Figure 44. Macroscopic view of a lung affected by TB. Caseous necrosis is extensive, and significant bronchogenic dissemination is also observed.
Figure 45. Macroscopic view of a lung affected by miliary TB. The miliary pattern can be seen throughout the lung parenchyma.
Non-conventional methods for diagnosing tuberculosis: new techniques

This topic will be discussed in detail in the following chapter. However, it should be mentioned that despite the many techniques that have been developed in the past 20 years, almost none are currently indicated for the routine diagnosis of TB in low- and middle-income countries. Despite the advantages offered by some of these techniques, most are very expensive and complicated to use. Consequently, the conventional or routine diagnosis of TB must be based on the five major sections in this chapter.

Conclusions regarding the diagnosis of tuberculosis with conventional methods

Section summary

Diagnosis of TB:

1. **Certain**: positive sample culture with the identification of the *M. tuberculosis* complex.
2. **Highly probable**: justifies the start of treatment and acceptance of the case as constituting TB in the framework of an NTP:
   - Smear-positive results. No need for culture.
   - Caseous necrosis in a biopsy sample (must be cultured).
3. **Exclusion**:
   - Based on clinical, radiological, and laboratory criteria.
   - Samples must always be processed for smear microscopy and culture.

Based on the topics dealt with in this chapter, the only way to establish a firm diagnosis of TB is to obtain a positive culture, with identification of the mycobacteria as belonging to the *M. tuberculosis* complex. In countries with low or middle incomes, culture should be performed in solid media, preferably Löwenstein-Jensen medium, with identification using biochemical tests.

A highly probable diagnosis of TB comprises those cases offering smear-positive results, or patients who for different reasons have required the performance of a biopsy, with histological study showing granulomas with caseous necrosis suggestive of TB. However, on the basis of its high specificity, rapidity, simplicity, and low cost, a definite diagnosis may be accepted (within the framework of an NTP) if positive staining using the Ziehl-Neelsen technique reveals the presence of acid-fast bacilli. Thus, a
smear-positive result represents a case of TB, and such a case should be registered with the NTP, and treatment is recommended.

In low- and middle-income countries, not all smear-positive samples should be subjected to culture since over 99% of such cases effectively correspond to TB, and culture examination would only add complexity and cost to case management at the peripheral level of health care. In these countries, culture is only indicated in those cases where several serial smear microscopy studies spaced at least 2 to 3 weeks apart prove to be negative, the symptoms persist, and the possibility of other diseases cannot be ruled out. Culture is also advised in the instance of suspected drug resistance or when the disorder may be caused by other mycobacteria. However, all biopsy specimens from cases of suspected TB should undergo culture examination.

Lastly, patients with clinical and radiological signs that are highly suggestive of TB may also be accepted as disease cases, even when microbiological studies have yielded negative results, other possible diseases have been ruled out, and the patient has been cured with antituberculous treatment. This constitutes what is known as an exclusion diagnosis, which requires microbiological confirmation, even if the results later prove to be negative. Such patients never exceed 10% of the total cases. In short, a diagnosis of TB should not be accepted based only on clinical and radiological criteria, without the performance of microbiological studies. Unfortunately, such diagnoses are still accepted too frequently.
Chapter summary

The last 10 to 15 years have seen a great increase in research on the diagnosis of tuberculosis (TB), with the introduction of numerous new techniques. However, practically none of these techniques are indicated for the routine diagnosis of TB in countries with low- or middle-income levels. Despite the advantages afforded by some of these novel techniques, they have not been able to replace smear microscopy and culture in their respective indications. Further, most of these techniques are very expensive and complicated to perform.

Of all the methods described in this chapter, the following should be pointed out:

1. Smear microscopy involving fluorochrome staining (auramine) offers the advantage of rendering the bacilli fluorescent, allowing the mycobacteria to be seen at lower magnification levels and allowing the assessment of many more microscopic fields in less time. This time-saving benefit makes the technique cost-effective in laboratories that process more than 25 to 30 smears per technician each day.

2. Liquid culture media offer superior sensitivity versus solid media and, above all, faster detection of microbial growth, which can shorten the time to results by 2 to 3 weeks. However, their main limitations include increased contamination levels, difficulty in identifying mixed cultures, and inability to observe the morphology of the bacterial colonies.

3. While simple and fast, the new techniques developed for mycobacterial species identification are expensive. Moreover, 99% of all disease caused by mycobacteria is attributable to *M. tuberculosis*, and the identification of species other than those pertaining to the *M. tuberculosis* complex is of low epidemiological relevance in countries with a high prevalence of TB. As a result, such techniques are not indicated for use in low- and middle-income countries.

4. Many new methods have been developed for drug susceptibility testing, some of which yield rapid results and are simple to perform. However, their high cost, the lack of reproducibility, and the need for standardisation explain why none of them are recommended for routine use in countries with low- or middle-income levels.

5. The techniques based on the amplification of nucleic acids are fast (yielding results in less than 1 day) and highly sensitive, but they are also very expensive, may yield false-negative results, and require careful clinical interpretation in the event of a positive result.
6. Molecular biological studies can be useful in the field of epidemiology, especially for: i) determining the general epidemiological pattern of strains in a given population and in the control of epidemics; ii) differentiating between relapses and exogenous reinfections; and iii) studying cross-contamination in the laboratory. However, these are expensive and time-consuming techniques, and the results obtained must be evaluated in combination with conventional epidemiological practices.

7. The determination of adenosine deaminase in TB-affected serosal fluids has been sufficiently validated to recommend its use in middle-income countries, but not in the poorest parts of the world.

The present chapter will address the newer techniques for diagnosing tuberculosis (TB), although it may be more correct to refer to them as non-conventional techniques, since some of them have been in use for a number of years. Each technique will be reviewed in terms of its methodology, indications, advantages, and limitations that render it unsuitable for the routine diagnosis of TB. For increased convenience, the chapter has been divided into eight sections: seven addressing microbiological techniques—this perhaps being the field in which most research has been conducted—and one section on the remaining methods.

Reflecting the sequence used in the diagnosis of TB, the sections on new or non-conventional bacteriological techniques have in turn been divided as follows: smear microscopy, culture, identification, drug susceptibility testing, genetic amplification, serological diagnosis, and the potential contribution of these microbiological techniques to the understanding of the epidemiology of the disease.

Non-conventional smear microscopy techniques

**Section summary**

While fluorochrome staining (auramine) is comparable with the Ziehl-Neelsen technique in terms of advantages and limitations, it should only be indicated in laboratories that process more than 25 to 30 cases of smear microscopy per technician per day. The technique offers the advantage of rendering the bacilli fluorescent (more visible) and allowing the assessment of many microscopic fields at lower magnification levels and in less time.

Although the Ziehl-Neelsen method is the most widely used and is recommended as the standard procedure, fluorochrome staining (auramine) is equally effective and is based on the same principle as acid-fast staining.
The advantage of this method is that the bacilli appear fluorescent (Figure 46); as a result, they can be seen much more easily and the examiner can work at lower magnification levels—allowing the assessment of many more microscopic fields in less time. While Ziehl-Neelsen staining requires operating at x1000 magnification and spending at least 10 to 15 minutes on observation, fluorescent staining makes it possible to work at only x200 to x400 magnification, as a result of which the preparation can be studied in only 2 to 3 minutes. However, positive readings with this technique must be confirmed by Ziehl-Neelsen staining, since Ziehl-Neelsen staining allows improved visualisation of certain details of bacterial morphology that, in the hands of an expert microbiologist, can provide clues to the identity of the *Mycobacterium* responsible for the disease.

Saving time is the main advantage of the fluorochrome technique. However, its limitations include the need for more costly technical equipment and materials that are difficult to maintain. A continuous electrical supply is also important, which can be a problem in very poor areas.

This fluorescence technique can be recommended for use in laboratories that process more than 20 to 30 smears per technician per day, where the resulting savings in time would compensate for the increased costs associated with equipment and materials. In practice, this would pertain to very few laboratories in countries with low- or middle-income levels.

![Figure 46. Direct sputum smear microscopy using the auramine-rhodamine technique. *M. tuberculosis* appears as small, fluorescent, yellow filaments over a green background.](image-url)
The sample extensions can also be prepared with a cytocentrifuge, which allows mycobacteria to be concentrated in a smaller space on the slide. After staining, this affords increased sensitivity and observation convenience.

**New mycobacterial culture methods**

*Section summary*

The radiometric Bactec® system must be regarded as one of the most important diagnostic advances in the past 20 years. It offers important savings in time—both in detecting growth and in performing drug susceptibility testing—as well as superior sensitivity. Disadvantages include having to work with radioactive materials and the high cost. The new liquid culture methods have increased in usefulness, replacing the need for radioactive materials and affording full automatic performance, thereby facilitating manipulation. However, these methods are very expensive and would only be indicated for use in reference laboratories in wealthy countries.

Although the biphasic systems are more sensitive than the radiometric systems and require no radioactive installations, they are not as time saving. This disadvantage, in addition to high cost and problems related to identification and drug susceptibility testing, largely limits their use. The use of blood culture techniques is not recommended in low- and middle-income countries, since their clinical manipulation is very specialised. Based on the high prevalence of TB in such areas, when TB is suspected (even if involving only fever of unknown origin) in an HIV-positive patient, the initiation of empirical antituberculous treatment is advised. In short, in places with limited resources, it is not acceptable to spend money on costly techniques considering the negligible public health benefit that may be derived.

In an attempt to overcome the main limitations associated with cultures, intense research in the last two decades has focused on the development of faster techniques that offer the added advantage of improved sensitivity performance. Consequently, three important advances were introduced in clinical laboratories: 1) liquid culture media; 2) biphasic culture media (MB-Septi-Check®); and 3) techniques for isolating mycobacteria from blood.

**Liquid culture media**

All such media offer the important advantage of increased sensitivity over solid media, particularly faster detection of bacterial growth (shortening the
time to results by 2-3 weeks). However, their most important limitations are an increased contamination rate, the difficulty of identifying mixed cultures, and the inability to observe colony morphology.

Two liquid culture media systems have been developed: radiometric (Bactec® 460 TB) and non-radiometric (e.g., MGIT, ESP, MB/Bact).

**Radiometric methods (Bactec® system)**

For reference laboratories, the Bactec® system is undoubtedly the most useful diagnostic advance in clinical microbiology in the past 20 years. The technique automatically detects mycobacterial growth, measuring the amount of \(^{14}\)CO\(_2\) generated by the metabolisation of \(^{14}\)C-radiolabelled substrate (fatty acids). The vials used contain 4 ml of Middlebrook 7H12 medium and the radiolabelled fatty acids, admitting inocula of up to 0.4 ml.

In comparison with the traditional culture systems, the Bactec method offers the following advantages:

1. Time saving (15-20 days) for the detection of growth.
2. Increased sensitivity both for detecting *M. tuberculosis* as well as for identifying other mycobacteria.
3. The possibility of identifying *M. tuberculosis* in 4 to 5 days, and of performing drug susceptibility testing for front-line drugs (isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide) in 3 to 6 days instead of the 21 to 42 days required by solid media. Pyrazinamide requires special Bactec medium.
4. The possibility of identification and drug susceptibility performance without having to perform subcultures.

However, the Bactec system also has several disadvantages, such as:

1. The need to use radioisotopes.
2. The high cost of equipment, reagents, and maintenance.
3. The need to use syringes, which can cause possible cross-contamination between samples, as well as the potential formation of aerosols.
4. Laborious performance, since a semi-automated system is used.

The main inconvenience of the Bactec system is the need for radiolabelled \(^{14}\)C-fatty acid, which means that the laboratory must obtain authorisation for the use and storage of radioactive material.

Based on the above considerations, use of the Bactec system appears to be justified only in reference laboratories in industrialised countries. In countries with low- or middle-income levels, the system is not warranted, since its main advantage (time savings) does not compensate for the increase in
Use of the Bactec system may be considered in reference laboratories in middle-income nations, but never in poorer countries, where the priority continues to be diagnosis by smear microscopy. A subject with smear-negative results and positive culture would already be receiving treatment, and a few additional weeks to confirm the diagnosis would have no effect on the treatment outcome of the patient or on the extent of the disease in the community.

Non-radiometric automated systems

In recent years, and with the purpose of resolving the disadvantages of the Bactec system, new non-radiometric liquid media involving automatic reading have been designed and manufactured. Manipulation of the vials is simpler, since reading is totally automatic, thereby saving more time for the technical personnel. These new systems nevertheless retain all the advantages of the Bactec system, with the added benefit of continuous readings of mycobacterial growth (because of automated operation). However, they are associated with high cost, which limits their use in most low- and middle-income countries.

The systems that have been used most widely in recent years are the MGIT (Mycobacteria Growth Indicator Tube System®); the Bactec 9000 MB System®; the ESP Culture System II-Mycobacteria Detection System®; and the MB-BacT Mycobacteria Detection System®. All these systems are based on the Bactec methodology, with the exception that radioactive labelling has been replaced by the incorporation of a ruthenium compound (which emits detectable fluorescence as the partial O₂ pressure in the medium decreases as a result of microbial metabolism) or other substances. Culture positivity is therefore based on the detection of mycobacterial oxygen consumption, the decrease in atmospheric pressure within the vial, or CO₂ release from the culture medium.

Non-radiometric biphasic culture media (MB-Septi-Check®)

A biphasic system was introduced in the market a few years ago for use with mycobacterial culture (MB-Septi-Check®), with the aim of affording a rapid and sensitive technique similar to the Bactec procedure, although without the need for radioisotopes. The system uses bottles containing 20 ml of Middlebrook 7H9 broth to which a device containing different solid media can be attached at the upper end.

This system has several advantages over the Bactec technique, such as the use of greater seeding inocula as well as somewhat greater sensitivity.
Moreover, MB-Septi-Check allows growth in the solid phase, thereby allowing identification tests to be performed without having to re-seed, as well as allowing identification of mixed colonies. The main inconveniences are that growth detection is slower than with the Bactec system, *in vitro* sensitivity tests are not possible, and the presumptive *M. tuberculosis* identification system incorporated in the solid phase often fails.

**Systems for mycobacterial culture in blood**

The increase in disseminated infections caused by *M. avium-intracellulare* and *M. tuberculosis* in AIDS patients has prompted the development of techniques capable of detecting mycobacteria in blood. Among these systems, those best suited for this purpose are based on the lysis-centrifugation principle in the radiometric system, or on the new automated culture systems.

Both offer similar sensitivity. The main advantage of lysis centrifugation is that it allows the quantification of the number of bacteria per millilitre of blood, with serial control of the efficacy of the prescribed treatment. In contrast, direct seeding of blood in 13A medium, with subsequent control by the radiometric system, avoids many of the hazardous steps that are involved when using the lysis-centrifugation technique.

Use is indicated in AIDS patients with CD4+ lymphocyte counts of less than 50 cells/mm³ who present with fever of unknown origin, and only in high-income countries.

**New techniques for identifying mycobacteria**

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<td>The relevance of identifying species other than those pertaining to the <em>M. tuberculosis</em> complex in regions with a high prevalence of disease, where over 99% of all mycobacterial diseases are caused by <em>M. tuberculosis</em>, is of little significance. Moreover, despite the simplicity and rapidity afforded by these new species identification techniques, their high cost and low clinical and public health utility make them unsuitable for use in countries with low- or middle-income levels.</td>
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The obvious limitations of the standard biochemical identification tests—complexity, slowness, and lack of reproducibility—have stimulated the development of rapid identification methods, including the NAP test in Bactec 12B, chromatography, and identification based on molecular techniques.
**NAP test in Bactec 12B**

NAP (\(p\)-nitro-alpha-acetylamino-beta-hydroxypropiophenone) is a precursor of chloramphenicol synthesis that inhibits the growth of *M. tuberculosis* complex, but not of the environmental mycobacteria. The test strain is inoculated in two vials: one corresponding to Bactec 12B and the other to Bactec 12B containing NAP. If the mycobacteria grow in the Bactec 12B vial, but not in the vial that also contains NAP, then the test microorganism effectively belongs to the *M. tuberculosis* complex. The test result is obtained in less than 1 week, without the need for re-seeding in solid media for conducting standard biochemical identification tests.

**Chromatography**

As has been commented, mycobacteria are characterised by a cellular wall with an extraordinary high complex lipid content (e.g., wax, mycolic acids, long-chain fatty acids, glycolipids). The lipid content of the wall is stable and specific to each mycobacterial species.

Among the most extensively studied mycobacterial lipids, mycolic acids and other cell wall fatty acids should be mentioned because of their taxonomic value. Mycolic acids can be separated with relative ease in the form of methyl esters via thin-layer chromatography (TLC) in silica gel. The TLC-based study of mycolic acids divides mycobacteria into groups, and definitive identification is achieved by gas chromatography.

Despite the high specificity of chromatography and the fact that there already are automatic systems that can perform the technique, both the equipment and materials are very expensive. As a result, they can only be recommended for use in reference laboratories in industrialised countries with experience in the field.

**Genetic probes**

Advances in molecular biology have allowed the identification of DNA or RNA sequences specific to each mycobacterial species. In order to allow hybridisation with these sequences, genetic probes have been developed, comprising fragments of complementary nucleic acids labelled with radioactive isotopes or chromogenic substances.

A probe is a biological reagent composed of a DNA fragment possessing a base sequence complementary to that of a genomic fragment of the microorganism. The probes are in turn labelled with different indicators that
are easy to detect: radioactive isotopes (hot probes) or chromogenic substrates (cold probes).

When the nucleic acid of a microorganism is freed, and the released DNA is subjected to denaturation (i.e., the two DNA molecular strands are separated by physical procedures at a temperature of 90-140°C), the probe is able to bind (hybridise) with its homologous fragment, provided such fragment exists. Probe hybridisation with its homologous fragment is easily detected via means of the incorporated marker.

At present, several cold probes are available on the market, such as Gen-Probe® and Syngene®, for the identification of *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. gordonae*. There are no commercially available probes yet for the other mycobacterial species.

The main advantages of genetic probes include simplicity of manipulation, adaptation to any laboratory, rapidity of the technique (identification in only 2 hours), and high specificity. The major disadvantages are that these methods do not identify species within the *M. tuberculosis* complex, and they are associated with high cost. Consequently, their use is limited to reference hospitals in industrialised countries.

**Other molecular techniques**

Polymerase chain reaction (PCR), which involves multiplication of a given DNA fragment millions of times, can be used for species identification in three ways:

1. Amplification with the appropriate primers, followed by detection of a specific fragment of a specific species via electrophoresis and ethidium bromide staining.
2. Amplification of a DNA fragment common to all mycobacterial species, followed by recognition of the product amplified with species-specific probes.
3. Amplification of a DNA fragment common to all mycobacterial species, followed by lysis of the product with restriction enzymes, and visualisation of the restriction fragments in agarose gel after staining with ethidium bromide. This methodology, known as PCR with restriction fragment length polymorphism analysis (PCR-RFLP or PRC), is very useful for rapidly identifying species belonging to the genus *Mycobacterium*.

Characterisation of the hypervariable regions of the 16S ribosomal RNA molecule specific to mycobacterial species has allowed the development of a mycobacterial identification protocol based on the sequencing of
their nucleic acids. After amplifying a genomic fraction via PCR, sequencing is performed, and comparison of the results identifies the species obtained with the known specific regions of each mycobacterial species. With this technology it has been possible to identify and describe new species belonging to the genus *Mycobacterium*.

**New techniques for drug susceptibility testing**

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| All methods presented in this section are only indicated for use in very few reference laboratories in industrialised countries. Despite the attractiveness of many of these methods, and the fact that they are fast and sometimes also easy to use, their high cost, frequent lack of reproducibility, and need for standardisation exclude them from use in low- and middle-income countries. Once again, it is necessary to emphasise that the priority concern of these countries is the diagnosis and treatment of cases with positive smear microscopy findings. As will be addressed in the chapter on re-treatment (Chapter 10), in these regions the management of cases with possible resistances must be included in the standardised treatment plans of the tuberculosis control programmes. Under these circumstances, a savings of 2 to 3 weeks spent in determining the drug susceptibility results affords no benefit, because in many instances the logistical problems also lead to important delays. In view of the above, re-treatment, when necessary, will have to be based on a detailed history of the drugs administered in the past (discussed in Chapter 10).

The World Health Organization and the International Union Against Tuberculosis and Lung Disease recommend the method of proportions or other standardised methods, with the antibiotic concentrations established for each medium. They also advise the establishment of internal quality controls (control of each medium and antibiotic batch, with the use of reference strains) and international controls (the minimum requirement being a standard concordance for isoniazid and rifampicin of 90%).

