed (by either type of laboratory method), patients can be treated with:

- a ***standard MDR regimen*** (standardized approach); or
- an ***individually tailored regimen***, based on DST of additional drugs.

For NTPs using conventional DST methods, there is often a delay of months before results are available to confirm or exclude MDR. These countries need to consider MDR treatment at two stages: when MDR is suspected but laboratory confirmation is pending, and once MDR is confirmed. While awaiting

results, patients who are highly likely to have MDR-TB (such as those whose prior treatment has failed) need ***an empirical MDR regimen***. If MDR is confirmed, this regimen may be continued, or it may be tailored on the basis of susceptibility to drugs other than isoniazid and rifampicin.

NTPs using rapid molecular-based DST will be able to confirm MDR-TB within 1−2 days, 1 and then can initiate treatment with a standard MDR regimen immediately, or may tailor the regimen later when DST results for second-line drugs become available.

Standardized and individualized approaches each have advantages. Standard MDR-TB regimens make it easier to estimate drug needs, to order, manage and distribute drug stocks, and to train personnel in the treatment of MDR-TB patients. Even when standard regimens are used throughout treatment, patients experiencing severe adverse effects will need to have their MDR treatment individualized. Thus all programmes need some capacity to individualize treatment.

General principles for designing MDR-TB treatment regimens

Assess information from:

• History of drugs used: 1 month of monotherapy or single drug intake over a failure regimen could be a strong predictor of resistance,

• DST: Most reliable for R and H; also reliable for Km and FQs; less reliable for E and Z; very low reliability for Group 4 drugs,

• Perform HIV test. If positive, initiate cotrimoxazole prophylactic therapy and antiretroviral therapy as soon as possible.

Use at least 4 drugs certain to be effective. The more of the following factors are present, the more likely it is that the drug will be effective:

• Resistance to these drugs is known from surveys to be rare in similar patients.

• DST results show susceptibility to drugs for which there is good laboratory reliability: injectable agents and fluoroquinolones.

• The drug is not commonly used in the area.

• For decisions about an individual patient – no prior history of treatment failure with the drug; no known close contacts with resistance to the drug.

Do not use drugs for which there is the possibility of cross- resistance. Many antituberculosis agents exhibit cross-resistance both within and across drug classes.

Eliminate drugs that are not safe. Quality of the drug is unknown. For decisions about an individual patient – known severe allergy or unmanageable intolerance; high risk of severe adverse drug effects such as renal failure, deafness, hepatitis, depression and/or psychosis.

Include drugs from Groups 1–5 in a hierarchical order based on potency:

• Use any of the first-line oral agents (Group 1) that are likely to be effective.

• Use an effective aminoglycoside or polypeptide by injection (Group 2).

• Use a fluoroquinolone (Group 3).

• Use the remaining Group 4 drugs to complete a regimen of at least four effective drugs.

• For regimens with fewer than four effective drugs, consider adding two Group 5 drugs. The total number of drugs will depend on the degree of uncertainty, and regimens often contain five to seven.

The lenths of treatment. In the treatment of patients with MDR-TB, an intensive phase of 8 months is suggested for most patients, and the duration may be modified according to the patient's response to therapy. In the treatment of patients newly diagnosed with MDR-TB (i.e. not previously treated for MDR-TB), a total treatment duration of 20 months is suggested for most patients, and the duration may be modified according to the patient's response to therapy.

In the treatment of patients with MDR-TB, regimens should include at least pyrazinamide, a fluoroquinolone, a parenteral agent, ethionamide (or prothionamide), and either cycloserine or PAS if cycloserine cannot be used.

In the treatment of patients with MDR-TB, a later-generation fluoroquinolone rather than an earlier-generation fluoroquinolone should be used.

In the treatment of patients with MDR-TB, ethionamide (or prothionamide) should be used.

In the treatment of patients with MDR-TB, four second-line antituberculosis drugs likely to be effective (including a parenteral agent), as well as pyrazinamide, should be included in the intensive phase.

Changing to an **individualized regimen** (once DST results are available for additional drugs beyond isoniazid and rifampicin) is advantageous because it:

• Allows clinicians to design a regimen with knowledge of resistance to particular injectables and fluoroquinolones, which is especially important if patients have received second-line drugs in the past. This knowledge helps in avoiding the use of toxic and expensive drugs to which the patient's *M. tuberculosis* is found to be resistant.