The reference drug susceptibility testing methods involving solid media were developed in the 1960s. There were no major advances until the 1980s, with the introduction of the Bactec® system. However, the advances in the 1990s have been impressive, partly because of the HIV epidemic and the need to detect resistances in such patients as quickly as possible. The list of these methods is extensive and includes flow cytometry, rRNA detection, redox indicators, MGIT, MB redox, latex-alpha antigen, E-test, sequencing, ESP
Mycosystem, PCR-SSCP, MycoBact/Alert 3D, Inno-Lipa, ATP detection, heteroduplex PCR, luciferase gene expression, and chromatography.

However, most of these techniques have not yet been adequately validated, and further studies are needed to evaluate them in comparison with the reference methods. To date, only four methods are accepted as references: three are based on solid media (resistance ratio, absolute concentrations, and proportions method), with visual reading after 21 to 28 days, while the fourth uses a liquid medium (the Bactec 460 radiometric technique), with semi-automatic reading and results after 3 to 5 days. To these times we must also add the time required to obtain a sample culture. The proportions method is the most widely used reference technique and is the only recommended option in low- and middle-income countries. Its main limitation is slowness in yielding results. Industrialised countries tend to employ faster techniques involving liquid media, although to date only the semi-automatic Bactec 460TB system is accepted as offering good correlation with the reference method. The fully automatic (non-radiometric) systems, which are as fast as the Bactec method, should be regarded as still being in the development phase. They represent the future trend in countries that do not have economic restrictions.

*M. tuberculosis* drug susceptibility studies can be made based on phenotypic or genetic techniques, as will be described below.

**Drug susceptibility testing based on phenotypic techniques**

The study of *M. tuberculosis* resistance based on phenotypic methods can be made using solid or liquid media, or using new technologies that are still in the evaluation phase.

**Drug susceptibility testing of M. tuberculosis in solid medium**

The methods most widely used in solid medium are:

1. *Method of proportions in Löwenstein-Jensen medium*, via either the indirect technique (culture of various centesimal dilutions) or the direct technique (in samples with high bacterial loads). This is the only recommended option in low- and middle-income countries.

2. *Sensitivity testing in Middlebrook media (7H10 or 7H11)*, using plates with Middlebrook semi-synthetic media. Results can be obtained after 2 to 3 weeks.

3. *E-test system*. This method involves the use of strips impregnated with increasing concentrations of different antimicrobial agents. The mycobac-
Drug susceptibility testing of M. tuberculosis in liquid medium

The great advantage of this type of medium is the savings in time (results in 4-14 days) and its simpler manipulation. However, its high cost, the use of radioactive material (in the case of the Bactec system), and the fact that most of these techniques have not been sufficiently compared contraindicate their use in low- and middle-income countries. The most widely used methods involving liquid media are the following:

1. **Bactec 12B system.** This is an adaptation of the solid-medium proportions technique. It is the only liquid-medium procedure to have undergone extensive evaluation and that has been validated in relation to the reference techniques. The method is widely used in industrialised countries because of its rapidity, simplicity, reproducibility, and concordance with the classic proportions technique.

2. Other liquid systems (**ESP II, MB-BacT, MGIT, Bactec 9000MB**). Despite their very promising future, most of these techniques have not been sufficiently compared or validated.

3. **Broth microdilution method.** Some authors have proposed broth microdilution methods to determine the MICs of chemotherapeutic agents against *M. tuberculosis*, and have suggested the use of Alamar blue or diphenyltetrazolium bromide to facilitate MIC reading. This allows the detection of resistance to rifampicin in only 3 days.

4. **Macrodilution method.** This is an adaptation of the Bactec 60 system to determine the MIC and minimum bactericidal concentration of the strains of *M. tuberculosis* and other mycobacteria in relation to each of the drugs analysed. The system is easy to use in drug-association synergy studies.

In vitro susceptibility studies of M. tuberculosis using new technologies

The appearance of multidrug-resistant TB has increased the need for methods capable of rapidly determining resistance to antituberculous drugs. These technologies, which are still in the evaluation phase and thus have not been adequately validated, comprise the following:
1. **Luciferase reporter phage assay.** In this method, live mycobacteria are infected with TM4 mycobacteriophages expressing the luciferase gene. After adding luciferin substrate, light photons are emitted. The amount of light is proportional to the number of viable bacilli present. If the mycobacteria come into contact with an antituberculous drug to which they are sensitive, bacterial destruction will result and the phages will be unable to infect them, thereby resulting in the cessation of light emission. Results are obtained in a few days.

2. **Phage amplified biologically (PhaB) system.** This technique uses a mycobacteriophage presenting a lytic cycle within *M. tuberculosis* and *M. smegmatis*. The advantages of this method are its simplicity, rapidity (4 days), reduced cost, and biosafety (since many mycobacteria are destroyed by the phage), and it does not require the use of costly equipment. At present, the concordance of the technique with the proportions method is good for rifampicin (95%), but insufficient for isoniazid (85%).

3. **Flow cytometry.** The advantages of this system are its rapidity (24-30 hours), simple performance, and use of inexpensive reagents. However, its disadvantages include the high cost of the flow cytometer and the need to work under high biosafety conditions. The system has been used with good results in *in vitro* studies of environmental mycobacteria susceptibility (*M. avium, M. marinum, M. fortuitum*, and *M. gordonae*) to many antibiotics.

**Drug susceptibility testing based on genetic techniques**

These methods, especially those relating to genetic polymorphisms, require the availability of a specialised infrastructure that is very costly, and thus tests are restricted to research laboratories in industrialised countries. The most frequently used genetic techniques are described below.

**DNA probes**

The use of commercial DNA probes has been proposed in the study of drug resistance of *M. tuberculosis* strains to rifampicin and isoniazid. The sensitivity and specificity of this test after 5 days of incubation is 95% for isoniazid and 100% for rifampicin.

At present, attempts are being made to develop techniques that can allow early identification of resistance to different drugs based on the detection (either directly from the clinical sample or based on culture) of the point mutations responsible for generation of resistant phenotypes. This requires
extensive knowledge of the genetic targets involved in the resistance mechanisms. The most important targets of such resistance to isoniazid and rifampicin are reviewed in Chapter 11.

**Single-strand conformation polymorphism**

The study of resistance based on cultures has been proposed, focusing on four target genes: \textit{katG}, \textit{inhA}, and \textit{ahpC} for isoniazid, and \textit{rpoB} for rifampicin. The methodology is based on the use of the single-strand conformation polymorphism technique, which allows the detection of a single mutation in a region of single-strand DNA from its altered secondary structure with respect to the single-strand DNA of wild strains. At present, this method can be standardised and can be used in laboratories possessing the required infrastructure.

**INNO-LipA solid-phase hybridisation**

Of the methods based on genetic procedures developed in the last few years, special mention should be given to Innogenetic’s INNO-LipA solid-phase hybridisation kit, which can detect over 80% of the mutations responsible for resistance to rifampicin in the \textit{rpoB} gene. The system uses nitrocellulose strips containing a series of probes that, upon hybridisation with the amplified product, perform two functions: 1) verification that the strain belongs to the \textit{M. tuberculosis} complex; and 2) full coverage of the \textit{rpoB} gene, thereby confirming the existence of up to 86% of all mutations responsible for resistance to rifampicin. The technique can be applied directly to the clinical sample or culture, and offers excellent correlation with the traditional methods. This is a rapid method that, in the event of resistance to rifampicin, allows us to suspect that the strain is multidrug resistant, since 90% of all strains resistant to rifampicin are also resistant to isoniazid.

**Diagnosis of tuberculosis using genetic amplification techniques**

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<td>The basis of genetic amplification techniques is to produce millions of identical copies of a specific and known nucleic acid sequence (DNA or RNA). These techniques are rapid (results available in less than 1 day) and highly sensitive, although they also have several limitations.</td>
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In smear-positive samples, these techniques offer very high sensitivity (>98%) and specificity (>98%), although in such cases the diagnosis has already been established by staining. Variability is greater in samples with smear-negative results, with the techniques that amplify RNA offering increased diagnostic possibilities. However, in these cases, a positive amplification result does not confirm the diagnosis of TB (1-5% false-positive results), and a negative reading does not rule out the diagnosis (sensitivity, 50-80%). Therefore, the result must be interpreted for each case in such situations.

The main disadvantages of these amplification systems are the false-negative results generated by reaction-inhibiting substances—particularly in non-respiratory samples—as well as the clinical interpretation of a positive result. Based on these limitations and considering the high cost of these techniques, their use is absolutely contraindicated in countries with low- or middle-income levels.

In the past decade, a series of molecular biological techniques have been developed that are able to amplify the specific DNA and RNA sequences of the *M. tuberculosis* complex. Based on a single copy of DNA or RNA, and using an enzymatic process, they can generate millions of copies of the target nucleic acid, thereby facilitating the detection of *M. tuberculosis*. This technology has made it possible to overcome the main problems inherent in conventional microbiological techniques, allowing rapid diagnosis (between 2-8 hours) and improving the sensitivity of the traditional culture methods.

After solving some of the problems prevalent in the early days of these techniques, it is now possible to find many commercially available amplification systems that provide all the reagents needed and that are able to function under standardised conditions. The development of PCR technology has been followed by the development of second-generation genetic amplification systems, such as strand displacement amplification, ligase chain reaction (LCR), Q beta replicase, and transcription-mediated amplification. In general, these systems involve three fundamental steps: 1) preparation of the sample to eliminate inhibitor substances of amplification; 2) amplification of the nucleic acid specific to the *M. tuberculosis* complex, with different formats capable of amplifying both mycobacterial DNA and RNA; and 3) detection of the amplified product using various methods.

The evaluations made with clinical samples involving smear-positive results have yielded excellent results in terms of sensitivity (>98%) and specificity (>98%) for both DNA and RNA amplifying systems. Variability is greater in samples with smear-negative results, with the techniques that amplify RNA offering increased diagnostic possibilities. This finding is easily explained, since by amplifying ribosomal RNA, these techniques increase
the amplification target 2000 to 3000 times, whereas in the case of techniques that use DNA, the target is present in the form of only a few copies in the microbial genome. Thus, with the AMTDT-2 system (one of the most extensively evaluated RNA amplification systems), sensitivity ranges from 83% to 85% in samples with smear-negative microscopy, and from 65% to 77% in non-respiratory clinical samples. In turn, PCR and LCR have been the most widely evaluated DNA amplifying systems. The studies based on PCR have yielded sensitivities of 50% to 60% in samples with smear-negative microscopy, with a significant reduction in the case of non-respiratory samples, owing to the number of reactions that are inhibited. This phenomenon does not occur with LCR, thus reflecting improved sample processing. The sensitivity and specificity performances of LCR in respiratory samples with negative smear microscopy findings vary from 53% to 72%, and from 71% to 78% in non-respiratory samples.

Based on the above, these techniques would have excellent applications in samples with smear-positive results, where, owing to high sensitivity and specificity, a positive reading would establish the diagnosis of TB, whereas a negative reading would strongly support the existence of mycobacteria not belonging to the *M. tuberculosis* complex. However, it should be pointed out that in these samples the diagnosis of TB has already been established by a more economical and simpler technique (i.e., smear microscopy). Moreover, over 99% of positive smear microscopy findings correspond to *M. tuberculosis* in countries with a high prevalence of TB disease, causing such sophisticated techniques to lose their potential use in low- and middle-income countries. Further, in samples with negative smear microscopy results, a positive amplification reading does not ensure the diagnosis of TB (1-5% of false-positive results), while a negative reading does not rule out the diagnosis (sensitivity of 50-80%). In these situations, the result must be interpreted with caution for each case.

One main disadvantage of these amplification systems is the false-negative results generated by interference by substances that inhibit reactions, particularly in non-respiratory samples. Another limitation is the clinical interpretation, which may involve a positive reading. For example, in most studies evaluating these systems, a small proportion of samples (1-5%) have a positive amplification result lacking culture confirmation and without clinical justification. In such cases, the presence of very few bacteria can result in a positive reading. In sites such as the pleura and meninges, a positive reading is always indicative of disease, although this is not the case in the lungs, where persons with inactive residual TB or healthy infected individ-
uals always carry a significant number of bacilli. These false-positive readings due to the different amplification reactions reflect the problem posed by such sensitive techniques, and they are considered a major limitation. Another limitation is the fact that many of these techniques are not fully automated, thereby complicating work and increasing the risk of contamination by amplicons during manual processing of the samples.

Based on these limitations, it must be accepted that these techniques simply represent one more available diagnostic tool, whose results must be interpreted in the context of each individual patient—as a result of which they cannot be introduced for the routine diagnosis of TB. Moreover, their high cost only allows them to be used in rich countries; utilisation is absolutely contraindicated in low- and middle-income countries.

In summary, the amplification techniques currently available for the diagnosis of TB can be classified according to the nature of the amplified nucleic acid component (DNA or RNA).

**Techniques that amplify mycobacterial DNA**

*Polymerase chain reaction*

PCR has been by far the most widely used DNA amplification technique for diagnosing TB. The method involves the serial repetition of three well-differentiated steps (Figure 47): 1) denaturation of the double-strand DNA molecule to form single-strand DNA; 2) alignment of the primers (synthetic oligonucleotides) to their complementary sequences; and 3) extension from the primers of a DNA chain complementary to the template DNA region to be amplified. This reaction is mediated by a DNA polymerase (TagDNA polymerase). These three steps represent a single amplification cycle, and the repetition of each cycle implies an exponential increment in the amplification product (Figure 47). In order to perform this technique, it is necessary to have: 1) the DNA fragment to be amplified (present in the clinical sample); 2) primers (synthetic oligonucleotides) that bind to the denaturalised DNA and frame the sequence to be copied; and 3) TagDNA polymerase, which is very stable at high temperatures. Specific probes have been developed for the detection of *M. tuberculosis*, *M. avium*, and *M. intracellulare*.

An automatic PCR technique has been developed, offering not only the attractive feature of automation, but also two new features: the introduction of an internal amplification control, and the use of magnetic particles bound to the specific probe for the capture of amplicons.
Figure 47. DNA amplification by PCR.

_Ligase chain reaction_

LCR comprises the same steps as PCR, although the technique uses four initiators or primers designed to flank the region to be amplified, arranging them in adjacent positions. Thus, the probes can be joined enzymatically via DNA ligase, after the action of DNA polymerase, to form the amplified product, which in turn serves as a template for subsequent amplification cycles (Figure 48). LCx MTB is a semi-automatic system that detects the amplified product via a microparticle enzymoimmunoassay technique.

_Strand displacement amplification_

This system uses specific primers, DNA polymerase, and a restriction endonuclease to yield approximately 10-fold amplification of the original target
DNA after 2 hours of reaction. Although the system is complex, the reaction steps take place individually and, once the reaction has started, require no further monitoring. The technique offers the advantage of being isothermal (except for the initial denaturalisation step at 95°C), and requires no specialised laboratory. Moreover, it can be applied to a single or double DNA strand.
Techniques that amplify mycobacterial RNA

Transcription-mediated amplification

The AMTDT-2 system (Amplified M. tuberculosis Direct Test) is based on the amplification of ribosomal RNA via the synthesis of complementary DNA and RNA, using an enzyme mixture composed of reverse transcriptase and RNA polymerase (Figure 49). This is an isothermal and autocatalytic process designed to amplify mycobacterial ribosomal 23S RNA. The system is completely manual, requires no sophisticated instruments or installations, and can be easily used in any laboratory. Rapid results are obtained (in less than 4 hours), with high sensitivity and specificity. In turn, the AMTDT-3 system, similar to the above, offers the advantage of full automation of the amplification and amplified product detection processes, as well as the incorporation of an internal amplification control.

Initially described in 1988, this technique is based on the incorporation of an oligonucleotide designed to bind specifically to the target nucleic acid (23S rRNA). The system is fully automated, and detection of the amplified product is carried out via fluorimetric testing—the amount of fluorescence generated being proportional to the amount of RNA amplified.

Nucleic acid sequence—based amplification

This system constitutes a commercial development of the previously described transcription-mediation amplification method, and is very similar to self-sustained sequence replication. Amplification (under isothermal conditions) of ribosomal 16S RNA is achieved by the concerted action of three enzymes: reverse transcriptase, RNA polymerase, and RNAase.

Serological diagnosis of tuberculosis

Section summary

There is no justification for spending scarce resources on serological tests in poor countries. Despite the many studies published on the subject, the attempts to develop a serological technique to help in the diagnosis of TB have failed. Major problems related to sensitivity (similar to those of smear microscopy), specificity, and the interpretation of a positive result (inactive residual TB and disease by other atypical mycobacteria) have prevented the technique from being recommended for standardised use in the diagnosis of TB, even in countries with more economic resources. Only in very specific cases of TB with smear-negative results, extrapulmonary TB, and TB in children can this methodology be somewhat useful in diagnosis (despite its low sensitivity), although the result should always be interpreted in the clinical context of the patient involved.
Figure 49. Schematic representation of DNA/RNA transcription-mediated amplification. RT = reverse transcriptase.

Although more than 100 years have passed since the first attempt was made to diagnose TB using blood tests, and despite the many advances in microbiology in recent years, it must be recognised that failure has beset the field of TB serology. Studies evaluating different antigens and techniques have increased in the last two decades, and yet it has not been possible to resolve the problems of sensitivity and specificity. No doubt there have been several
significant advances, but progress has been for the most part unsatisfactory. In short, serological testing has so many limitations that it cannot be used for the routine diagnostic assessment of TB.

The factors that have contributed to the recent advances in the serological diagnosis of TB include the use of more sensitive techniques and more specific purified antigens. From the technical perspective, testing by enzyme-linked immunosorbent assay (ELISA) appears to be the most useful, since it is rapid, can be automated, provides reproducible results, and offers optimum sensitivity performance. The best-known and most widely used purified antigens in recent years have been of a protein and lipid nature.

Current data indicate that the diagnosis of TB in the context of clinical practice, using ELISA with the best antigens available, would require a specificity of close to 100% in order to ensure good test predictive values. To this effect, it would be necessary to adopt cut-off points (to yield a positive result) equal to the averages obtained in the serum of normal individuals plus 3 standard deviations—in which case the sensitivity usually does not exceed 50%.

At present, knowledge is still limited with regards to the dynamics of the appearance of immunoglobulins and their half-lives in the course of TB, and to the antigens that may prove most useful. It is also not clear why some patients with active TB do not have detectable antibody titres at the time of diagnosis. Until these questions are answered, the usefulness of serology in the diagnosis of TB will remain uncertain and the technique will be inapplicable in the context of general use.

Although many of the studies published on this subject have served to define antigens capable of affording very high specificity, the corresponding sensitivity depends on the site and severity of the disease. The best sensitivity corresponds to pulmonary TB with positive smear microscopy (65-85%), but this performance is only slightly better than that afforded by staining. Moreover, sensitivity decreases considerably in the presence of negative smear microscopy results (<50%) and in patients with extrapulmonary TB (<25-30%). Despite such low sensitivity, the best performance could correspond to cases in which smear microscopy cannot contribute to obtaining a rapid diagnosis. Likewise, in children, serological testing has very low sensitivity (<25%), but may be useful in the few cases in which the technique proves positive—particularly since smear microscopy will almost always be negative. Moreover, a positive result in children almost always indicates the presence of TB disease.

The interpretation of a positive result is complicated. It is necessary to emphasise the number of positive results recorded in healthy individuals
with inactive residual TB—regardless of whether they have received treatment or not—and in those with mycobacteriosis. The former group of patients is extremely common in TB endemic regions (comprising all patients healed in the past), in whom a positive result indicates that the disease had existed and has been healed. This phenomenon can be explained by the dynamics involving the appearance and disappearance of TB antibodies. Up to as long as 20 years after TB healing, circulating antibodies may persist with the capacity to respond to serological testing. The same applies to the other mycobacteria involving antigenic determinants crossed with those of \textit{M. tuberculosis}. Thus, the existence of a large pool of infected individuals with inactive latent TB further limits the possibility of employing serological diagnostic tests in low- and middle-income countries.

**Microbiological techniques as an aid in tuberculosis epidemiology**

<table>
<thead>
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<th>Section summary</th>
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<td>Recent studies involving molecular biological techniques have been very beneficial with regards to certain aspects of the epidemiology of TB. The most widely used marker, in view of its great discriminative capacity, has been the study of restriction fragment length polymorphism (RFLP), using the insertion sequence IS6110. The genome of \textit{M. tuberculosis} contains on average 5 to 20 copies of IS6110, located at various positions along the chromosome. These studies have contributed valuable information for: 1) determining the general epidemiological pattern of TB strains in a given population; 2) outbreak investigations and TB control; 3) differentiating between relapses and exogenous reinfections; and 4) studying cross-contamination in the laboratory. The results obtained with this technique must always be assessed in combination with the information afforded by conventional epidemiology.</td>
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Traditionally, the study of the epidemiology of TB has been complicated by the absence of adequate strain markers. The only marker used until recently—the study of susceptibility to mycobacteriophages—has significant limitations owing to its low discriminating capacity. Other methods investigated, such as the enzyme type, serotype, and drug susceptibility pattern, have been unable to find practical application.
At present, the application of molecular techniques in epidemiology makes it possible to precisely determine the strains circulating in a given population. Such techniques include genomic enzyme restriction followed by field electrophoresis, restriction-hybridisation with probes complementary to repeated sequences in the genome of *M. tuberculosis*, and PCR-mediated amplification polymorphism. The most widely used, in view of its excellent discriminative capacity, is the study of restriction fragment length polymorphism (RFLP), using the insertion sequence IS6110. A standardised protocol has been developed, allowing comparisons of the results obtained from different laboratories, as well as the establishment of large-scale databases. This marker can be used for: 1) determining the general epidemiological pattern in a given population; 2) controlling epidemics; 3) differentiating between relapses and exogenous reinfections; and 4) studying cross-contamination in the laboratory.

The genome of *M. tuberculosis* contains a large number of copies (between 5-20 copies) of the IS6110 insertion sequence, located at various positions along the chromosome. Strains that are not related epidemiologically present their own restriction-hybridisation patterns and exhibit a high degree of polymorphism. In contrast, strains that are related epidemiologically exhibit identical patterns, and a clonality relation can easily be established. In strains with low or no presence of the IS6110 insertion sequence, it is necessary to use other molecular markers associated with increased polymorphism. These studies can be very useful for establishing the transmission dynamics in the community. However, the results obtained must be interpreted in the context of the study setting, and must always be assessed with the information provided by conventional epidemiology methods. Despite the attractiveness of these methods, they are very expensive to carry out and require expertise for interpretation. This disadvantage, together with the high rate of TB transmission in countries with low or middle incomes, make the application of these techniques impractical in these geographical settings.

**Other non-microbiological techniques**

**Section summary**
Of all the new techniques that have been developed in recent years in relation to the analysis of serosal fluids from patients with suspected TB, adenosine deaminase determination has shown great usefulness, with a sensitivity and specificity in excess of 95% in studies from highly endemic regions. The technique has been
sufficiently validated to allow it to be used in cases of uncertainty (i.e., pleural TB) in middle-income countries, but not in poorer parts of the world.

In countries with high disease burdens, when pleural or other serosal TB disease is suspected, antituberculous treatment should be initiated if performing a biopsy is not possible. A series of simple laboratory tests applied to the fluid may also be helpful.

Many of the studies of pleural fluid and other serosal components in patients with TB serve to guide diagnosis. With few exceptions, unless there is positive smear microscopy of sputum or pleural fluid, a biopsy is needed to confirm the diagnosis of pleural TB. Samples obtained by this method must always be processed for smear microscopy, culture, and histological study.

**Interpretation of pleural fluid testing**

Serosal fluid affected by TB can be characterised as an exudate with a high protein concentration and glucose levels generally in excess of 60 mg/dl (3.3 mmol/l). Low glucose levels are associated with concomitant empyema, rheumatoid arthritis, delayed diagnosis, or advanced disease with pleural fibrosis and pachypleuritis. The fluid is generally acidic, and the total leucocyte count is usually less than 5000 cells/l. The typical finding involves more than 50% of mature lymphocytes, although polymorphonuclear cells may predominate in the early stages of the disorder. Mesothelial cells are scarce, with generally fewer than 10 cells per 1000 leucocytes. This absence of mesothelial cells has been attributed to the fibrinous layer covering the pleural surface, which prevents these cells from desquamating.

**Adenosine deaminase**

The determination of adenosine deaminase (ADA) in pleural fluid and other serosal fluids has been shown to be very effective in diagnosing pleural TB in recent years. This enzyme, which is involved in purine catabolism and whose main physiological activity takes place in lymphoid tissue, affords great sensitivity and specificity (>95% in highly endemic countries), although false-positive results have been detected in subjects with metapneumonic effusion, empyema, rheumatoid arthritis, lupus erythematosus, or lymphoma.

The usefulness of determining this enzyme has been sufficiently validated by numerous studies, thus allowing it to be incorporated in the
diagnostic algorithm for suspected TB effusions. The sensitivity of microbiological techniques with regards to serosal TB is very low; as a result, the diagnosis of TB can be accepted in the case of a patient with suggestive clinical manifestations, radiographic evidence, and a positive ADA assay. This is particularly valid in places where a pleural biopsy cannot be performed, or when meningeal, peritoneal, or pericardic TB is suspected. The positivity cut-off points for this technique must be established by the laboratory performing the test, and depend on the origin of the fluid. Thus, the cut-off points for pleural or peritoneal fluid usually range between 40 to 45 international units, whereas in the case of cerebrospinal fluid the range is usually 9 to 10 international units.

The technique is neither complex nor expensive, as a result of which certain reference centres (depending on the population) in middle-income countries may benefit from using the procedure. The technique is not indicated in poorer regions where, because of the limited resources available and the high endemic rates, the initiation of empirical TB treatment is recommended instead.

**Other determinations in serosal fluid with suspected tuberculosis**

Another test that has been shown to be useful in studying pleural TB is the determination of lysosyme (muramidase) in the fluid, and especially the ratio of lysosyme to the equivalent assay in serum. A ratio of over 1.2 has been shown to afford excellent sensitivity and specificity, with some false-positive results attributable to empyema and rheumatoid arthritis. However, further studies are needed to validate the technique before it can be recommended for routine use.

Since in tuberculous pleural fluid the proportions and absolute numbers of T lymphocytes are considerably increased versus blood values, the number of lymphokines caused by these cells will also be increased. One such lymphokine, interferon, has been shown to be a useful parameter in the diagnosis of pleural TB.

Lastly, there is evidence suggesting that levels of some tumour markers, such as immunosuppressive acidic protein (IAP) and alpha1-acid glycoprotein, are significantly higher in tuberculous pleural fluid compared with in neoplastic pleural effusions. However, the measurement of these markers is not indicated in low- and middle-income countries.
Recommended reading for the chapter


Chapter 9 - Treatment of tuberculosis

Chapter summary
The treatment of tuberculosis (TB) is based on two bacteriological considerations: the combination of drugs to avoid the selection of drug resistance, and the need for prolonged treatment to ensure that all bacteria in their different phases of metabolic growth are effectively destroyed. Clinical and microbiological research between 1950 and 1970 led to the conclusion that the best treatment for a patient with sensitive TB is 2HRZ/4HR. However, considering that in many parts of the world pharmacological treatments have been used indiscriminately, resistance to isoniazid (H) is usually more than 4%. Thus, to prevent treatment failure and further drug resistance in this setting, ethambutol (E) should always be used during the first 2 months of therapy. It has also been demonstrated that if strict supervision of observed therapy is not established for the full duration of treatment, there is a high risk of developing resistance to rifampicin (R). For this reason, in areas where supervision of drug administration cannot be guaranteed in the second phase, ethambutol should be prescribed instead of rifampicin during the second phase, and treatment should be extended to 8 months. These inconveniences with rifampicin are clearly obviated if several drugs are used in combination (especially H+R and H+R+Z), at fixed doses, and incorporated in the same tablet—an approach that should always be recommended. Thus, the treatment scheme advised for all initial patients should be 2HRZE/4HR or 2HRZE/6HE.

Although all first-line drugs (isoniazid, rifampicin, pyrazinamide [Z], ethambutol, and streptomycin) are well tolerated, personnel treating and caring for patients should be aware of the few associated side effects. It is important to know how to proceed in the event of side effects, to deal with food-drug interactions, and to manage patients presenting with special conditions, such as renal failure, severe liver impairment, or pregnancy. All these aspects of management will be analysed in detail in this chapter, and must always be dealt with by specialist physicians in the field.

Lastly, it must be emphasised that extrapulmonary TB should be treated in the same way as pulmonary TB, and that it is essential to know when surgery is indicated, as well as when the use of corticoid therapy is warranted. Hospitalisation of patients with TB disease should be based on the severity of the disease, and occasionally to ensure adherence to therapy; hospitalisation should never be based only on the patient having TB.
A brief history

Section summary

The history of TB treatment comprises two major periods: the chemotherapeutic period, corresponding to the last 50 years during which TB became a curable disease, and the period covering all previous attempts to overcome this terrible disease. The pre-chemotherapeutic period of TB management made use of many different resources over the centuries, although practically none were particularly efficacious. The early years of the chemotherapeutic period gave rise to the currently accepted bacteriological bases for the treatment of TB, including the need to combine several drugs to counter the development of drug resistance. During the very early periods of the chemotherapeutic era (the late 1940s), it became apparent that resistance to antituberculous drugs was the result of flawed patient management on both the individual and community level.

Chapter 3 provides a detailed explanation of the efforts to combat TB throughout history. Up until the introduction of chemotherapy, most of these attempts were futile. All this began to change with the introduction of streptomycin by Waksman and Schatz in 1943, and its use against TB since 1944. This antibiotic led to clinical and radiological improvement, with negative conversion of smear microscopy in diseased individuals. Its only disadvantages were toxicity (as the drug was not very purified at the time) and the fact that after 2 to 3 months of treatment a significant proportion of patients suffered a relapse with TB that was no longer susceptible to streptomycin. Drug resistance, another means of M. tuberculosis to defend itself, was becoming known. Thus, the main disadvantage associated with streptomycin was the appearance of bacilli resistant to the drug in patients who had already received streptomycin therapy for some time (e.g., routinely for 2 to 3 months).

In 1944, therapeutic testing began with para-aminosalicylic acid, which was shown to have similar efficacy as streptomycin, in addition to the same toxicity and resistance problems. However, in 1949 it was seen that para-aminosalicylic acid, combined with streptomycin, also delayed or prevented the development of resistance to streptomycin. Still, a treatment offering efficacy, low cost, easy administration, and no side effects remained elusive until the introduction of isoniazid, or isonicotinic acid hydrazide, a substance that had been first synthesised in 1912 but that was not experimentally tested against TB until 1951. In view of its advantages, isoniazid was referred to as the “miracle drug” against TB, and up until today there is no drug that surpasses it. However, it was soon realised that isoniazid alone could not
resolve the problem of TB, and bacteria resistant to the drug rapidly appeared. Treatment was then shifted towards combining isoniazid with streptomycin and para-aminosalicylic acid in 1955. This long-term combination therapy became for the first time the only treatment capable of completely curing TB, without encouraging the emergence of drug-resistant mutants.

Based on the above considerations, the first and most important microbiological principle for TB treatment was established: the combination of drugs to counter the development of drug resistance. It also became evident that very prolonged treatment was required to prevent disease relapse. This second major microbiological principle was established a few decades later, based on universally accepted theoretical models. It is therefore essential to review these microbiological considerations in order to understand the current management requirements recommended for TB.

Microbiological bases for the treatment of tuberculosis

Section summary

The microbiological fundamentals of TB therapy were established between 1950 and 1970. The first principle attempts to respond to the large number of multiplying bacilli present in the tissue of a diseased host, and to the ability of \textit{M. tuberculosis} to mutate after multiple divisions. Thus, to counteract this property of the tubercle bacilli, it is essential to know that several drugs must always be used in combination in order to avoid the development of drug-resistant mutants that can undermine the efficacy of a medication.

The second principle attempts to respond to the variable growth capacity of \textit{M. tuberculosis} in different locations within lesions, which varies depending on metabolic status. For this reason, extended treatments are needed, to allow treatments to act upon the latent bacterial populations that divide very little during treatment because the prevalent surrounding environmental conditions are not conducive for proliferation.

At present, it is widely accepted that TB chemotherapy should be based on two important microbiological considerations: the combination of drugs to avoid the development of resistance, and the need for prolonged chemotherapy to prevent disease relapse.

Prevention of resistance: the need for drug combinations

If treatment is started in a patient with cavitary TB using only one drug, the patient experiences a first phase during which most of the bacteria are elimi-
nated and the symptoms improve. This initial phase is followed by a second phase in which treatment selects the resistant bacteria, which in a short time become the dominant microbial population—the “fall and rise” phenomenon. In addition, the drug will lose its efficacy for the rest of the life of the patient, since TB resistance is chromosomal, definitive, and irreversible. In effect, although all the bacilli present in a colony originate from a single cell, the bacilli do not show a homogeneous behaviour against the different antituberculous drugs. Thus, beyond a certain number of microorganisms, spontaneous natural mutants arise during the successive bacillary divisions, which will be resistant to some of the drugs used. Such mutation is random and independent of the environment involved, but is closely related to the number of bacilli present and the type and concentration of the medication administered. The approximate number of bacilli needed for the appearance of a natural mutant resistant to each of the drugs is shown in Table 5. Table 6 describes the bacterial populations calculated for each of the different types of TB lesions. Thus, in a culture of \textit{M. tuberculosis}, spontaneous natural mutation gives rise to one microorganism resistant to isoniazid for every \(10^5\) to \(10^6\) bacilli. This mutation is independent for each of the different drugs used, since different genetic targets are involved. The probability that resistance to two drugs may develop is equal to the product of their respective mutation rates.

\begin{table}[h]
\centering
\begin{tabular}{|c|l|}
\hline
\textbf{Drug} & \textbf{Number of bacilli} \\
\hline
Isoniazid (I) & \(1 \times 10^5\text{-}10^6\) bacilli \\
Rifampicin (R) & \(1 \times 10^7\text{-}10^8\) bacilli \\
Streptomycin (S) & \(1 \times 10^5\text{-}10^6\) bacilli \\
Ethambutol (E) & \(1 \times 10^5\text{-}10^6\) bacilli \\
Pyrazinamide (Z) & \(1 \times 10^5\text{-}10^6\) bacilli \\
Quinolones & \(1 \times 10^5\text{-}10^6\) bacilli \\
Others & \(1 \times 10^3\text{-}10^6\) bacilli \\
\hline
\end{tabular}
\caption{Number of bacilli required for the appearance of a mutant resistant to different drugs}
\end{table}

Thus, all monotherapeutic regimens (real, or masked by combination with drugs to which resistance has previously been established or which prove ineffective) inevitably lead to treatment failure and to the development of resistance. When two or more drugs are administered, the risk of resistance is practically zero, since the volume of this bacillary population is not usually attained in the human body (\(10^{13}\) for H+R and \(10^{19}\) for H+R+E).
Table 6. Estimated bacterial populations within different TB lesions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Bacillary Population</th>
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<tbody>
<tr>
<td>Smear-positive TB</td>
<td>$10^7$-$10^9$ bacilli</td>
</tr>
<tr>
<td>Cavitary</td>
<td>$10^7$-$10^9$ bacilli</td>
</tr>
<tr>
<td>Infiltrating</td>
<td>$10^4$-$10^7$ bacilli</td>
</tr>
<tr>
<td>Nodules</td>
<td>$10^4$-$10^6$ bacilli</td>
</tr>
<tr>
<td>Adenopathies</td>
<td>$10^4$-$10^6$ bacilli</td>
</tr>
<tr>
<td>Renal TB</td>
<td>$10^4$-$10^6$ bacilli</td>
</tr>
<tr>
<td>Extrapulmonary TB</td>
<td>$10^4$-$10^6$ bacilli</td>
</tr>
</tbody>
</table>

The need for prolonged treatments.

Bacillary populations of *M. tuberculosis*

As has been mentioned, *M. tuberculosis* is a strict aerobe whose growth and metabolic activity is proportional to the surrounding oxygen partial pressure and pH. The ideal conditions for the bacilli comprise a pH of 7.40 and an oxygen pressure of 110 to 140 mm Hg. Based on the different characteristics of the environment in which *M. tuberculosis* is found, four tubercle bacilli growth modalities have been established that condition the bases of the currently used drug combinations and the duration of treatment. The bacillary populations can be described as follows:

*Metabolically active and under conditions of continuous growth*

This population is also referred to as emergent flora and represents most of the bacilli, with a population of $10^7$ to $10^9$. These bacilli are easily detected in the expectorations of diseased individuals, and are located within the cavitary walls, where the oxygen pressure and pH are ideal for growth. These bacilli are located extracellularly and are responsible for the failure of anti-TB treatment and for the development of resistance if they are not homogeneously eliminated. This population is rapidly exterminated by the bactericidal action of isoniazid, and less rapidly by streptomycin and rifampicin. The ability of a treatment regimen to eliminate a particular bacterial population is referred to as its bactericidal activity, which can be assessed by the percentage of negative conversion of cultures at the end of the second month of treatment. Early bactericidal activity refers to the capacity of the drug to kill bacteria within the first 2 months of therapy. Some consider that negative conversion of cultures after 2 months may also be an indication of the sterilising capacity of the drug (see below).
**Bacilli in the acid-inhibition phase**

This is a scanty numerous population of about $10^3$ to $10^5$ bacilli. Their growth is inhibited by the acid medium contained within the phagolysosomes of the macrophages in the case of bacteria located intracellularly, or by the acidity in the necrotic inflammatory zones of the cavitary wall. The deficient oxygenation in the necrotic tissue also helps to inhibit their growth. Thus, since these bacilli lack metabolic activity, they are unlikely to be eliminated by the administered drug. For this reason, this population and the bacteria in sporadic multiplication phase constitute the so-called persistent bacterial flora, which represent the main source of microbiological TB relapse. The most active drug against this particular bacterial population is pyrazinamide. The action of isoniazid and rifampicin decreases by almost 50% when the pH of the environment decreases from 6.6 to 5.4, whereas the activity of pyrazinamide increases with increased acidity. The ability of drugs to eliminate this bacillary population and its sporadic multiplication is referred to as its sterilising activity, and can be quantified by the number of relapses that follow treatment. The sterilising capacity of pyrazinamide has been shown to reduce the duration of treatment to 6 months.

**Bacilli in the sporadic multiplication phase**

This population comprises approximately $10^3$ to $10^5$ bacilli, often located in solid caseum, where the pH is neutral. These bacteria undergo long dormant periods, with occasional and brief metabolic periods that last for hours. As a result, the administered drug is only able to destroy these bacteria during the brief metabolic periods, which may not occur during the course of therapy. Therefore, this population, together with bacilli in the acid-inhibition phase, are responsible for microbiological relapses after the conclusion of therapy. On the other hand, the limited and occasional activity of these bacteria prevents them from developing resistances. The drug of choice for eliminating this population is rifampicin, because of the rapid onset of its sterilising action (15-20 minutes, versus 24 hours as in the case of isoniazid).

**Persistent or totally dormant populations**

These bacteria lack metabolic activity, as a result of which anti-TB treatment is not effective against them. Probably only the individual host defence mechanisms are able to have some effect on this population. One hypothesis is that these bacteria are one of the populations responsible for relapse in patients with severe immunodeficiency.
Rationale for an ideal initial treatment regimen

Section summary
The microbiological bases for TB treatment indicate that the combination 2HRZ/4HR is ideal in all initial cases of the disease where sensitivity to all the drugs can be guaranteed. However, the high initial resistance rate to isoniazid (H) that is found in many parts of the world makes it necessary to add a fourth drug (ethambutol [E]) to this initial phase of therapy. Indeed, this resistance to isoniazid is found in almost all low- or middle-income countries, and these nations should recommend the use of a fourth drug in their respective National Tuberculosis Control Programmes (NTPs). Moreover, administering these drugs either daily or 2 to 3 times a week has equivalent efficacy, which makes the 2HRZE/4H_R or 2HRZE/4H_R regimens equally recommendable. However, in order to be able to recommend regimens with rifampicin (R) in the second phase, it is essential to ensure adherence to treatment in both phases, for in the event of non-compliance, there is a serious risk of developing resistance to rifampicin—which is the drug requiring protection at the present time.

The regimen 2HRZE/6HE (or 2HRZE/6HT in areas where the prevalence of HIV infection is very low), although somewhat less effective than 2HRZE/4HR, should be recommended as the initial regimen in low- and middle-income countries where strict supervision of administration cannot be guaranteed in the second phase—a situation that is much more frequent than admitted by the different NTPs.