• Allows clinicians to tailor the regimen in settings with high rates of resistance to second-line drugs where it may be difficult to find a standard regimen that is appropriate for all patients.

• Provides flexibility if patients experience adverse effects related to one drug.

In some settings, individualized regimens may achieve higher cure rates than standard MDR regimens.

Standart regimen for MDR-TB treatment:

8ZCmLfxEt(Pt)Cs(±PAS)/12ZLfxEt(Pt)Cs(±PAS)

Selection of individualized MDR-TB regimens

Individually designed regimens are based on the patient's history of past drug use and on DST to isoniazid, rifampicin, the second-line injectable agents and

a fluoroquinolone. Every effort should be made to supplement the patient's memory of treatment with objective records from previous health care providers. A detailed clinical history can help suggest which drugs are likely to be ineffective. Although resistance can develop in less than 1 month, if a patient has used a drug for more than 1 month with persistently positive smears or cultures, the strain should − as a general rule − be considered as "probably resistant" to that drug, even if DST results indicate that it is susceptible.

DST results should complement, rather than invalidate, other sources of data on the likely effectiveness of a specific drug. For example, if a history of prior anti-TB drug use suggests that a drug is likely to be ineffective, that drug should not be relied on as one of the four core drugs in the regimen, even if DST shows the strain to be susceptible. Alternatively, if the patient has never taken a particular drug and resistance to that drug is extremely uncommon in the community, DST results that indicate resistance may be the result case of a laboratory error or of the limited specificity of DST for some second-line drugs.

Another important limitation is the turn-around time for DST results: the patient may have already received months of treatment by the time DST results become available from the laboratory. The possibility of further acquired resistance developing during this time must be considered. If there is a high probability of acquired resistance to a particular drug after collection of the specimen for DST, that drug should not be counted as one of the four drugs in the core regimen (but can be included as an adjunctive agent).

Examples of treatment regimens in depending on drug susceptibility spectrum.

- *Resistance* to HR(±S) (Z): *Used* Z+injectable drug+Fq+two drugs from 4 group±E;
- *Resistance* to HRSE (Z): *Used* Z+injectable drug+Fq+three drugs from 4 group;
- *Resistance* to HRSE (Z) Km: *Used* Z+injectable drug+Fq+three drugs from 4 group;

- *Resistance* to HRSE (Z) KmOfx: *Used* Z+injectable drug+Fq+three drugs from 4 group+drugs from 5 group.

The role of surgery in the treatment of patients with drug-resistant tuberculosis

Surgery may be indicated in concrete cases for managing sequelae or complications of pulmonary TB. For patients with extra-pulmonary TB, surgery may be acceptable for obtaining samples for study and treating some situations such as constrictive pericarditis, vertebral abscesses compressing the spinal cord or superfi cial and accessible abscesses in cases of osteoarticular TB. Note that in pulmonary TB, surgery should not be considered as a viable option for therapy, in view of the excellent performance of pharmacological treatments.

A historical review of TB treatment during the fi rst half of the twentieth century shows that surgery played a major role. Reduction of the bacillary burden achieved by the different surgical procedures in the prep harmacotherapy era produced a higher cure rate than that of the natural evolution of the disease. Surgery nonetheless fails to entirely eradicate bacilli from lesions and involves high morbidity and mortality. With the discovery of effective anti-TB drugs, the indication for surgery was progressively abandoned and had virtually disappeared from case management by the 1970s. The question then emerged again for patients with MDR/XDR-TB and resistance to multiple other drugs, when practically no available pharmacotherapy regimen ensured a cure. Under these circumstances, many patients today face situations very similar to those in the prepharmacotherapy era.

Despite the absence of randomised trials assessing the role of surgery in the treatment of patients with MDR-TB, virtually all available guidelines and specifi c recommendations on the subject mention surgery, although it is assigned only a secondary role. Surgery should be considered for treating DR-TB only in patients meeting the three following conditions:

1) a fairly localised lesion,

2) an adequate respiratory reserve, and

3) a lack of sufficient available drugs to design a regimen potent enough to ensure cure.

The strongest advocates of surgical treatment recommend scheduling surgery at the time of the lowest possible bacillary load, preferably after sputum smears and culture have become negative, and suggest continuing a predetermined pharmacotherapy regimen of 18 to 24 months. It would be useful to evaluate the clinical outcome of these patients with negative cultures if chemotherapy was continued without surgery, considering that pharmacological treatment has demonstrated effi cacy in sputum conversion, bearing in mind that the bacillary load is already much lower. It should be kept in mind that surgery performed on these patients, even by the most experienced surgeons, has high rates of morbidity and mortality.

Consequently, surgery should only be considered for the management of MDR/XDR-TB for patients meeting the three conditions mentioned above and must be performed only by experienced surgeons with the support of effi cient postoperative care units. Such settings are available mostly in developed countries. Of course surgery may be indicated more often in patients with XDR-TB in settings where the third condition−a lack of sufficient available drugs−is seen more frequently.

Collapse Therapy

It is a reversible surgical therapy which involves collapse of lung by artificial pneumoperitonium or pneumothorax used for cavity containing diseased lung with concept that compression of the cavity will change the local environment in manner which will inhibit the mycobacteria. This therapy does not appear to be of general utility, although artificial pneumoperitonium and pneumothorax may be helpful in highly selected cases. However, controlled studies are lacking.

Laser Therapy

This has also been tried as an adjunct to chemotherapy in some countries such as Russia for the treatment of drug resistant TB. This is effective in multicavitary disease with heavy bacterial loads particularly when there is an increased chance of failure of medical treatment. It is thought to have a role in the rapid killing of bacteria, increases and improves penetration of antitubercular drugs in walled off lesions and helps in early closure of cavities and is of proven benefit in tracheal and bronchial stenosis due to endobronchial growth. It also reduces the trauma of surgery and post-operative complications.

Immunotherapy or Immunomodulation

Therapeutic modulation of the immune system to enhance the host's immunity to control tuberculosis and to shorten the durations of chemotherapy required to «cure» patients with drug susceptible disease has been tried with some success. Mycobacterium vaccae have shown transiently favourable results when given to drug resistant tuberculosis who had failed chemotherapy. Immune modulation can be affected by enhancing proinflammatory cytokines such as IL-2, IL-12, IFN-γ, TNF-α, inhibiting the antiinflammatory cytokines such as IL-4, IL-5, IL-10, addition of serum to enhance humoral factors or diverting the harmful Th2 immune pathway to the beneficial Th1 response by vaccination utilizing M. vaccae.

However these therapies are adjuncts and have proved useful in selected cases of drug resistant tuberculosis and randomized control trials, have failed to confirm the utility of this therapy. Beneficial effect of parenterally used IFN-γ have been reported in disseminated disease attributable to mycobacteria other than tuberculosis that was refractory to chemotherapy. Favourable results were reported following one month use of inhaled IFN-γ, 500 microgram thrice weekly. Cytokine therapy has been shown to have clinical utility in modifying the inflammatory manifestation of the lepromatous type of disease. IL-2 was used to restore antigen responsiveness, presumably via enhancing IFN- γ production. Thalidomide has

been shown to inhibit the in-vitro release of TNF-α from peripheral blood monocytes.

In patients with active tuberculosis it induces a significant gain in weight. However the possibility that thalidomide agents may ameliorate tissue injury in tuberculosis needs further study. The potential role of diverse agents such as transfer factor, indomethacin, and levamisole is yet to be established. Levamisole as adjunct to drug treatment has been reported to cause more rapid radiological clearing in the treated group. However it did not significantly affect the clinical outcome. *Mycobacterium w (*commercially available as Immuvac) has been extensively studied as an effective immunomodulator for treatment of leprosy. It enhances bacterial killing and lesion clearance when used as an adjuvant to multi-drug therapy for leprosy. *Mycobacterium w* shares antigens with *M. leprae* as well as *M. tuberculosis* suggesting its application in treatment of drug resistant tuberculosis. A randomised control study has demonstrated that this drug may be responsible for overall reduction of duration of therapy, with no change in sputum conversion rate compared with the traditional short course chemotherapy in new as well as re-treatment cases of tuberculosis. Another advantage though not proven, may be that Immuvac effect would be longer lasting and could take care of defaulters more meaningfully than chemotherapy alone, leading to a reduction in relapse rate and the emergence of MDR TB. Recently, a randomised control trial has been initiated in 2007 and is under progress in order to establish its efficacy and safety as an adjunct therapy in New Pulmonary Tuberculosis (Category I) Patients.