Drug formulations containing H+R, H+R+Z, H+R+Z+E, H+E, and H+T, in fixed-dose combinations in the same tablet, should be recommended for standard use in the context of control programmes. These formulations facilitate the supervision of patient adherence to therapy, improve compliance, and especially prevent partial default of treatment. This last factor is associated significantly with the emergence of drug resistance. The other drug combinations for which bioavailability studies are not available should be avoided.

Therefore, unless strict supervision of the administration of the entire treatment in the first and second therapeutic phase is guaranteed, the ideal regimen for use in low- and middle-income countries is 2HRZE/6HE. Only when such supervision can be guaranteed can 2HRZE/4HR be considered, regardless of whether the second phase includes daily or intermittent administration. Protection against the selection of resistance to rifampicin, and the possible invalidation of this drug, are the reasons for these differences in treatment recommendation.

The need to combine drugs in the fight against TB has already been mentioned. The drugs that selectively act upon the different bacterial populations are isoniazid (H), rifampicin (R), and pyrazinamide (Z); these three drugs
should constitute the basis for an effective TB treatment regimen. The best treatment for TB tested to date uses isoniazid, rifampicin, and pyrazinamide during the first 2 months of treatment, followed by isoniazid and rifampicin for another 4 months. This regimen offers potent bactericidal and sterilising action, with few relapses (less than 1-2%) and side effects. Pyrazinamide should only be administered for 2 months, since after this period the great majority of lesions and cells presenting acidic conditions would have disappeared (i.e., the conditions of preferential action for this drug).

Thus, in all cases of initial TB in which sensitivity to all the drugs can be guaranteed, the ideal treatment is 2HRZ/4HR. However, there are important conditioning factors—microbiological (possible initial resistance to some of these drugs), logistical (impossibility of guaranteeing supervision of administration), and cost (some drugs are very expensive)—that make it necessary to consider a series of variables in relation to this theoretically ideal regimen. These recommendations can be outlined using a series of questions:

**Why the need for a fourth drug in the initial phase?**

Isoniazid and streptomycin (S) have been used extensively worldwide in the past decades, although sometimes inappropriately (e.g., frequent or masked monotherapies). This has caused the initial resistance rates to these two drugs to be high in many parts of the globe. The situation particularly affects isoniazid, which remains the basis for initial treatment. In the event of initial resistance to isoniazid, and considering the high proportion of natural mutants resistant to pyrazinamide that is in all bacterial populations (Table 5), it can be assumed that rifampicin is the sole remaining agent that can be used against very large microbial populations. For this reason, in areas with a high initial resistance to isoniazid, a fourth drug must be added during the first 2 months of treatment when the bacillary population is very high. By the second treatment phase, this population would have been reduced to such low levels that even with initial resistance to isoniazid, the number of surviving bacilli would be too small to generate a mutant resistant to rifampicin (Table 5). At present, unless the initial resistance rate to isoniazid is known (based on well-designed and conducted studies) and is shown to be less than 4%, a fourth drug should always be added during the first 2 months of therapy. This criterion is applicable to practically all low- and middle-income countries, as well as to many wealthier nations.
What is the ideal drug to add to isoniazid, rifampicin, and pyrazinamide in the initial phase?

Having accepted the need to add a fourth drug in the first phase of TB treatment, the choice may centre on streptomycin or ethambutol (E). The latter is preferable for both microbiological and practical reasons. The microbiological reason is that streptomycin has been as extensively used as isoniazid and its initial resistance rate in much of the world is therefore also high. Since the reason for adding a fourth drug is to prevent the emergence of resistances, streptomycin should be rejected in favour of ethambutol, a drug to which very few initial resistances have been described. In turn, the practical reason is that streptomycin must be administered via the intramuscular route, which requires the availability of a nurse to perform injections. This complicates the administration of treatment at the most peripheral levels of health care, i.e., in areas where the person responsible for treatment may be a less qualified health worker or perhaps someone who does not work in health care (e.g., community leaders, teachers). Furthermore, in very poor areas where disposable syringes are unavailable, the risk of HIV transmission must also be considered.

Can the drugs be administered only 2 to 3 times a week?

Intermittent treatments

At present, unless the initial resistance rate to isoniazid is known (based on well-designed and conducted representative studies) and is shown to be less than 4%, a fourth drug should always be added during the first 2 months of therapy. *M. tuberculosis* multiplies very slowly (approximately once every 14-24 hours), which allows drugs to be effective even when administered in a single daily dose. Moreover, for over two decades it has been known that the administration of a single dose of isoniazid is able to inhibit bacterial growth for 4 days, as a result of which it is equally effective to administer either two weekly doses or a daily dose. However, when the interval between doses exceeds 4 days, efficacy decreases. With rifampicin and ethambutol, growth inhibition is found to persist for more than 8 days, although the bactericidal action of rifampicin makes the latter much more effective. In any case, the efficacy of these two drugs is seen to be similar when administered daily or once a week. Similar considerations apply to pyrazinamide, which, at a pH of 5.6, inhibits mycobacterial growth for 9 days, following 24 hours of bacterial exposure to the drug. This inhibitor effect, which pertains to isoniazid, rifampicin, ethambutol, and pyrazinamide, is not applicable when
the combination includes ethionamide (Eth) or thiacetazone (T). Although streptomycin is bactericidal and the other two drugs exert bacteriostatic effects, when the three are combined efficacy decreases dramatically when not administered on a daily basis, particularly in the case of thiacetazone. Therefore, if regimens including isoniazid, rifampicin, ethambutol, and pyrazinamide are recommended, an intermittent style of administration, in the form of two weekly doses, can be used with the same therapeutic safety margin as with daily dosing—the sole requirement being an increase in the amount of isoniazid, ethambutol, and pyrazinamide in each dose (Table 7). The amount per dose of rifampicin should not be increased. However, in order for efficacy to be the same, a minimum of two doses per week is required. Consequently, National Tuberculosis Control Programmes (NTPs) that recommend such intermittent regimens should first ensure strict supervision of administration of the medication.

The fact that some programmes recommend administration 3 times a week (when it has been determined that twice a week is enough) can be attributable to practical reasons. It is in the second phase of therapy (with daily dosing in the first phase) when nonadherence to treatment increases. Hence, if a single weekly dose were to be missed, 2 weekly administrations would suffice to ensure therapeutic efficacy. However, if the problem of missing a dose occurs in the twice-weekly treatment regimen, the patient in effect would have received only a single weekly dose. This is a very dangerous situation, since rifampicin inhibits mycobacterial growth for 8 days and isoniazid does so for only 4 days. In effect, a single weekly dose of these two drugs means that the patient is actually receiving sequential monotherapy with rifampicin for 3 days of the week, with the risk of selecting for rifampicin-resistant mutants.

Furthermore, the use of an intermittent treatment regimen makes supervision of treatment adherence easier. In this case, supervision is necessary only twice a week instead of daily. This option is much less expensive (in the case of rifampicin, the most costly drug, it is not necessary to increase the dose at each administration), and toxicity is similar to that associated with daily dosing. Moreover, it has been suggested that by achieving greater peak concentrations in blood, the selection of resistant mutants is less likely.

Although the drugs could be administered intermittently from the start of treatment (inhibition of mycobacterial growth being achieved from the first dose), it is generally advised to begin therapy with a daily administration phase lasting 1 to 2 weeks, when the maximum bactericidal action takes place. Nevertheless, some studies have shown intermittent administration to be effective from the start, although involving four drugs in initial phases.
Should regimens without rifampicin be used in the second phase?
When are they advisable?

The ideal therapeutic regimen for all initial TB cases comprises 2 months with isoniazid, rifampicin, ethambutol, and pyrazinamide, plus 4 additional months with isoniazid and rifampicin—2HRZE/4HR. However, rifampicin and isoniazid are among the most suitable drugs for treating TB. Considering that the initial resistance rates to isoniazid are already high in many parts of the world, a top priority at present is to protect rifampicin from the development of mutants resistant to it. As has been explained in the previous section, if intermittent medication is provided in the second phase of treatment, each failure in dosing conveys a potential danger of rifampicin monotherapy for several days. If this happens occasionally, no problems are likely and the treatment will still be effective. However, the reality is that the most frequent practice among noncompliant patients is not that of abandoning treatment completely, but that of intermittent discontinuation of one or more of the drugs, which poses the serious risk of the development of resistances to rifampicin. For this reason, rifampicin should never be administered in the second phase of treatment unless strict dosing supervision can be guaranteed.

Economic and operational considerations led the International Union Against Tuberculosis and Lung Disease (IUATLD) to advocate a second treatment phase of 6 months with isoniazid and thiacetazone, administering both drugs in the same tablet, without the need for direct supervision of dosing. This regimen (2HRZE/6HT) is not only much less expensive than the previously mentioned treatment regimen, but also protects rifampicin against possible resistances (since rifampicin is administered only in the first phase, along with three other drugs). This scheme offers a highly bactericidal first phase requiring strict dosing supervision, and a self-administered second phase that could be described as sterilising. Its extensive use in many parts of the world has confirmed its efficacy under TB control programme conditions.

Still, in the past 10 years serious problems have been reported as a result of the frequent and severe toxicity of thiacetazone in AIDS patients. As a result, thiacetazone has been replaced with ethambutol in treatment recommendations. This change still protects rifampicin from resistances, although the initial cost advantage is now lost because the new regimen is similar in cost to the one using rifampicin in the second therapeutic phase.
Should preparations of various drugs at fixed doses in the same tablet be recommended?

For years it has been discussed whether the bioavailability of drugs administered together in the same tablet is the same as when each drug is given separately. The controversy has been particularly intense in relation to rifampicin. Many studies have shown that the combinations of H+R, H+R+Z, and H+T can be used without any problems. The combinations of H+R+Z+E and H+E have also recently been manufactured, and bioavailability studies have reported favourable results thus far.

These formulations, which contain several drugs at fixed doses in the same tablet, should be recommended for use under TB control programme conditions, since they facilitate the supervision of patient adherence to therapy, improve compliance in patients for whom strict supervision is not possible, and especially prevent selective discontinuation of one or more drugs. This last point, in addition to causing treatment failures, promotes the selection of drug-resistant mutants. In theory, the problem of selective discontinuation of treatment should never occur if strict supervision of therapy is ensured. The achievement of strict patient adherence to therapy should therefore be a goal of all NTPs.

Drug combinations other than those specified above should be avoided. In some countries, it is common to find combinations, prepared by local companies, that are different from those specified. These products should strictly be avoided since they lead to the development of resistance owing to the low bioavailability of some of their active substances. The best strategy is to ensure that each country only purchases the drugs and combinations recommended by the respective NTPs, avoiding the acquisition of other products, even in private practice. While this objective is difficult to achieve in many free market—oriented economies, it should constitute a priority aim.

What are the recommended regimens for new TB cases?

Based on all the above considerations, the ideal treatment regimen should always include a first phase of 2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol. The approach in the second phase will depend on whether or not strict supervision of administration is possible. If adherence to treatment can be guaranteed, then the second phase should comprise isoni-
azid and rifampicin for 4 months, administered on a daily basis or intermittently to facilitate supervision. The recommended regimens would therefore consist of 2HRZE/4HR, 2HRZE/4H₂R₂, or 2HRZE/4H₃R₃. However, it must be re-emphasised that the use of these regimens requires strict supervision of drug dosing, which calls for the existence of a well-established, well-run NTP.

If adherence to treatment cannot be guaranteed, then the second phase should consist of 6 months of isoniazid and ethambutol combined in the same tablet. This prevents the selection of rifampicin-resistant mutants, as well as lowers the risk of augmenting the problem of multidrug resistance.

**First-line antituberculous drugs: action and side effects**

<table>
<thead>
<tr>
<th>Section summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first-line drugs for the treatment of TB are isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin. This section discusses these drugs, which are the most effective and well-tolerated treatment options available, and which are associated with fewer adverse reactions as well as lower costs. Other drugs should not be used prior to using these first-line agents. Moreover, in order to facilitate patient adherence and improve tolerance, these medications should be administered together in a single daily dose.</td>
</tr>
</tbody>
</table>

Table 7 describes the mechanism of action, target bacterial population, most common side effects, and most frequent pharmacological interactions of the five first-line antituberculous drugs: isoniazid (H), rifampicin (R), pyrazinamide (Z), ethambutol (E), and streptomycin (S). These medications are the most effective and well-tolerated treatment options available. They are associated with fewer adverse reactions or side effects, and they are also the least expensive. As a result, the use of other drugs is not justified without first using these five agents.

These five drugs are to be administered simultaneously in a single dose, in order to facilitate patient adherence (and therefore efficacy), as well as to ensure improved tolerance and less toxicity. The only exception to this rule is represented by rifampicin and pyrazinamide, which should be administered at least eight to eleven hours apart.
Table 7. First-line antituberculous drugs: target bacterial population, dosage, frequent side effects, and pharmacological interactions

<table>
<thead>
<tr>
<th>Drug</th>
<th>Daily dose</th>
<th>Twice-weekly dose</th>
<th>Side effects</th>
<th>Control</th>
<th>Interactions</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>5 mg/kg</td>
<td>15 mg/kg</td>
<td>Neuritis</td>
<td>SGOT</td>
<td>Phenotoin</td>
<td>Extra+ intracellular bactericide</td>
</tr>
<tr>
<td></td>
<td>Up to 300 mg/kg</td>
<td></td>
<td>Hepatitis</td>
<td>SGPT</td>
<td></td>
<td>Bactericidal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypersensitivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>10 mg/kg</td>
<td>10 mg/kg</td>
<td>Hepatitis</td>
<td>SGOT</td>
<td>Inhibits oral contraceptives</td>
<td>Quinidine</td>
</tr>
<tr>
<td></td>
<td>Up to 600 mg/kg</td>
<td></td>
<td>Febrile reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Purpura</td>
<td>SGPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>15-30 mg/kg</td>
<td>50 mg/kg</td>
<td>Hyperuricaemia</td>
<td>Uric acid</td>
<td></td>
<td>Intra cellular bactericide</td>
</tr>
<tr>
<td></td>
<td>Up to 2 g</td>
<td></td>
<td>Hepatitis</td>
<td>SGOT</td>
<td></td>
<td>Sterilising</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SGPT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>15-20 mg/kg</td>
<td>50 mg/kg</td>
<td>Optic neuritis</td>
<td>Red-green discrimination</td>
<td>Extra+ intracellular bacteriostatic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>15-20 mg/kg</td>
<td>25-30 mg/kg</td>
<td>VIII cranial nerve damage</td>
<td>Vestibular function</td>
<td>Neuromuscular blocker</td>
<td>Extracellular bactericide</td>
</tr>
<tr>
<td></td>
<td>Up to 1 g</td>
<td>Up to 1 g</td>
<td>Hypersensitivity</td>
<td>Audiogram</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Creatinine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvate transaminase

Alternative regimens when first-line drugs cannot be used

Section summary
In all treatment regimens, attempts should be made to use as many first-line drugs as possible. If a drug that has been rendered ineffective is correctly identified, then the following regimens can be recommended:

- If pyrazinamide (Z) cannot be used 2HRE/7HR
- If isoniazid (H) cannot be used 2REZ/10RE
- If rifampicin (R) cannot be used 2HEZ(S)/10HE
- If ethambutol (E) cannot be used 2HRZS/4HR

Before starting this section, it should be commented that treatment becomes greatly complicated when any of the first-line drugs cannot be used. For this reason, patients in whom these drugs cannot be used must only be cared for by specialist physicians with extensive experience in the use of second-line drugs and combinations. Unfortunately, many specialist physicians lack this knowledge and experience, and should therefore avoid treating such patients, since there is the risk of worsening the problem as a result of increased intol-
erance or resistance. Consequently, this section should only serve as a guide for physicians who are experts in the field.

The first premise in this section should be the advice to use as many first-line drugs as possible in any treatment regimen. An account is provided of the regimens that should be recommended in the event that only one first-line drug cannot be used. These schemes constitute an effective guide for resolving the problems associated with serious adverse reactions to antituberculous drugs and demonstrated resistance to a single drug. Most adverse reactions are attributable to a single drug, and can be resolved by designing a treatment regimen to exclude that agent.

Prior to the introduction of rifampicin (R), the different drug combinations had to be administered for at least 18 to 24 months to ensure cure. The duration of treatment can now be shortened to 9 months owing to the bactericidal and sterilising action of rifampicin. With the subsequent “rediscovery” of pyrazinamide (Z), this duration has been shortened further, to only 6 months.

If, for some reason, pyrazinamide cannot be used in the initial treatment phase (e.g., due to a lack of availability, serious adverse reactions, or demonstrated resistance), a 9-month regimen should be advised with isoniazid and rifampicin, with supplementation with ethambutol (E) during the first 2 months of therapy (2HRE/7HR).

If isoniazid (H) cannot be used, because of toxicity or demonstrated resistance to the drug, then rifampicin and ethambutol should be given for at least 12 months, with supplementation with pyrazinamide during the initial 2 months (2REZ/10RE).

On the other hand, if rifampicin cannot be used, then the isoniazid and ethambutol regimen is again advised for a minimum of 12 months, likewise with pyrazinamide supplementation during the first 2 months (2HEZ/10HE). In countries with high initial resistance rates to isoniazid, supplementing with streptomycin (S) may also be appropriate in the first 2 months.

In turn, if ethambutol cannot be used, the treatment should be the same as that previously mentioned for 6 months, but using streptomycin instead of ethambutol in the 2 months of the first treatment phase (2HRZS/4HR).

The inability to use streptomycin does not affect the initial treatment scheme, since this should be the same as that indicated above, i.e., 2HRZE/4HR.

In all these treatment regimens, the drugs can be administered intermittently in the second phase, correcting only the dose per administration. The doses to be administered for each drug, in both the daily and intermittent dosing options, are the same as those specified in Table 7.
Management of adverse antituberculous drug reactions

Section summary

The risk groups for adverse antituberculous drug reactions are the elderly; malnourished patients; pregnant or nursing women; alcoholics; patients with chronic renal or liver failure, HIV infection, disseminated and advanced TB, atopy, anaemia, or diabetes mellitus; patients with a family history of adverse anti-TB drug reactions; patients receiving irregular TB treatment; and individuals who in addition to TB treatment are taking drugs for other disorders. In all these groups, close follow-up is required during treatment, with periodic clinical controls and laboratory tests.

A physician with extensive experience in the field should always carry out the management of adverse reactions. Such therapy cannot be standardised; each case must be dealt with individually. Initially, in the event of mild or moderate reactions, attempts should not be made to suppress treatment, although in the case of serious or severe toxic reactions, urgent hospitalisation is indicated, with suspension of the entire treatment until improvements in clinical and laboratory tests are observed. Subsequently, attempts should be made to reintroduce the treatment, except in the case of serious adverse reactions, such as purpura, anaphylactic shock, acute renal failure, severe hepatitis, haemolysis, retrobulbar optic neuritis, exfoliative dermatitis, and agranulocytosis. In such an event, a different treatment scheme should be designed, excluding the drug implicated in the observed reaction. In the rest of cases, such progressive drug reintroduction should be attempted, beginning with those substances least likely to have been implicated in the reaction. The ideal approach is to begin administering a sixth of the total dose, followed by gradual increments of one sixth each day, thereby ensuring full reintroduction in 1 week.

At the start of this section, emphasis should again be placed on the recommendation that only expert physicians with extensive experience should deal with such patients. If no such personnel are available, it is best not to become involved in the management of these complicated cases.