Glutoxim (a commercial immunostimulator) fully converts Isoniazid into its active form in the presence of hydrogen peroxide. Glutoxim lowered the H MIC by a factor of 1,6-2 in sensitive MBT H37Rv, and also in clinical isolates with strong/weak drug-resistance caused by various genetic mutations.

Gene Therapy

The decoding of the human genome provides another fascinating aspect in the future therapeutic intervention of tuberculosis. By identifying resistance genes,

it will be possible to detect drug resistance before start of therapy and also to develop drugs that target these specific genes, enabling us to considerably reduce the duration of therapy.

Role of Steroids

The adjuvant use of corticosteroids in DR-TB patients has been shown not to increase mortality and can be beneficial in conditions such as severe respiratory insufficiency, severe drug induced rashes and central nervous system or pericardial involvement. Prednisone is commonly used, starting at approximately 1 mg/kg and gradually decreasing the dose to 10 mg per week when a long course is indicated.

Corticosteroids may also alleviate symptoms in patients with an exacerbation of obstructive pulmonary disease or when patient is in a very low general condition. In these cases, prednisone may be given in a short course, tapering over 1–2 weeks, starting at approximately 1 mg/kg and decreasing the dose by 5–10 mg per day. Injectable corticosteroids are often used initially when a more immediate response is needed.

10. MANAGEMENT OF ADVERSE DRUG REACTIONS

The key principles in the management of adverse drug effects in MDR-TB treatment are:

1. If minor adverse effects occur, be supportive, consider administration of ancillary drugs and reassure patients.

2. In case of major adverse effects that are life-threatening or can potentially cause damage to vital organs, identify and discontinue the offending drugs. Once adverse drug reactions are identified, they must be addressed promptly and effectively:

1) Ancillary drugs (such as metoclopramide for gastrointestinal disturbance, vitamin B6 for peripheral neuropathy, non-steroidal anti-infl ammatory drugs for arthralgia and headaches, antihistamines for hypersensitivity reactions, levothyroxine for hypo thyroidism, potassium and magnesium replacement for electrolyte wasting) may be helpful.

2) If ancillary drugs do not solve the problem or are not useful (i.e., cases of hepatitis or renal failure), the suspected drug(s) should be identified. At times, reactions may not be caused by anti-TB drugs. Other potential causes must be dealt with as well. Careful clinical assessment and differential diagnosis is critical. For example, depression may be due to chronic TB or socioeconomic issues rather than to Cs. In such cases, suspending Cs may not be helpful.

3) Reducing the dosage of the offending drugs may resolve the problem in some cases, such as with gastrointestinal disturbance, but the dosage must be increased to adequate levels gradually at a later point in time if the patient can tolerate it. In other cases, suspected agents should be temporarily or permanently stopped. Drugs that are c ompletely intolerable or cause major adverse effects that are lifethreatening or damaging vital organs (such as hepatitis, renal toxicity, optic neuritis, severe neurological and psychiatric disturbance) should be discontinued. In cases where there are two or more suspected agents, a procedure re-challenging

with these suspected agents one by one after symptoms minimise should be conducted to identify the offending drugs.

4) Permanent discontinuation of one or more drugs may be required. However, discontinuation of drugs, especially FQ and injectables, may compromise the effi cacy of the regimens. Permanent discontinuation of FQ is rarely needed. The decision to permanently discontinue FQ should be made only after very careful clinical assessment, as it may substantially increase the risk of treatment failure. Secondline injectables are also critical in the intensive phase and should not be discontinued too early. An average 10 % of patients (5 %−15 %) cannot tolerate Et/Pt or Cs or PAS. If a higher proportion of patients (25 % or higher) has Et/Pt withdrawn from the treatment regimen due to adverse drug effects, investigation is required to ensure that health-care workers have not unnecessarily stopped Eth/Pto due to minor adverse effects. This also applies to PAS and Cs.

5) Replacement with other drugs should be considered if discontinuation of one or more of the drugs may compromise the effi cacy of the regimen. This is particularly important in the intensive phase.

6) Permanent discontinuation of MDR-TB treatment due to adverse drug effects is rarely required. If 5 % or more patients have permanent discontinuation of MDR-TB treatment due to adverse drug effects, assessment is needed to fi nd out whether this can be improved.

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