Although generally well tolerated, the first-line antituberculous drugs may cause side effects or adverse reactions, as described in Table 8. Some of the reactions may be serious and, in exceptional cases, can even lead to death. Much has been written about the possible fatal outcomes of liver toxicity associated with isoniazid.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse reaction</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>Symptomatic hepatitis</td>
<td>Treatment interruption and evaluation of transaminase and bilirubin levels</td>
</tr>
<tr>
<td></td>
<td>Peripheral neuropathy</td>
<td>Supervision and pyridoxine administration (B6) 25-50 mg/day</td>
</tr>
<tr>
<td></td>
<td>Skin hypersensitivity (rare)</td>
<td>If serious, interrupt treatment. Desensitise if drug is essential for treatment</td>
</tr>
<tr>
<td></td>
<td>Pellagra</td>
<td>Treat with nicotinamide</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Asymptomatic hepatitis: transient bilirubin elevation without cell damage and no transaminase level increase; subsides spontaneously and rapidly</td>
<td>Supervision</td>
</tr>
<tr>
<td></td>
<td>Symptomatic hepatitis</td>
<td>Drug suspension</td>
</tr>
<tr>
<td></td>
<td>Skin hypersensitivity and photosensitivity</td>
<td>Drug suspension and supervision</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal alterations</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>Reduced efficacy of oral contraceptives, anticoagulants, and oral hypoglycaemic drugs</td>
<td>Proceed according to medical criterion</td>
</tr>
<tr>
<td></td>
<td>“Flu” syndrome similar to influenza, presenting after 3-6 months</td>
<td>More frequent in intermittent treatment. Often corrected by daily treatment</td>
</tr>
<tr>
<td></td>
<td>Thrombocytopenic purpura</td>
<td>Symptomatic treatment and supervision. Drug suspension</td>
</tr>
<tr>
<td></td>
<td>Dyspnoea similar to asthma</td>
<td>Immediate and definitive drug suspension</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Haemolytic anaemia</td>
<td>Drug suspension</td>
</tr>
<tr>
<td></td>
<td>Acute renal failure</td>
<td>Drug suspension</td>
</tr>
<tr>
<td></td>
<td>Arthralgia</td>
<td>Suspend if arthralgia is intense</td>
</tr>
<tr>
<td></td>
<td>Gout</td>
<td>Definitive suspension</td>
</tr>
<tr>
<td></td>
<td>Nausea and anorexia</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>Symptomatic hepatitis</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>(asymptomatic hyperuricaemia—very frequent—does not require treatment suspension)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypersensitivity</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>Hypersensitivity, skin, and generalised reactions</td>
<td>Drug suspension</td>
</tr>
</tbody>
</table>
### Drug Adverse reaction Recommended action

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse reaction</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>Vestibular alterations, deafness. (These reactions may be permanent; most often found in young children and in patients over age 45 years)</td>
<td>Drug suspension</td>
</tr>
<tr>
<td></td>
<td>Vertigo and numbness (related to serum concentration)</td>
<td>Total and definitive suspension</td>
</tr>
<tr>
<td></td>
<td>Aplastic anaemia, agranulocytosis (rare)</td>
<td>Total and definitive suspension</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Optic neuritis</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td>Drug suspension, if permanent</td>
</tr>
<tr>
<td></td>
<td>Peripheral neuropathy, hypersensitivity (rare)</td>
<td>Symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medical criterion (drug should be avoided in patients with severe renal problems during pregnancy). In serious cases, total and definitive suspension</td>
</tr>
</tbody>
</table>

### General considerations regarding adverse drug reactions

Since adverse antituberculous drug reactions can be serious and even life threatening, early detection is essential and will clearly affect the associated morbidity and mortality. In this context, the application of strictly supervised TB therapy will also allow earlier clinical control and prompter detection of adverse drug reactions. Once again, it must be emphasised that physicians with expertise in the field should manage these difficult cases.

When a person experiences a serious adverse reaction, all treatment should be suspended until the affected organ or system is normalised (usually within 2-3 weeks). During this period, it will be necessary for the patient to undergo adequate clinical evaluation and for the physician to assess the risk-benefit ratio of these drugs.

The continuation of treatment in a patient who has suffered an adverse reaction cannot be standardised, and each situation must be evaluated individually. With regards to the antituberculous treatment regimen following an adverse reaction, as many first-line drugs should be tried as possible, since these are more effective and less toxic than the second-line agents.

### Risk groups for adverse drug reactions. Action recommendations

Different groups at risk of adverse reactions have been identified (Table 9). Such patients must be specifically evaluated according to their risk profile.
Table 9. Risk groups/factors for adverse antituberculous drug reactions

<table>
<thead>
<tr>
<th>Risk Group/Factor</th>
<th>Reason for Higher Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced age</td>
<td></td>
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<tr>
<td>Malnutrition</td>
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<tr>
<td>Pregnancy or nursing</td>
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<tr>
<td>Alcoholism</td>
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<tr>
<td>Liver failure</td>
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<tr>
<td>Chronic renal failure</td>
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<tr>
<td>HIV infection</td>
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<tr>
<td>Disseminated and advanced TB</td>
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<tr>
<td>Atopy</td>
<td></td>
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<tr>
<td>Anaemia</td>
<td></td>
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<tr>
<td>Diabetes mellitus</td>
<td></td>
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<tr>
<td>Family history of adverse antituberculous drug reactions</td>
<td></td>
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<tr>
<td>Patients receiving irregular antituberculous treatment</td>
<td></td>
</tr>
<tr>
<td>Patients receiving medication for other disorders, in addition to antituberculous drugs</td>
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</tbody>
</table>

In all the groups listed in Table 9, and in patients who in the course of treatment develop signs and/or symptoms suggestive of adverse reactions, careful evaluation must be carried out, with close follow-up and strict laboratory test assessments. This will facilitate the early detection of adverse reactions.

The reasons contributing to the possibility of adverse antituberculous drug reactions in the groups listed in Table 9 are the following:

1. **Ageing.** Ageing is responsible for changes in drug metabolism and excretion, and for increased interindividual variability.

2. **Malnutrition.** A high prevalence of fatty liver exists in such situations, resulting in a reduction in hepatocyte glutathione. Consequently, there is little neutralisation of the toxic metabolites originating from drug acetylation. Likewise, hypoalbuminaemia is observed, which causes an increase in the plasma free (i.e., unbound) fraction of drugs, thus increasing the risk of drug-induced toxicity.

3. **Pregnancy.** As in the case of malnutrition, fatty liver with hypoalbuminaemia may be observed during pregnancy. Moreover, the use of aminoglycosides has been associated with the development of hearing problems in newborns of women taking this class of medication. Growth cartilage abnormalities have been associated with the use of quinolones in pregnant women. Likewise, ethionamide should be avoided, since it has been shown to have teratogenic effects in animals.

4. **Liver or kidney dysfunction.** Anti-TB drugs can cause liver or kidney toxicity; consequently, special caution should be exercised when administering such substances to patients with impaired function of these organs.
5. **Treatment with other drugs.** The cytochrome P450 has been frequently associated with the production of hepatotoxic reactive metabolites. Its involvement in drug metabolism and effects on bioavailability should be considered when combinations of drugs are used.

6. **Disseminated or advanced TB.** In this setting, adverse reactions are probably a consequence of malnutrition or liver deterioration attributable to the disease itself.

7. **Patients previously subjected to antituberculous therapy.** There is an increased risk of damage attributable to rifampicin, which in turn is associated with hypersensitivity reactions that lead to the release of immune complexes.

8. **Atopy.** Atopy has been linked to the existence of other cases of adverse anti-TB drug reactions in family members; thus, the clinical history of the patient should be carefully evaluated before treatment is started.

9. **Sex.** Women are at a comparatively greater risk of developing drug-induced liver reactions. This effect must be considered when providing TB treatment.

10. **HIV infection.** Adverse reactions are more frequent in HIV-infected patients. The risk of such reactions increases with the degree of host immunosuppression.

In groups at risk of developing adverse reactions, baseline examinations should be requested, adapted to the clinical picture. Routine supervision based on laboratory tests is not necessary in patients given antituberculous treatment. The baseline examinations to be requested should include measurement of the following: a complete blood count with haematocrit, platelet count, prothrombin time, glucose, urea, creatinine, uric acid, transaminases, total bilirubin, conjugated and unconjugated bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase. Hepatitis serology should also be performed in areas endemic for viral hepatitis.

Controversy persists regarding the role of fast acetylators in the metabolism of isoniazid. It is known that the rate of acetylation of isoniazid and monoacetylhydrazine is genetically determined and there is a bimodal distribution of persons who acetylate them either slowly or rapidly. Since individuals acetylate isoniazid at the same speed that they convert monoacetylhydrazine to diacetylhydrazine, which is not toxic, it is very likely that this pattern does not influence hepatotoxicity reactions.

Drug dosage should be adjusted to body weight in all cases, and the personnel, patient, and relatives must be instructed on the possibility of
developing an adverse reaction, highlighting the need to report any such reactions immediately to the physician.

In special cases, the patient at high risk of adverse reactions may require hospitalisation; unnecessary prolonged stays should be avoided.

**The management approach to adverse drug reactions**

Success in the management of an adverse reaction depends on the establishment of an early and correct diagnosis. The patient and relatives must be instructed on how to recognise the most common adverse effects and to report them to the health personnel. It is equally important to question the patient about possible adverse reactions each time the patient reports for treatment.

The first step involves evaluating the severity of the adverse reaction and establishing whether it is dose dependent (a very common situation), and making the necessary dose adjustments. If the adverse effect is due to some factor other than dose, then the physician should advise suspending treatment with all drugs, or only the drug suspected of causing the reaction, and should assess if treatment suspension be permanent or temporary. It is important to try to establish a cause-effect relation between the adverse event and the suspect drug. The management of any adverse anti-TB drug reaction must always be carried out by physicians with great expertise in the field.

In situations of mild or moderate adverse reactions, the initial consideration is to not suspend the medication. The recommended approach is to provide symptomatic treatment for the reaction, adjust the drug dose, or change the timing of administration. If these measures prove to be unsuccessful, suspension of treatment should then be considered.

However, in patients with serious or severe toxic reactions, urgent hospitalisation is required, with suspension of the entire treatment until clinical and laboratory test improvement is achieved. Once the reaction has improved, the patient should be discharged, and after clinical and laboratory test amelioration, a waiting period of approximately 2 to 4 weeks is advised, to allow stabilisation of the immune system. During this time, the patient should be monitored for TB evolution. After 2 to 4 weeks of normalised clinical and laboratory test results, reintroduction of therapy may be considered, with possible desensitisation attempts. However, if during these 2 to 4 weeks the patient suffers a serious complication or develops a more severe form of TB, urgent hospitalisation is necessary, with the evaluation of an alternative treatment scheme—which may involve second-line drugs—or the reintroduction of therapy with immediate desensitisation.
Reintroduction of treatment and desensitisation

The reintroduction of therapy and desensitisation should not be attempted in patients who have developed severe toxic reactions, such as purpura, anaphylactic shock, acute renal failure, haemolysis, retrobulbar optic neuritis, severe hepatitis, exfoliative dermatitis, or agranulocytosis. In such situations that seriously compromise the patient’s life, a new treatment scheme should be used, excluding the drug implicated in the reaction. Treatment reintroduction is likewise contraindicated in HIV-infected individuals.

Before attempting to reintroduce treatment and perform desensitisation, a plan should be established on how to proceed in the event the adverse reaction reoccurs. Some experts recommend that the patient should receive prednisone (1-2 mg/kg body weight) 3 days before reintroduction of antituberculous treatment, continuing with this dosage for up to 2 weeks after reintroducing treatment, followed by gradual tapering of the dose. Concomitantly, the attending physician may consider using an H₁-blocker (e.g., loratadine 10 mg/day) or an H₂-blocker (e.g., ranitidine 300 mg/day), administered preferably at 18:00, in view of the relation of gastric acidity to the circadian cycle.

The subsequent steps indicated for reintroducing treatment and desensitisation are detailed below:

1. Based on a detailed clinical history and pertinent laboratory tests, attempt to identify the possible drug responsible for the observed reaction.
2. Determine whether it is necessary to continue administering the drug suspected of causing the adverse reaction.
3. Identify what other additional drugs are to be used in the reintroduction of antituberculous treatment.
4. Reintroduce the treatment drug by drug, in progressively increasing doses.
5. Progressive drug reintroduction should begin with the substance that is least likely to have been implicated in the observed reaction. This serves to increase physician and patient confidence, and the possibility of a new adverse reaction is postponed. This should be followed by the continued reintroduction of drugs in order of their likelihood of being related to the adverse reaction (from least to most likely).
6. If it is not possible to identify the culprit medication before reintroducing therapy, referral to the adverse anti-TB drug reaction frequency studies made in the country is required, in order to decide the best approach.
7. The timing of reintroduction of each drug will depend on the severity of the adverse drug reaction and on patient tolerance.

8. The ideal approach is to begin administering a sixth of the total dose, followed by gradual increments of one sixth each day, thereby ensuring full reintroduction of the drug in 1 week—this length of time being too short for a monotherapeutic regimen to select mutants resistant to that particular drug.

9. Once one of the antituberculous drugs has been reintroduced in the course of 1 week, an additional drug should be reintroduced each week in the same way as the first drug. Since a single week is not enough for the selection of resistances to the drugs, this recommendation decreases the likelihood of resistance development.

10. In relation to management after drug reintroduction, it must be taken into account that the regimen designed cannot be subject to the treatment norms established for the majority of patients. The patient subjected to treatment reintroduction must complete therapy according to the regimen designed, and the doses must be rigorously adjusted to body weight. The second phase of such treatments must always be administered on a daily basis.

11. It is extremely important for all severe adverse reactions to be managed and supervised by a specialist physician with extensive experience in these cases.

Drug and food interactions in tuberculosis

<table>
<thead>
<tr>
<th>Section summary</th>
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</table>
| The most important drug interactions that may be encountered with antituberculous medication occur at the level of absorption and metabolism of these agents. Isoniazid, rifampicin, and ethambutol require an acid medium for absorption; as a result, their absorption will be worse in the presence of drugs that increase the gastric pH, or in patients with achlorhydria (a common situation in HIV-infected patients).

Particularly important is the effect of food on the absorption of antituberculosis drugs. Isoniazid, rifampicin, and cycloserine must be administered with the stomach empty, whereas rifapentine, clarithromycin, clofazimine, and paraaminosalicylic acid are better absorbed with food. In turn, the bioavailability of pyrazinamide, ethambutol, ethionamide, rifabutin, and fluoroquinolones is only minimally affected by food, and can therefore be administered with meals. |
The rifamycins (rifampicin more so than rifabutin) induce cytochrome P450 enzyme activity, as a result of which they may produce interactions with drugs such as oral anticoagulants, contraceptives, glucocorticoids, oral antidiabetic drugs, immune suppressors (cyclosporine), and methadone—thereby reducing their therapeutic efficacy. In the case of rifampicin, the maximum inducing effect appears after 9 to 12 days. Rifampicin also interacts with protease inhibitors, the latter being the basis of HIV treatment, significantly reducing their plasma concentrations.

Drug interactions consist of alterations in the response of a drug due to the action of some other drug, food, beverage, or environmental contaminant. Such interactions may have beneficial effects (e.g., increasing the plasma concentrations of low bioavailability drugs) or deleterious consequences, which lead to treatment inefficacy (e.g., increased plasma concentrations with the risk of toxicity, or a reduction of such concentrations).

Interactions may take place:
– At the drug absorption level.
– At the distribution level. This is generally of little importance, unless the metabolism of the drug involved is also inhibited, or its renal elimination is reduced.
– At the metabolic level. These are the most important interactions.
– Pharmacodynamic interactions. These are modifications in target organ response and may give rise to addition phenomena, drug synergy, or antagonism.

As mentioned above, the most important drug interactions are those taking place at the metabolic level, although in the case of antituberculous therapy, those occurring at the drug absorption level can also be important—particularly in view of the possible effect of food on these drugs. TB can cause physiological and immune alterations that may affect drug absorption, metabolism, and binding to plasma proteins, and thus alter the magnitude of the interaction.

**Interactions at the drug absorption level. The effect of food**

These interactions may consist of an alteration in drug absorption rate, changes in the total amount of drug absorbed, or both. The causes of such interactions can be attributed to changes in the pH of the gastrointestinal contents, effects on gastric emptying and gastrointestinal motility, the fixation or chelation of drugs to form soluble complexes, and transport via glycoprotein P.
Isoniazid, rifampicin, and ethambutol require an acid medium for absorption; as a result, their absorption is worse in the presence of drugs that increase the gastric pH, or in patients with achlorhydria (a common condition in HIV-infected patients). It is therefore advisable to administer these drugs at least 1 hour before taking antacids.

Food exerts important effects on the absorption of antituberculous drugs. Table 10 details the most important interactions of all antituberculous drugs (first- and second-line) with food and antacids. Isoniazid, rifampicin, cycloserine, and azithromycin should be administered with the stomach empty, whereas rifapentine, clarithromycin, clofazimine, and para-aminosalicylic acid are better absorbed with food. In turn, the bioavailability of pyrazinamide, ethambutol, ethionamide, rifabutin, and fluoroquinolones is only minimally affected by food, and these medications can therefore be administered with meals. However, in the case of fluoroquinolones, it is important to avoid administration with dairy products, since the high calcium content of dairy products can reduce drug absorption as a result of the formation of complexes. In turn, isoniazid is unstable in the presence of sugars such as glucose and lactose, as a result of which drinks or drugs containing substantial amounts of these sugars (e.g., zalcitabine) should be avoided when taking isoniazid. In such situations, it is advisable to space both administrations at least 1 hour apart.

On the other hand, HIV-infected individuals may present with malabsorption due to gastrointestinal mucosal disorders (the *M. avium* complex), infectious diarrhoea (cryptosporidiosis or microsporidiosis), hypochlorhydria or achlorhydria, lactose intolerance, pancreatic insufficiency, or mucosal atrophy due to caloric-protein malnutrition. Such malabsorption has been shown to affect certain drugs, including rifampicin and ethambutol. In turn, isoniazid and pyrazinamide are well absorbed in patients with HIV infection, although absorption decreases in the presence of diarrhoea.

**Interactions at the drug metabolism level**

Metabolism-inducing drugs reduce the amount of substrate, with the potential risk of treatment failure. This action can be countered by increasing the substrate dose or removing the enzyme inducer. In contrast, metabolism-inhibiting drugs increase the substrate concentration, as a result of which toxicity may be enhanced.
Table 10. The most clinically significant interactions of antituberculous drugs with food and antacids

<table>
<thead>
<tr>
<th>Drug</th>
<th>Effect of food</th>
<th>Effect of antacids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>To be administered on an empty stomach, since absorption is reduced by 57% in the presence of food, particularly carbohydrates. Avoid administering with liquids containing abundant glucose or lactose. Isoniazid can inhibit monoamine oxidase, as a result of which the drug should not be administered with food containing abundant tyramine or alcohol, since disulfiram-type reactions may occur, with an increased risk of liver toxicity.</td>
<td>Antacids reduce AUC by 0-19%. Contradictory information. Avoid combined administration as a measure of caution.</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>To be administered on an empty stomach, since absorption is reduced by up to 26% in the presence of food.</td>
<td>Can be administered with ranitidine, although coadministration with antacids should be avoided.</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>Food exerts minimum effects on bioavailability.</td>
<td>Can be administered with antacids.</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>Food exerts minimum effects on bioavailability. Cmax is reduced by 16%, with no changes in AUC.</td>
<td>Reduces Cmax by 28% and AUC by 10%. Avoid combined dosing.</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Food exerts minimum effects on bioavailability. However, absorption is reduced by foods with abundant calcium (dairy products) and other ions; administration should thus be made 1 h before or 2 h after meals</td>
<td>Important reduction in absorption. Avoid combined dosing.</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>Food exerts minimum effects on bioavailability.</td>
<td>Can be administered with antacids.</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>Food reduces Cmax by 30% and prolongs Tmax 3.5-fold. Orange juice (and probably also other acid beverages) reduces Cmax by 15%. If possible, administer with water and between meals.</td>
<td>Antacids do not significantly modify AUC or Cmax.</td>
</tr>
<tr>
<td>Para-aminosalicylic acid</td>
<td>Acidic drinks or yoghurt prevent release in stomach, reducing the incidence of nausea. Food increases its absorption (-52% AUC). Administer with water, orange juice, or fatty foods.</td>
<td>Antacids do not significantly modify AUC or Cmax.</td>
</tr>
</tbody>
</table>
The rifamycins (rifampicin more than rifabutin) induce cytochrome P450 enzyme activity; consequently, they may reduce the therapeutic efficacy of drugs such as oral anticoagulants, contraceptives, glucocorticoids, oral antidiabetic drugs, immune suppressors (cyclosporine), and methadone, among other substances. In the case of rifampicin, the maximum inducer effect occurs after 9 to 12 days. Rifampicin also interacts with cotrimoxazole, reducing the area under the curve for trimethoprim by 63% and for sulphamethoxazole by 23%.

Protease inhibitors, the basis of anti-HIV therapy, generally act as metabolism inhibitors. These drugs constitute CYP3A4 substrates, and when combined with inducers of this enzyme (e.g., rifamycins), their plasma concentrations can be reduced significantly—thus compromising their therapeutic efficacy and facilitating the development of resistance. This is why the combination of rifampicin with protease inhibitors is contraindicated, with the exception of ritonavir, the combination of ritonavir and saquinavir, and the non-nucleosides delavirdine and nevirapine—due to the risk of causing treatment inefficacy and promoting resistances to these drugs. When rifampicin is used initially, at least 2 weeks must elapse after suspension of this drug before starting protease inhibitor therapy. Rifabutin is easier to use in patients requiring antiretroviral therapy; it can be combined with most of these drugs. Due to the mechanism of action and difficult use of these substances, such treatments must always be prescribed by specialist physicians with the required expertise.

Due to the type of metabolism involved, it is unlikely for interactions to occur between protease inhibitors and isoniazid, pyrazinamide, ethambutol, aminoglycosides, para-aminosalicylic acid, and quinolones. Isoniazid can

<table>
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<tr>
<th>Drug</th>
<th>Effect of food</th>
<th>Effect of antacids</th>
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</thead>
<tbody>
<tr>
<td>Clofazimine</td>
<td>Fatty foods increase C&lt;sub&gt;max&lt;/sub&gt;.</td>
<td>Can be administered with antacids.</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>Food exerts minimum effects on bioavailability.</td>
<td>Not known. Didanosine does not affect absorption. Avoid administering with antacids</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>Food exerts minimum effects on bioavailability.</td>
<td>Important reduction in absorption.</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Bioavailability increases by 25%.</td>
<td>Avoid combined dosing.</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Administer 1 h before or 2 h after meals. Food reduces absorption by 50%.</td>
<td>Although data are limited, combined dosing seems possible.</td>
</tr>
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</table>

AUC = area under the curve; C<sub>max</sub> = maximum concentration; T<sub>max</sub> = time to maximum concentration.
increase the concentrations of phenytoin and carbamazepine, probably as a result of inhibition of the liver metabolism of these antiepileptic drugs. Isoniazid can also alter the metabolism of ethanol and paracetamol, increasing the production of a toxic metabolite of the latter. High paracetamol doses should therefore be avoided when administering isoniazid. In turn, clarithromycin demonstrates clinically important interactions with carbamazepine and theophylline, as a result of which the plasma levels of these drugs should be monitored.

**Pharmacodynamic interactions**

Drugs that tend to worsen renal function, such as the aminoglycosides, can reduce the elimination of antiretrovirals, which are mainly eliminated through the kidneys, such as 3TC, d4T, and ddC.

Pyrazinamide can induce episodes of gout in patients at risk, since it competes with uric acid for renal elimination. This effect is more evident in patients receiving allopurinol, since allopurinol reduces the elimination of the main metabolite of pyrazinamide, which also reduces uric acid secretion.

Ethambutol can cause optic neuritis, while rifabutin can cause uveitis. Patients who simultaneously receive several drugs capable of causing ocular toxicity must be closely monitored.

In patients administered aminoglycosides on a continuous basis, periodic hearing evaluation is indicated, particularly among those individuals receiving other ototoxic agents in combination (clarithromycin, ethacrynic acid, furosemide). Aminoglycosides can enhance the effects of muscle blockers and can trigger neuromuscular block in patients with myasthenia gravis.

Cycloserine should be administered with caution to patients with a history of depression or psychosis, in view of its adverse effects on the central nervous system.

Ethionamide can cause peripheral neuritis; consequently, caution is advised when combining it with antiretrovirals that also exhibit such toxicity. It can also cause liver toxicity and goitre, with or without hypothyroidism. Periodic monitoring of the concentrations of thyroid-stimulating hormone is required. Para-aminosalicylic acid can enhance such toxicity at the thyroid level. Para-aminosalicylic acid can cause diarrhoea, affect the pharmacokinetics of other drugs, as well as cause different forms of malabsorption (e.g., steatorrhoea, vitamin B₁₂, folic acid, xylose, and iron absorption).

Clofazimine can cause changes in skin colour, while amiodarone and rifabutin can worsen this side effect.
In patients who do not respond adequately to antituberculous treatment, monitoring of plasma concentrations may be helpful. A number of studies have reported low plasma concentrations of antituberculous agents in HIV-infected patients undergoing antiretroviral therapy.

Therefore, emphasis must be placed on the importance of closely monitoring the possible side effects caused by these different drugs.

Special situations in the treatment of tuberculosis

**Section summary**

It is not necessary to modify the initial treatment regimens in HIV-infected patients, individuals with some form of immune deficiency, pregnant or nursing women, children, or infants. The only requirement is to adjust the corresponding doses and ensure close follow-up. When malabsorption is involved, or the patient is unable to take medication orally, the same regimens are provided, although via the parenteral route.

In advanced liver failure, the administered regimen should always include ethambutol (E) and streptomycin (S), with a third drug that should be isoniazid (H) if laboratory evidence indicates cholestatic conditions (2HES/10HE), or rifampicin (R) in the event of necrosis (2RES/10RE).

In patients with advanced kidney disease, it is best to avoid (or at least monitor the blood levels to ensure dose adjustment) all drugs with potential nephrotoxic effects, such as ethambutol, streptomycin, kanamycin, cycloserine, viomycin, and capreomycin. The 2HRZ/4HR regimen, with close supervision and follow-up, should suffice.

In patients with immune deficiency, very close patient follow-up is necessary, with each patient evaluated on an individual basis, even though the standard treatment regimens indicated above should be used. Studies involving large series of diabetic or patients with malignancies, or those who have undergone gastrectomy, have reported similar results as in healthy individuals.

In HIV-infected subjects, the same treatment regimens are indicated, with the only additional requirement of closer follow-up because of the increased risk of adverse reactions, intolerance, and drug interactions.

During pregnancy, no changes to the recommended regimens are necessary, for although isoniazid (H) and rifampicin (R) cross the placental barrier, they have not been associated with teratogenic actions. However, due to its potential foetal toxicity, streptomycin (S) should be avoided, along with ethionamide (Eth) and the other aminoglycosides. During nursing, all antitu-
Berculous agents can be used. Although part of the dose is secreted in human milk, the amount is insufficient to either have a therapeutic effect or cause adverse effects.

In children and infants, the same regimens as in adults apply, although maximal caution should be exercised and the dose should be adjusted according to body weight.

In patients with malabsorption problems, or who are unable to take medication orally, the same regimens defined above apply—employing the same doses, but administered parenterally.

In patients with advanced chronic liver failure, the recommended treatment regimen should always include those first-line agents that are preferentially eliminated by the kidneys (i.e., ethambutol [E] and streptomycin), taking care to avoid drugs selectively metabolised in the liver (pyrazinamide [Z], ethionamide, and para-aminosalicylic acid [PAS]). The drug to be added to ethambutol and streptomycin in turn depends on the predominant biochemical alteration involved:

- When the patient exhibits cholestatic alterations (increased bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase levels), isoniazid should be added—thus yielding the regimen 2HES/10HE, with frequent monitoring of liver function.
- When the observed pattern is necrotic (increased liver transaminase levels), it is better to add rifampicin, since liver toxicity generally occurs in combination with isoniazid (rifampicin being an enzyme inducer). The recommended alternative regimen is therefore 2RES/10RE, likewise with frequent liver function tests.
- When the predominant biochemical pattern is not defined, it is better to use 2RES/10RE.
- In patients undergoing antituberculous therapy who have drug-induced liver toxicity, the points detailed above should be followed when reintroduction of all the drugs is not possible, and depending on the predominant biochemical pattern observed.

In cases of advanced kidney disease, it is advisable to avoid (or at least monitor) the blood levels to adjust the doses of all drugs with a potential for nephrotoxicity or that are eliminated by the kidneys. These drugs are ethambutol, streptomycin, kanamycin, viomycin, capreomycin, and cycloserine. Thus, the regimen 2HRZ/4HR would suffice, provided there is very close monitoring and follow-up since there is a possibility of selection of resistances attributable to not being able to administer a fourth drug in the initial
phase of treatment. In such cases, it is advisable to administer the second treatment phase on a daily basis. All these patients must be subjected to full clinical evaluation, with a 24-hour urine creatinine clearance test. Based on the test result, it may be advisable to reduce the dose or increase the dosing interval. If creatinine clearance exceeds 50 ml/min, no such changes in dose or dosing interval are needed. However, if clearance is less than 50 ml/min, the therapy specified in Table 11 is indicated. In no event is it necessary to modify the dose or dosing interval of rifampicin. In patients undergoing dialysis, treatment should be provided at the end of the dialysis session.

**Table 11.** Dose adjustment for antituberculous drugs in patients with chronic renal failure, according to the 24-hour urine creatinine clearance test results

<table>
<thead>
<tr>
<th>Creatinine clearance (ml/min)</th>
<th>Drug</th>
<th>Dose Interval</th>
<th>Dose Interval</th>
<th>Dose Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>H, E, Z, S</td>
<td>&lt; Reduce to half</td>
<td>Every 48 h</td>
<td>–</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>–</td>
<td>–</td>
<td>Reduce to half</td>
<td>Every 18-24 h</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

E = ethambutol; H = isoniazid; S = streptomycin; Z = pyrazinamide.

**Treatment of extrapulmonary tuberculosis**

**Section summary**
Under conditions of a TB control program, there should be no differences in the treatment of extrapulmonary TB versus pulmonary TB.

Regarding the treatment of extrapulmonary locations of TB, the trials conducted to date have not been as thorough as treatment trials for pulmonary TB. Nevertheless, there appear to be no theoretical or practical bases for not using the same treatment regimens as those indicated for pulmonary TB (in terms of the drug associations and durations involved). Thus, the localisation of the disease should not imply any exception to treatment, and the treatment regimens described above should be used. Some authors and scientific societies recommend extending the duration of therapy to 9 months in patients with meningeal, osteoarticular, and lymphatic TB—although there is no firm evidence supporting this recommendation.
The drugs with the greatest distribution in cerebrospinal fluid are pyrazinamide (80% of the plasma concentration), isoniazid (50%), and ethambutol (20%). Only streptomycin and rifampicin cross the blood-brain barrier and afford therapeutic drug levels in the presence of meningeal inflammation.

**Surgery, corticoids, and other therapeutic measures**

**Section summary**

Surgery is only indicated in specific cases for managing the sequelae or complications of pulmonary TB, and in very exceptional cases of multidrug-resistant TB in which the lesions are localised and there are no other drugs to treat the disease. In patients with extrapulmonary TB, surgery may be acceptable for obtaining samples for study and for treating certain situations such as constrictive pericarditis, vertebral abscesses that may compress the spinal cord, or superficial and accessible abscesses in cases of osteoarticular TB.

Corticoid treatment should only be contemplated in four situations: meningeal TB, serious miliary TB, pericardial TB, and TB involving ganglionicbronchial perforation.

At present, surgery lacks indications in the management of TB. In lung TB, surgery should be passed over in view of the excellent performance afforded by pharmacological treatment. Only some believe that surgery should be considered in the treatment of localised lesions in which the bacterial population is resistant to practically all drugs. This condition is very rare parti-

cularly regarding localised lesions with good lung function. Often, when surgery is performed in such patients, the procedure is used more because of economic constraints or lack of access to effective second-line drugs than because of any genuine surgical indication. Moreover, even when radiographs reveal the presence of localised lesions, it should be remembered that TB is in fact a disseminated disease.

A different situation is presented in the management of TB complications or sequelae, including massive haemoptysis, bronchiectasis, and bronchopleural fistulae, where surgery may indeed prove useful.

In extrapulmonary TB, surgery may be useful, particularly for obtaining samples for diagnostic purposes, and for treating certain conditions, such as constrictive pericarditis or vertebral abscesses that may compress the spinal cord. Surgery should also be evaluated in cases of superficial and accessible abscesses in the context of osteoarticular TB. Apart from unique cases, sur-
gery is not indicated for use in peripheral adenopathies affected by the disease. Surgery should only be considered in the event of mechanical complications and possible sequelae.

In turn, corticotherapy should only be considered in three situations:
1. **Meningeal TB.** Some paediatricians recommend corticotherapy to prevent the development of an internal hydrocephalus.
2. **Miliary TB.** Use in seriously ill patients to provide symptom relief, although corticotherapy appears to have little effect on the prognosis.
3. **Pericardial TB.** To reduce the risk of constrictive pericarditis and the need for subsequent surgery.

The use of corticosteroids is not justified in pleural TB, where it has been shown that while these drugs shorten the time to symptom and effusion resolution, they have no effects on preventing possible complications. Similar considerations apply to endobronchial TB, where it is not clear whether corticoids reduce complications. However, in the IUATLD’s recommendations regarding children with TB, corticosteroids are advised when ganglio-bronchial perforations are present.

Lastly, vitamins and protein supplements are not helpful. Pyridoxine can interfere with the activity of isoniazid, whereas nicotinamide can interfere with pyrazinamide. Such substances should only be used in the setting of deficiencies, which is a fairly common situation in poorer countries.

### Hospital admission criteria

**Section summary**
At present, only five conditions warrant hospitalisation:
- Disease severity. Admission is due to the seriousness of the patient’s condition, not due to the fact that the patient has TB.
- Complications of the disease or its sequelae. Admission is likewise due to complications, and not due to merely having TB.
- Management of serious adverse drug reactions.
- Re-treatment of TB with second-line drugs.
- Due to social reasons (rare).
- Uncomplicated initial TB is not a criterion for hospital admission.

Rest and sanatorium care are no longer used, both from the treatment and the patient isolation perspective. Thus, there are presently no indications for admission of initial TB cases simply due to the fact that the patient has the disease. However, in some instances admission is warranted—because of the
seriousness of the patient’s condition (e.g., respiratory failure, massive haemoptysis, severe malnutrition, terminal condition) or because of complications of the disease or its sequelae (e.g., empyemas, bronchopleural fistulae, haemoptysis, respiratory failure). It is necessary to point out, however, that admission in these situations is due to the seriousness of the patient’s condition, not to the fact that the patient has TB.

Additional indications for admission are: 1) serious adverse drug reactions, which may be life threatening; and 2) the first few weeks of re-treatment with second-line drugs, due to the increased risk of intolerance and adverse antituberculous drug reactions.

Lastly, social factors may sometimes also support admission, such as extreme poverty, excessive distance between the home of the patient and the health care centre (a relatively common situation in very poor countries), and repeated treatment default.

**Recommended reading for the chapter**

Chapter 10 - Re-treatment of tuberculosis

Chapter summary

The re-treatment of tuberculosis (TB) constitutes one of the most difficult challenges in the management of the disease; consequently, in theory it should only be carried out by expert physicians. This aspect is all the more important considering that in many instances, re-treatment is the last chance for the patient to get cured. Unfortunately, however, many professionals with limited knowledge of TB re-treatment undertake such management, thereby making the problem worse.

The reasons for re-treatment comprise a variety of factors as distinct as microbiological relapse, pharmacological failure, patient abandonment, and poor adherence to therapy.

The truly worrying aspect is the selection of resistant mutants to the drugs used—a situation almost always seen in treatment failure and partial abandonment of therapy. These are situations that must be well known to the physician facing the problem. The health professional must also be aware of the limited utility of drug sensitivity testing, and of the great importance of a detailed history of the drugs used in the past, which are essential when devising a re-treatment strategy. With a sound knowledge of all the drugs offering action against TB, a re-treatment scheme can be designed that includes a minimum of three drugs that have never been used by the patient. There are presently 13 drugs for which such action has been demonstrated; consequently, the problem is often more a matter of limited drug availability in a given country than of a true lack of alternative second-line drugs.

However, re-treatment is extremely expensive, particularly when second-line drugs are needed. This is why priority should be given to the management of initial patients, both in view of the superior cost-effectiveness of initial TB therapy and because correct management of the initial disease is the only way to prevent the need for re-treatment. Hence, at least in theory, no resources should be spent on re-treatment until it is confirmed that all initial disease patients have been granted free access to a short-duration treatment regimen.

Once the above has been taken into account, even the poorest countries with the greatest TB problems should attempt to offer at least one standard 8-month re-treatment regimen involving first-line drugs (2HRZES/1HRZE/5H3R3E3). This regimen is inexpensive and very practical. Not only does it heal over 90% of patients included in National Tuberculosis Control Programmes (NTPs) as re-treated cases, but it also identifies the true failures (i.e., patients with drug resistance) who may thus be able to receive more individualised care at a higher level within the health care system. The great majority of countries with low-income levels should not spend further resources on re-treatment.
However, in middle-income countries and in some low-income countries, the acquisition of a second-line drug reserve may be advisable in order to offer a standard treatment regimen with these agents: 3(Z, Kn, Eth, ofloxacin)/15(Z, Eth, ofloxacin). Such drug reserves should always be managed by the Central Unit of the corresponding NTP.

The possibility of individualised re-treatment according to drug susceptibility results is perhaps only advisable in richer countries, and in some middle-income countries.

As its name indicates, the term tuberculosis (TB) “re-treatment” refers to treatment in a patient who has already been treated with antituberculous drugs in the past. These therefore are patients who have already had the opportunity to receive treatment and cure their disease. Accordingly, in most patients in whom re-treatment is being considered, there were errors in the administration of the previous treatment. In short, “the best re-treatment is the therapy that should have been administered several years earlier, with the application of a good initial treatment scheme.” This statement would only exclude specific cases of disease relapse, a concept that will be dealt with later on. Thus, in order to minimise the number of individuals requiring re-treatment, two fundamental premises apply: the need to prescribe standardised initial treatment protocols, and the use of strict supervision measures to guarantee that patients adhere to treatment.

TB re-treatment is often the last chance the patient has of cure, particularly in low- and middle-income countries where the availability of second-line drugs is limited. For this reason, re-treatment schemes must always be managed by experts in the field, even in the case of those schemes that are administered on a standard basis in the context of a National Tuberculosis Control Programme (NTP).

This chapter describes the bases for developing a re-treatment regimen, and discusses its possible use according to the resources available in different countries.

**Different situations requiring re-treatment: relapse, failure, default, and poor adherence to therapy**

**Section summary**
The most important consideration when dealing with a patient subjected to re-treatment for TB is to attempt to identify whether disease relapse, treatment failure, partial or total treatment default, or poor patient adherence to medication is
involved. It is essential to try to determine the reason for re-treatment because subsequent treatment is specific to each situation. However, the process of assessing the different situations requiring re-treatment is complex, and often times cannot be accomplished at the peripheral levels of the health care system.

Relapse is the appearance of new microbiological evidence of disease in a patient who has completed correct treatment and has been cured. Such situations do not usually condition the selection of drug-resistant mutants. Failure is defined as the appearance or persistence of microbiological activity in a given patient in the course of antituberculous treatment, either following initial negative conversion of the cultures or without negative conversion. In general, failure implies the selection of resistant mutants to all the drugs that are being used. On the other hand, if a patient completely abandons treatment, a situation similar to relapse will develop, with the isolates continuing to be sensitive to the drugs used. If abandonment is only partial, the resulting situation is more akin to treatment failure, with the possible selection of resistant mutants.

The first and most important consideration when dealing with a patient requiring re-treatment for TB is to determine whether the case corresponds to bacteriological relapse or to pharmacological failure, since the therapeutic connotations and prognoses differ greatly from one setting to another.

Bacteriological relapse is defined as the appearance of microbiological evidence of disease in a patient who has completed appropriate treatment and who has been cured. However, the truth is that only in cases of directly supervised treatment can it be ensured that the patient has effectively taken all the necessary medications. While relapse may occur in the early or later stages of the disease, it most often takes place during the first 12 to 24 months after the end of therapy. Such relapses are usually attributable to the persistent or dormant bacterial population that did not divide during the administration of initial treatment, and that therefore has not found the conditions required to favour drug-resistant mutants. This is why it is generally possible to restart treatment in these patients using the same initial therapeutic scheme. If relapse occurs several years after the initial treatment, the true situation may correspond to reinfection rather than to relapse—although this is difficult to demonstrate. In any case, relapse does not change the indication or affect the repeated use of the initial therapeutic regimen. Although, as has already been mentioned, microbiological relapse usually occurs at the expense of sensitive bacteria, and it is advisable to perform sensitivity tests of the first-line antituberculous drugs in these patients.

The concept of relapse and the presumed sensitivity similar to that found in the initial disease process is only applicable when it is certain that
the patient has received an appropriate treatment regimen. In the field, when drug resistance studies are performed, relapses tend to be associated with a significant increase in resistance compared with the available initial susceptibility results. This may be explained by poor patient adherence to therapy, or because patients presenting in the relapse group already had an initial primary resistance. This is why patients who relapse are assigned a reinforced longer primary treatment under the conditions of a TB control programme.

Pharmacological failure is the appearance or persistence of microbiological activity in a patient during the course of antituberculous treatment and following initial negative conversion of the cultures. Failure is also accepted in patients in whom there is no such negative conversion of cultures during the course of therapy. In the initial 6-month treatment course recommended in the present Guide (i.e., 2HRZE/4HR), failure can be considered when the sputum cultures at the end of the fourth month remain positive. In the 8-month treatment regimen (2HRZE/6HE), it is necessary to wait until the end of the fifth month to confirm possible failure. In this situation, treatment failure occurs at the expense of the bacillary population, which has been metabolically active and has divided during treatment, favouring the selection of naturally resistant mutants. For this reason, failure almost always involves resistance to all the drugs being administered. Hence, the essential requirement in such cases is drug susceptibility testing.

Drug resistance is the theoretical explanation underlying treatment failure. In actual practice, however, up to 50% of all failures show sensitivity in the in vitro tests, and implementing a new or reinforced first-line drug regimen ultimately cures such patients. The so-called programme failures, i.e., failed treatment due to treatment irregularities, should be resolved if the standard therapeutic guidelines of the NTP are followed. Another influencing factor of treatment failure is the lack of correlation between the laboratory drug susceptibility test results and the clinical evolution of the patient.

Chronic TB patients who have undergone multiple treatments often represent multidrug resistance (MDR) and are the main therapeutic problem. In an important percentage of such patients, the ‘culprit’ in the development of drug resistance is the physician who recommends incorrect treatment regimens. When patients show poor adherence to therapy, they typically abandon all the drugs at an early stage, as a result of which the subsequent relapses occur at the expense of the initial bacterial population that has not developed drug resistance. In these situations, it suffices to reintroduce the initial treatment regimen to achieve cure. These cases are similar to relapse...
cases, with the same resistance behavioural pattern. Only when the patient selectively abandons treatment (as in the case of masked monotherapies) does the risk of developing drug resistance occur. This risk does not exist when the patient ceases taking all medications, which is a more common occurrence.

Based on the above considerations, it may be accepted that a patient undergoing re-treatment, without drug resistance, either corresponds to a case of microbiological relapse or to a case of complete treatment default. When drug resistance is demonstrated, the problem is almost always attributable to the physician who recommended the wrong initial treatment regimen or, infrequently, to partial or selective patient default of therapy.

**Methods for detecting drug resistance**

A detailed history of the drugs taken in the past is the best method for detecting drug resistance and for designing a re-treatment regimen. In practice, this approach is superior to sensitivity testing, which, although compulsory in all patients subjected to re-treatment for TB, affords very late information. Drug susceptibility test results must always be compared with the corresponding history of drugs taken by the patient.

When dealing with a patient requiring re-treatment for TB, the first requirement is to determine whether the patient has any drug resistance. Above any other method, review of the medical record is critical, as it will contain information indicating if the patient had relapsed, abandoned treatment completely, experienced treatment failure, or partially defaulted on treatment. In the case of relapse or treatment abandonment, it may be accepted that there are no drug resistances, and that it would suffice simply to reintroduce the initial treatment regimen, with close monitoring of the microbiological course.

However, if the case involves treatment failure, partial abandonment of therapy, or a chronic patient with multiple previous treatments (the most frequent situation), then it is very possible that there is resistance to one or more drugs. In these cases, a detailed and thorough history of the drugs previously used by the patient should be established, with evaluation of all the doses administered and the drug combinations used in each treatment protocol.

If this detailed and exhaustive history of the drugs used in the past is prepared by expert personnel and considerable time is spent on compiling
the history (no less than 30-60 minutes), a chronological representation can be obtained of the introduction and withdrawal of drugs in the past (Figure 50)—thus making it possible to identify either real or masked past monotherapies. It becomes possible to determine (with good accuracy) drug resistances, which should indicate which drugs should not be used again in the re-treatment regimen.

In practice, this drug history is superior to drug susceptibility testing, which may take more than 3 months to yield results (when conventional methods are used) and which can fail because of insufficient culture development. Moreover, it is important to consider that while the in vitro and in vivo correlation of susceptibility testing is very reliable for isoniazid and rifampicin, and even for streptomycin, the same cannot be said for the remaining drugs, in which reliability is far less. Drug resistance, as defined by susceptibility test results, refers to the inefficacy of a particular anti-TB drug in the culture medium. Sensitivity determined by such testing does not imply that the drug will be effective in a new treatment regimen. The patient drug history must be evaluated in these cases.

Drug susceptibility testing, while compulsory in all patients undergoing re-treatment, affords very late information. Thus, it is not acceptable to wait so long before starting re-treatment. Drug susceptibility results must always be compared with the corresponding history of drugs taken by the patient. Drug susceptibility tests have been addressed in detail in Chapter 7.

A drug resistance detection system based on the history of drugs taken in the past becomes all the more important in low- and middle-income countries, where sensitivity testing must be performed using the proportions technique in solid culture media, which implies a delay of about 4 to 5 months before information is received. This major inconvenience makes a detailed history of the drugs taken in the past essential for evaluating a re-treatment protocol.

### Bases for choosing a re-treatment regimen

<table>
<thead>
<tr>
<th>Section summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>When selecting a re-treatment regimen, a series of 10 premises must be taken into consideration. Treatment should include three drugs that the patient has never taken. At least two of these drugs must be maintained for 18 to 24 months if neither isoniazid nor rifampicin can be used. If use of these drugs is possible, then treatment for 12 months will suffice. Re-treatment must be carried out by</td>
</tr>
</tbody>
</table>
specialised personnel with experience with re-treatment regimens, since management with the great majority of second-line drugs is complicated and side effects are more frequent than with other drugs. At present, 13 drugs have been identified that offer activity against \textit{M. tuberculosis}. An effective re-treatment regimen needs only three. The most important limitations are determined by the availability of these drugs and the experience in designing adequate treatment regimens.

After establishing the past drug history of the patient, a re-treatment scheme adhering to the 10 premises reflected in Table 12 must be developed. Treatment should include three drugs that the patient is certain to have never taken. At least two of these drugs must be maintained for 18 to 24 months if it is not possible to include isoniazid or rifampicin. If such inclusion is possible, then treatment for 12 months will suffice. Re-treatment can be carried out in the ambulatory setting, with close outpatient follow-up by specialised centres and involving personnel with experience in the field, since the use of appropriate intensive treatment regimens with second-line drugs is often complicated and involves toxic adverse effects. If there is no adequate infrastructure or social environment for careful outpatient monitoring, initiate re-treatment with the patient admitted to a reference centre.

\begin{table}[h]
\centering
\begin{tabular}{p{12cm}}
\hline
\textbf{Table 12. Guidelines for the elaboration of a TB re-treatment regimen} \\
\hline
1. Initiate re-treatment in the ambulatory setting if there is adequate infrastructure and social environment for outpatient monitoring. Otherwise initiate re-treatment with the patient admitted to a reference centre. \\
2. The scheme should be designed by personnel with extensive experience in the handling of second-line drugs. \\
3. It is very important to establish a detailed history of the drugs used by the patient in the past. \\
4. Associate at least three drugs that have never been used by the patient or for which no drug resistance exists (i.e., well associated in earlier treatment regimens). \\
5. Use the maximum possible number of bactericidal drugs. \\
6. Always include an aminoglycoside or capreomycin. \\
7. Caution is required due to possible cross-resistance among drugs, especially: \\
   \begin{itemize}
   \item Aminoglycosides*: streptomycin $\rightarrow$ kanamycin $\rightarrow$ amikacin \\
   \item All quinolones \\
   \end{itemize}
8. Minimum treatment time: \\
   \begin{itemize}
   \item 18 months if isoniazid and rifampicin cannot be used \\
   \item 12 months if isoniazid or rifampicin can be used \\
   \end{itemize}
9. Strict supervision of treatment administration is required. \\
10. Never add a single drug to an ineffective or failing regimen. \\
\hline
\end{tabular}
\end{table}

* Resistances in aminoglycosides are considered to be unidirectional, and such drugs should be used sequentially as shown in the table. See explanation in text below.
**Figure 50.** Sample form for recording a detailed history of the antituberculous drugs used in the past. This drug history should serve as a basis for preparing an individualised re-treatment regimen. Complete one sheet for each patient and year.
Among the drugs to be included in a re-treatment regimen, and in relation to some of the premises indicated in Table 12, the following should be noted:

1. If feasible, combine the maximum possible number of available bactericidal drugs that act upon different targets in the microorganism. If few such drugs are available, an aminoglycoside must always be included. Although capreomycin does not belong to this group, it possesses a similar mechanism of action and should therefore be included in this group when selecting an aminoglycoside. Moreover, since capreomycin is less toxic and is well tolerated, many experts recommend its use when streptomycin cannot be used.

2. It is important to evaluate the possibility of cross-resistance with incorrectly administered drugs. Drug resistance among aminoglycosides is considered to be unidirectional, and such drugs should be used sequentially, as reflected in the Table. Thus, if a drug is resistant to streptomycin, it is very likely to remain sensitive to the rest. If it is resistant to kanamycin, it is very likely to remain sensitive to the rest, except streptomycin (to which it most likely will be resistant). Cross-resistance also exists between rifampicin and the ansamycin antibiotics or rifamycins.

3. Do not administer drugs with confirmed or suspected drug resistance. Their combination should only be evaluated in the treatment regimen if no other drugs are available.

4. Never add a single drug to, or associate it with, a regimen that has already been found to be ineffective. To do so would only create the conditions for masked monotherapy.

5. Careful assessment is required of the selected drugs, taking into account that they have important toxicities and side effects, and that they must be administered to persons who have already undergone multiple treatments in the past.

The list of all antimycobacterial drugs that can be used in a re-treatment regimen is provided in Table 13; the great majority of these second-line agents are very expensive and difficult to obtain. As can be seen in Table 13, there are 13 drugs with demonstrated anti-TB efficacy, although involving different bactericidal and sterilising capacities. For this reason, attempts should always be made to include as many first-line drugs as possible (isoniazid, rifampicin, ethambutol, streptomycin, and pyrazinamide), for in addition to being more effective, they are better tolerated and much easier to acquire. As has already been pointed out, all TB patients can be cured with at least three never previously used drugs. A sufficiently large therapeutic arsenal exists to ensure that cure can be achieved. Ultimately, the most important limitations are determined by the availability of second-line drugs and
the ability to acquire them. Success of re-treatment will depend on the experience in designing adequate treatment regimens and in the use of these drugs, which have increased toxicity and poorer tolerance profiles.

**Table 13.** Drugs with demonstrated activity against *M. tuberculosis*

| 1. Isoniazid          |
| 2. Rifampicin        |
| 3. Pyrazinamide      |
| 4. Ethambutol        |
| 5. Streptomycin      |
| 6. Capreomycin       |
| 7. Kanamycin         |
| 8. Amikacin          |
| 9. Ethionamide – prothionamide |
| 10. Cycloserine      |
| 11. Para-aminosalicylic acid |
| 12. Quinolones       |
| – Ciprofloxacin      |
| – Ofloxacin          |
| – Levofloxacin       |
| – Sparfloxacin       |
| – Moxifloxacin       |
| 13. Thiacetazone     |

**Second-line antituberculous drugs**

**Section summary**
The so-called second-line drugs include capreomycin, kanamycin, amikacin, ethionamide, cycloserine, para-aminosalicylic acid, thiacetazone, and the second-generation fluoroquinolones (ofloxacin and ciprofloxacin). All are more expensive, more difficult to obtain, less effective, much more toxic, and less tolerated than the first-line drugs. For this reason, attempts should always be made to introduce as many first-line substances as possible in the re-treatment scheme. The side effects and intolerances involved must be well known in order to ensure effective management of patients undergoing re-treatment.

Table 14 details the mechanism of action, target bacterial population, dosage, common side effects, and drug interactions of the most common second-line antituberculous drugs. In addition, the following should be considered:
1. Thiacetazone should be administered at a dose of 150 mg in a single administration. Its main complications comprise blood dyscrasias with the appearance of bleeding and petechiae due to thrombocytopenia. Aplastic anaemia has also been described. In HIV-infected patients, dermatological alterations are frequently seen, some of which can be fatal (Steven-
Johnson syndrome). The use of thiacetazone is therefore contraindicated in such patients.

2. While capreomycin is not exactly an aminoglycoside, its mechanism of action is similar. It is less toxic, however, and may therefore be the drug of choice in re-treatments when streptomycin cannot be used.

3. All drugs should be administered in a single dose and simultaneously, since this not only offers superior results, but in general also ensures improved tolerance with fewer toxic effects. The only exception is represented by rifampicin and para-aminosalicylic acid, which should be administered at least 8 to 11 hours apart. In the case of ethionamide, para-aminosalicylic acid, and cycloserine, dosing in two to three administrations should be considered because of the possibility of gastric intolerance.

4. Prothionamide is an ethionamide derivative; it possesses the same action but offers improved gastric tolerance. The dosage indicated is the same as for ethionamide. However, since there are more data on ethionamide, ethionamide is preferred.

5. The fluoroquinolone derivatives, dealt with more extensively below (under the section on new antituberculous drugs), are perhaps the drugs on which most research in TB re-treatment has been conducted in the last decade. These agents constitute one of the possible options in patients with MDR-TB.

6. Clofazimine is an antileprosy drug that is also used in MDR-TB, although its efficacy against TB is very low.

### Table 14. Daily dosage, side effects, and drug interactions of the major second-line antituberculous drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Daily dose</th>
<th>Side effects</th>
<th>Side-effect control test</th>
<th>Interaction</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capreomycin</td>
<td>15-30 mg/kg Up to 1 g</td>
<td>VIII cranial nerve lesion, nephrotoxicity</td>
<td>Vestibular function, audiometry, BUN</td>
<td>Neuromuscular blocker</td>
<td>Extracellular bactericide</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>15-30 mg/kg Up to 1 g</td>
<td>VIII cranial nerve lesion, nephrotoxicity</td>
<td>Vestibular function, audiogram, BUN</td>
<td>Neuromuscular blocker</td>
<td>Extracellular bactericide</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>15-30 mg/kg Up to 1 g</td>
<td>GI alterations, hepatotoxicity</td>
<td>SGOT</td>
<td></td>
<td>Extra- + intracellular bactericide</td>
</tr>
<tr>
<td>PAS</td>
<td>150 mg/kg Up to 12 g</td>
<td>GI alterations, hepatotoxicity</td>
<td>SGOT</td>
<td></td>
<td>Extracellular bactericide</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>10-20 mg/kg Up to 1 g</td>
<td>Psychosis, seizures, rash</td>
<td>Psychological tests</td>
<td>Alcohol</td>
<td>Extra- + intracellular bactericide</td>
</tr>
</tbody>
</table>

BUN = blood urea nitrogen; GI = gastrointestinal; PAS = para-aminosalicylic acid; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvate transaminase.
New drugs: future therapeutic perspectives in tuberculosis

**Section summary**

Despite the many studies continuously conducted to discover new drugs or therapies for TB, the great majority of possible options are still in the research stage and for the time being cannot be recommended in clinical practice. These alternatives include new antibiotics or derivatives of previously developed antimicrobials with known antimycobacterial properties. The drugs that are most widely used and that have demonstrated the best effects are the rifamycin derivatives, fluoroquinolone derivatives, some macrolides, the oxazolidinones, and the nitroimidazoles.

Of all the new antibiotics and other future therapeutic possibilities in TB treatment that are analysed in the present chapter, low- and middle-income countries can consider the incorporation of the second-generation fluoroquinolones (ciprofloxacin or ofloxacin) for the management of patients with MDR-TB as they are less expensive, well tolerated, and easily obtained. The remaining options have no current indications in these countries.

Many studies—the majority of which are in the experimental stage—have been carried out in an attempt to discover new drugs or therapies for *M. tuberculosis* disease. While most efforts have focused on the development of new antibiotics or derivatives of previously developed antimicrobials with known antimycobacterial properties, other substances or therapies have also been investigated. The present section will be divided into two parts: research in relation to new antibiotics, and future directions in TB management.

**New antibiotics with activity against M. tuberculosis**

Some agents have already been tested in clinical trials, while others have only been evaluated in animals or in *in vitro* studies. The level of knowledge gained is greatest in the case of the antimicrobials belonging to pharmacological groups similar to those already used, such as the derivatives of the rifamycins (e.g., rifabutin, rifapentine, FCE 22250, GCP 29861, R-76-1), isoniazid, clofazimine, thiacetazone, and mitronidazole. A second major group is represented by families of new antimicrobials, such as the quinolones (e.g., ofloxacin, ciprofloxacin, sparfloxacin, levofloxacin, moxifloxacin), the macrolides (e.g., roxithromycin, clarithromycin, azithromycin), folate reductase inhibitors, novel beta-lactams, and mycoplanecins, among others.

The drugs that are the most widely used and that have shown the greatest action have been the rifamycin derivatives, fluoroquinolone derivatives, some macrolides, oxazolidinones, and nitroimidazoles.
**Rifamycin derivatives**

Within the family of the rifamycins (and apart from rifampicin), rifabutin and rifapentine deserve mention since they have mechanisms of action and minimum inhibitory concentrations (MICs) very similar to those of rifampicin. However, rifabutin presents cross-resistance with rifampicin in 70% of cases, while rifapentine does so in 100% of cases. This eliminates their possible use in the treatment of patients with TB resistant to rifampicin.

Rifabutin has become a good drug for replacing rifampicin in the initial treatment of patients with HIV infection who need protease inhibitor therapy, since the drug causes less active induction of the cytochrome P450 pathways in the liver. It has also been found to be useful as one of the basic drugs in the treatment of disease caused by environmental mycobacteria.

The main advantage of rifapentine is that it is the rifamycin with the longest duration of action (24 hours, or five times longer than that of rifampicin), and it exhibits an increased macrophage penetration potential (25- to 50-fold that of rifampicin). Thus, rifapentine could be used in highly intermittent treatment schemes. This drug has been studied in a number of randomised clinical trials, almost all involving a single weekly dose of 600 mg, and always with isoniazid. Results from these studies have shown that regimens containing rifapentine demonstrate the same toxicity, tolerance, compliance, and therapeutic failure profile as those containing rifampicin, although the relapse rate is higher. This suggests that the weekly 600-mg dose is probably suboptimal. Regimens involving 900-mg and 1200-mg doses are currently being evaluated in patients without HIV infection, since in patients infected with the virus the failure rate has been found to be comparatively greater. Despite its possible future application, the role of rifapentine in the treatment of TB remains to be defined.

With regards to the other rifamycins, such as FCE 22250, GCP 29861, and R-76-1, there is little experience with them to date. Until there are further studies to assess their possible roles, none of them can be recommended.

**Fluoroquinolone derivatives**

These agents are carbonic acid derivatives, and their antituberculous action has been found to involve the inhibition of DNA gyrase. Since all the agents in this class act at the same level, they all present cross-resistance to each other. Consequently, if one of them cannot be used because of established bacterial resistance, the rest of the group is likewise rendered ineffective.
However, not all members of the family present cross-resistance with the rest of the antituberculous drug groups.

The first-generation fluoroquinolones, such as norfloxacin, are of little use, since they need to be given in very high doses. However, the second-generation drugs, such as ofloxacin and ciprofloxacin, have shown very good sensitivity in vitro, and there is sufficient clinical experience to propose them as one of the drug options of choice in TB re-treatment regimens—particularly because they are very well tolerated over the long-term and are easily available almost anywhere in the world. However, no randomised clinical trials have yet been conducted of these drugs.

In turn, some third-generation (e.g., levofloxacin) and fourth-generation (e.g., moxifloxacin) fluoroquinolones offer superior MICs than do the second-generation drugs, and may also have additional advantages. For example, it has been found that the sterilisation capacity of moxifloxacin in the lungs of infected mice is superior when the drug is used with isoniazid than when either isoniazid or moxifloxacin is used alone. However, these drugs are very expensive, and there is no information yet on their long-term tolerance and toxicity. Consequently, for the time being, the second-generation fluoroquinolones are preferred, as they are less expensive, well tolerated, and easily obtained.

Macrolide derivatives

Although some macrolides, such as roxithromycin, clarithromycin, and azithromycin, have demonstrated in vitro antimycobacterial activity with good MICs, this is not the case in all instances, and there is insufficient evidence to recommend their use in the clinical setting. Accordingly, because both clarithromycin and azithromycin have shown such good activity against the rest of environmental mycobacteria, they currently constitute the basis for treating diseases caused by practically all these mycobacteria.

Oxazolidinone derivatives

The oxazolidinones are antibiotics that are administered orally. Their mechanism of action involves early protein synthesis disruption. Both the most widely used representatives in this class (e.g., linezolid) and the rest of its members (e.g., U-100480 and esperezolid) have demonstrated antituberculous activity in vitro. Linezolid also demonstrates antituberculous activity in vivo, and has been used on an experimental basis in patients with MDR-TB. Little, however, is known about its toxicity, particularly when administered
for prolonged periods of time. To date, the main toxic effects identified involve induced anaemia and thrombocytopenia, and peripheral neuropathy. These agents do not present cross-resistance with the antituberculous agents. At present, the lack of knowledge about their possible toxicity and their very high price excludes these drugs from possible use, even as rescue medication.

**Nitroimidazole derivatives**

These compounds, which are related to metronidazole, have been shown to be bactericidal when used against *M. tuberculosis*, both *in vitro* and *in vivo*. Experiments involving the drug referred to as PA-824 have shown efficacy similar to that of isoniazid, although its spectrum of action is very restricted (highly specific for TB). Both isoniazid and PA-824 affect bacterial cell wall lipid synthesis, although at different stages of the process. PA-824 also inhibits protein synthesis. In the same way as isoniazid, PA-824 acts on the rapidly multiplying bacterial population. However, in a static anaerobic culture model, the drug has also appeared to act on bacteria that are not in the replication phase. PA-824 has been shown to be effective against *M. tuberculosis* strains that are resistant to the first-line drugs. Moreover, it appears to be less toxic than isoniazid. Although further studies are needed, this agent shows promise as a good alternative to the first-line medicines. Until then, it cannot be recommended for use.

**Other future therapeutic possibilities in tuberculosis**

Apart from the antimycobacterial agents, other substances that are not antibiotics have also been developed. These substances, which show inhibitory effects on mycobacterial growth, include the derivatives of vitamin K or coenzyme Q (gangamycin).

Research is also being conducted on substances that interfere with the biosynthesis of vital components of mycobacteria, such as the mycoside C synthesis inhibitors, arabinogalactan synthesis inhibitors, transmethylation inhibitors, magnesium chelating agents, membrane cation flow inducers, substances interfering with mycobactin synthesis, membrane receptor blockers, trehalose phosphate synthetase inhibitors, analogues of meso-diaminopimelic-D-alanine, mycobactin analogues, and inhibitors of muramic acid enzymatic glycosylation.

Another line of treatment research refers to the so-called products for re-sensitising previously resistant bacterial strains, such as membrane per-
meators, beta-lactamase inhibitors (amoxicillin with clavulanic acid), and inhibitors of aminoglycoside-inactivating enzymes.

Lastly, an important chapter in the development of antimycobacterial treatment comprises immunotherapeutic or immune-modulating agents, which, together with the above-mentioned substances, might improve TB therapy. Monoclonal antibodies, new vaccines, substances that improve opsonisation, cytokines, and immune-enhancing microbiological agents are the options that are currently being investigated the most intensively.

Another future therapeutic possibility is the use of antimicrobials contained within liposomes. These are phospholipids (double- or multi-layered phospholipid vesicles) that can encapsulate drugs and macromolecules (size: 0.2 nm to 2-3 nm). These liposomes are avidly phagocytosed by macrophages, which may help direct drugs towards the macrophage population and the rest of the organs of the reticuloendothelial system. Liposomes are administered via the intramuscular route, which would help to reduce the dose and number of injections required, as well as (hopefully) toxicity and cost. The effect may persist for up to 5 weeks after administration, which might facilitate improved patient adherence to therapy. Studies involving streptomycin have been conducted in mice, as well as with rifampicin and isoniazid contained in cholesterol and phosphatidyl choline as vehicles. Intracellular action has been demonstrated, particularly in the liver, spleen, and kidneys, and, to a lesser extent, in lung tissue and lymph nodes.

**Surgery for the management of multidrug-resistant tuberculosis**

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<td>Removing diseased portions of lung tissue for the management of MDR-TB is practically never indicated and should be rejected systematically. Lung surgery should only be contemplated in exceptional cases. Unfortunately, surgery is often ultimately used because of a lack of experience in the management of these patients or because of a lack of second-line drugs in certain countries. In such situations, it would be necessary to decide which approach is more reasonable: surgery in the face of such practical difficulties, or establishment of an adequate second-line drug bank to avoid surgical treatment.</td>
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With all the therapeutic resources and premises considered above, it is clear that efforts to remove diseased lung tissue for the management of MDR-TB should almost never be performed. The special circumstances that would
warrant surgery include the presence of localised lung lesions, patients with
good respiratory function, and the unavailability of drugs needed to design
a re-treatment regimen. It should be emphasised that lung surgery is associ-
ated with high morbidity and mortality; further, the procedure is not certain
to cure the disease. Thus, ultimately, TB re-treatment is a matter of adequate
management of second-line drugs.

Re-treatment as a strategy in an NTP: possibilities in low-
and middle-income countries

Section summary
No resources should be spent on re-treatment until it is certain that all initial
disease patients have been granted free access to a short-duration treatment
scheme.

Once the above has been taken into account, even the poorest countries with
the greatest TB problems should attempt to offer at least one standard 8-month
re-treatment regimen involving first-line drugs (2HRZES/1HRZE/5H3R3E3). This
regimen is inexpensive and practical. It has been shown to heal over 90% of the
patients included in NTPs as re-treated cases. It also identifies patients who have
drug resistances, allowing them to receive more individualised care at a higher
level within the health care system. The great majority of low-income countries
should not spend further resources on re-treatment.

However, in middle-income countries, as well as in some low-income coun-
tries, the acquisition of a second-line drug bank may be advisable in order to
offer a standard treatment regimen with these agents: 3(pyrazinamide, kanamy-
cin, ethionamide, ofloxacin)/15(pyrazinamide, ethionamide, ofloxacin). Such drug
banks should always be managed by the Central Unit of the corresponding NTP.

Individualised re-treatment according to the sensitivity results is only advis-
able in the wealthier countries and, as a last resort, in some middle-income coun-
tries.

It should be emphasised that prevention—with good initial drug schemes and
strict supervision of treatment—is the best way to avoid having to deal with
the complex problem of re-treatment.

The topics covered in this section offer a theoretical update in the field,
and point to easy applicability, provided the best economical, health care,
and epidemiological conditions are available. This is indeed the situation in
richer countries, which have few cases of TB and which have abundant eco-
nomical and health care resources. In these countries, there are more specialist
physicians than there are patients requiring re-treatment. This makes it pos-
sible for each patient to be cared for by expert personnel, with the added support of all the necessary diagnostic methods (rapid sensitivity testing) and therapeutic options, since there are no economic limitations. These expert physicians are able to identify relapses and cases of complete treatment abandonment, and can recommend returning to the same initial treatment scheme. They are also able to identify failures and cases of partial abandonment, and design re-treatment schemes that involve the appropriate second-line drugs. Thus, in the industrialised parts of the world, re-treatment is customised for each specific case. Nevertheless, these individualised re-treatments cost between US$5000 and US$10,000, and may even exceed US$100,000 if the costs of prolonged hospitalisations are included.

Need for a standard re-treatment regimen with first-line drugs at the peripheral level in low-income countries

The situation described for industrialised countries does not apply to most regions in the world. In TB endemic countries—all of which have limited economic resources—even with the institution of good NTPs it is estimated that 10% to 15% of initial patients will ultimately return to the programme after some time. This means that in a country where 10,000 cases of TB are diagnosed a year, 1500 would require re-treatment. Depending on the existing economic and health care level, this figure is 50 to 500 times greater than the number of specialist physicians available in the country. This makes it practically impossible for each patient who is readmitted to the programme for re-treatment to be cared for by an expert in the field. Without great experience, the risk of incorrectly determining the cause for re-treatment (i.e., treatment abandonment, relapse, treatment failure, or poor patient compliance) is very high. This is a very important point, since only treatment failure indicates the existence of drug resistance (although poor adherence to therapy may also explain resistance). In the remaining cases (about 90% of patients), relapse or treatment abandonment is more likely the explanation, and the development of drug resistance is thus not likely to be expected.

Owing to the large numbers of patients admitted for re-treatment in countries with high TB endemic rates, it is necessary to treat these patients at the peripheral levels of the health care network, where it is not possible to establish which of the above four reasons is responsible for the indication of re-treatment. Moreover, it would be absolutely impossible, from the economic perspective, to provide individualised re-treatment schemes for all these patients. In effect, while these treatments cost US$5000 to US$7000,
an initial treatment scheme, such as those recommended in the present Guide, costs only US$10 to US$20. In fact, the cost of a single individualised (and less effective) re-treatment scheme would be equivalent to the cost of treating 250 to 350 patients with initial disease—the group of patients that constitutes the true epidemiological priority in TB endemic countries. Furthermore, most countries with a high prevalence of TB can only afford to spend less than US$50 to US$100 per inhabitant for all personal health care needs.

These operational and economic limitations led the International Union Against Tuberculosis and Lung Disease to consider the introduction of a standard re-treatment scheme with well-tolerated first-line drugs and easy management characteristics as the best approach to large-scale re-treatment in NTPs. This 8-month re-treatment regimen is administered in three phases (2HRZES/1HRZE/5H_{3}R_{3}E_{3}), should be strictly supervised, and costs only about US$30. The scheme is run in a similar way as that for initial TB patients. Under field conditions, the scheme will heal all relapse cases and cases of treatment abandonment (which is the cause of the great majority of re-treatments), along with 50% of patients reporting treatment failure. In this way, at the peripheral level, the proposed regimen will serve to identify the true cases of resistance (i.e., the patients who do not heal), which represents only 1% to 2% of the total number of patients. This small patient group can then be treated at higher levels within the health care system by specialist physicians, and with the possibility of using second-line medicines.

Re-treatment with second-line drugs in low-income countries: individualised or standardised?

It has already been explained why in all low- and middle-income countries the corresponding NTPs should implement a standard 8-month re-treatment scheme involving first-line drugs for patients failing initial therapy. Such a scheme, conducted at the peripheral level and involving simple management, would enable the identification of true problem cases, i.e., patients with drug resistances. However, once these patients have been identified, what should be done with them? Is use of an expensive individualised regimen involving second-line drugs justified?

Two possibilities exist for the management of such patients: individualised re-treatment according to the resistance pattern observed, or re-treatment involving a standardised regimen of second-line drugs, without the need to perform sensitivity tests. There has been great controversy over which of the two is more appropriate. However, the fact is that second-line treatments are
used infrequently in the great majority of countries with low-income levels; consequently, very few resistances to such drugs may be expected. Furthermore, such countries generally have very few chances of performing susceptibility tests with second-line drugs. Even if testing were possible, reliability is much more limited than in the case of first-line agents. For this reason, the most practical approach in these cases is to provide a standard re-treatment regimen involving second-line drugs that are known to be relatively well tolerated and easy to obtain, and that may offer important savings. Such a regimen may include pyrazinamide, a drug that is always administered together with other agents and to which there is rarely drug resistance. If the regimen does not include either isoniazid or rifampicin, the duration of re-treatment would be a minimum of 18 months. The most recommended option would be 3 months with pyrazinamide, ethionamide, ofloxacin (or ciprofloxacin), and an aminoglycoside other than streptomycin (although kanamycin is easier to acquire in most parts of the world), followed by another 15 months with the same drugs but withholding the aminoglycoside. This second-line drug regimen costs approximately US$1000, which represents an important savings compared with the US$5000 to US$7000 associated with individualised re-treatment schemes.

Only when this third treatment step fails is it justified to move on to the fourth level of therapy—the most expensive and difficult in terms of management—which involves individualised re-treatment with second-line drugs lasting a minimum of 18 to 24 months.

Possibilities for re-treatment according to the available economic resources

It has already been emphasised that re-treatment is never an epidemiological priority. This is why, at least in theory, no resources should be spent on re-treatment until it is confirmed that all patients with initial disease have been granted free access to a short-duration treatment regimen. Unfortunately, this is not the case in many areas with serious TB problems; indeed, few resources are dedicated to sensitivity testing, second-line drugs, and other such concerns. This notion of maintaining cost-efficacy priorities is in conflict with the fact that caring for a patient with MDR-TB, without being able to offer the patient the necessary drugs, is almost the equivalent of a death sentence. This individualised clinical approach to the problem is the source of much controversy. As a result, the best approach may be to ensure treatment for all initial TB cases, under supervision, and to concurrently establish
a minimum second-line drug bank that is to be managed only by personnel with extensive experience.

Once the above considerations have been applied, the implementation of the indicated re-treatment protocol becomes a matter of available economic and health care resources. All countries, including the poorest nations with the greatest TB endemic problems, should attempt to offer at least one standard 8-month re-treatment regimen involving first-line drugs (2HRZE/1HRZE/5H$_{3}$R$_{2}$E$_{5}$). As has been commented, this scheme is not particularly expensive and can be administered to a good proportion of patients (15% of the total); as a result, it can be expected to have epidemiological repercussions. This regimen should at least be included in the respective NTP manuals. Perhaps the results afforded by such treatment can provide the basis for future cohort studies.

Most of the low-income countries should not spend more resources on re-treatment apart from those already mentioned. However, countries with middle-level incomes (and also some low-income countries), which should have already moved beyond these two steps of treatment (initial patients and standard re-treatment with first-line drugs), may be able to establish a second-line drug bank so as to be able to offer a standard re-treatment scheme with these agents. However, the great risk posed by acquiring these drugs is possible indiscriminate use, which could lead to the amplification of drug resistance and to possible treatment inefficacy of second-line drugs in the future. For this reason, when an NTP decides that it possesses the necessary resources for, and considers acquiring, a second-line drug bank, careful assessment should be made of the number of annual re-treatments that may be required. Once acquired, this drug bank should always be managed by the NTP’s Central Unit, which should not authorise any re-treatment without first confirming that sufficient amounts of medication exist for completion of the required 18 months of treatment. Moreover, before authorising such costly re-treatments, the prescribing specialist physician and the specialist physician working part- or full-time in the Central Unit should agree on the appropriateness of the course of therapy. This way, adequate management is guaranteed, resources are not wasted, and the validity (efficacy) of the drug is not compromised.

The last therapeutic step, which involves individualised re-treatment according to the sensitivity results obtained, is only advisable in richer countries and, as a last resort, in some middle-income countries.
Recommended reading for the chapter

Chapter 11 - Resistance of *M. tuberculosis* to antituberculous drugs

Chapter summary

The prevention of drug resistance must be the first and most important premise of tuberculosis (TB) management. A TB patient with multidrug resistance (MDR) is much more difficult and costly to cure. Consequently, all efforts should be made to prevent the development of drug resistance by adopting a series of basic measures that are easy to implement under control programme conditions. These measures can be summarised as follows:

1. Implementation of a good National Tuberculosis Control Programme for the entire country.
2. Directly Observed Therapy, Short Course (DOTS) for all initial TB cases.
3. Recommendation of directly observed treatment for all patients.
4. Administration of antituberculous drugs combined in the same tablet.
5. Minimisation of the influence of the private health sector on the management of TB.
6. Treatment without any financial cost to the patient.

It is necessary to clearly distinguish between the concept of initial or primary resistance and secondary or acquired resistance. The truth is that barely 50 years after the introduction of the first antituberculous drugs, the indiscriminate use of such agents has led to the appearance of many MDR-TB cases in many parts of the world. It is thus important to know the future extent of this problem. Current estimates suggest that the situation may reach catastrophic proportions if MDR bacilli are as capable of causing disease as drug-sensitive bacilli.

Fortunately, *M. tuberculosis* only acquires resistance through genomic alterations, and the mutation, which most frequently phenotypically expresses resistance to isoniazid, is linked to a vital gene (*katG*) that also encodes for enzyme activities (catalase and peroxidase), which in turn are integral for the survival and virulence of the microorganism. For this reason, the development of resistance is often associated with a fitness cost to the bacilli. Thus, transmission of MDR strains in the community probably will only have clinical importance in the future for severely immunosuppressed individuals, particularly those infected with HIV. In contrast, in immunocompetent individuals, only isolated cases of such resistance will continue to appear, as has occurred in the past decades with the contacts of chronic TB patients.
There is abundant evidence of the capacity of *M. tuberculosis* to adapt to adverse environmental conditions. One example, of a microbiological nature, has only become apparent in the last three to four decades, when the selective pressure exerted by antituberculous drugs revealed one of the many mechanisms used by *M. tuberculosis* to defend itself against treatment. In effect, only after the introduction of drugs for the treatment of tuberculosis (TB) was it realised that within the enormous population of microorganisms present in the diseased individual, many bacteria present genetic mutations that make them resistant to such drugs. Resistance of *M. tuberculosis* to antituberculous drugs was emerging—becoming in the last decade one of the most serious problems identified by the international organisations attempting to control this endemic illness. Fortunately, *M. tuberculosis* only acquires resistance through genomic alteration, a fact that has greatly facilitated the struggle against this problem.

**Basic concepts and definitions**

**Section summary**

It is necessary to distinguish among three different concepts in relation to the development of drug resistance. Natural resistance is resistance found in wild strains as a result of their continuous multiplication, which does not constitute a significant population. Such wild-type resistance must be selected by an antituberculous agent in order for that natural resistance to be expressed phenotypically. When this happens as a consequence of deficient therapy (i.e., genuine or masked monotherapy), so-called acquired or secondary resistance develops. If a patient with acquired resistance from prior therapy infects an individual who has not used antituberculous treatment in the past, the second subject may develop TB; in this case, the type of resistance is referred to as initial or primary resistance (i.e., patients not previously treated). When a patient shows concomitant resistance at least to isoniazid and rifampicin, he or she is said to have multidrug resistance.

Thus, resistances in the context of TB always reflect substandard treatment of the disease. Acquired resistance is a direct consequence of poor therapeutic practice, whereas primary resistance constitutes evidence of the transmission of resistant strains found within the community.

The natural resistance of *M. tuberculosis* to antituberculous drugs is a characteristic of the genetic plasticity of the microorganism, although it did not become apparent until antituberculous drugs were introduced. At present, and as has been commented before (Table 5), spontaneous natural mutation
in a wild-type TB culture yields one mycobacterium resistant to isoniazid for every $10^5$ to $10^7$ bacilli, and one strain resistant to rifampicin for every $10^7$ to $10^9$ microorganisms. The frequency for the rest of drugs is one mutant per $10^5$ to $10^7$ bacilli, with the exception of pyrazinamide, for which the ratio is one mutant bacterium per $10^2$ to $10^4$ bacteria. This rate of genetic alteration is different for each anti-TB drug. Consequently, the probability that resistance to two drugs may develop is equal to the product of their respective mutation rates. The bacillary population present in a host with cavitary lesions is $10^8$ to $10^9$, versus $10^3$ to $10^5$ in the case of caseum or nodular disease (Table 6). Therefore, if treatment is started with a single drug in a case of cavitary TB, the patient experiences a first phase in which most of the bacteria are eliminated and the symptoms improve. However, this treatment selects the resistant bacilli, which in a short time become the dominant microbial population. In addition, the drug in question will have lost its efficacy for that patient, since TB resistance is chromosomal, definitive, and irreversible. Thus, all monotherapeutic regimens (real or masked by combination with drugs to which resistance has been developed, or which prove ineffective) inevitably lead to treatment failure and to the appearance of drug resistance. Accordingly, the need to combine drugs that have never been used by the patient (or which have been correctly associated in the past) must be the first guiding principle in TB therapy. This principle is relatively simple to follow in the case of an initial patient, but can become difficult when the patient has already been subjected to various treatment schemes. On administering two or more antituberculous agents, the risk of developing resistance is practically zero, since the bacillary load required to select a wild-type resistant strain would be too large for the human body: $10^{14}$ for isoniazid plus rifampicin, $10^{10}$ for isoniazid plus rifampicin plus ethambutol, and $10^{10-12}$ when three second-line drugs are combined.

Based on the above considerations, three completely different situations can be identified within the concept of resistance. The first situation is represented by natural resistance, which is found in wild-type strains as a result of their continual multiplication. In effect, on reaching a certain number of bacilli, a genetic alteration takes place in one mycobacterium; this mutation by chance may specifically affect the target site of some antituberculous drugs. However, such resistance must be selected by the drug in question in order for natural resistance to be expressed phenotypically. When this happens as a consequence of deficient therapy (i.e., genuine or masked monotherapy), so-called acquired or secondary resistance develops. In all such cases, the underlying cause is human error, owing to incorrect treatment prescription
by the physician, or to patient selection of the drug being taken. If a patient with acquired resistance infects an individual who has not used antituberculous treatment in the past, this second subject may develop TB—resistance in this case is referred to as initial or primary resistance (involving patients not previously treated). The best term in microbiological terms is “primary” resistance, which is advocated by the World Health Organization (WHO) and by the International Union Against Tuberculosis and Lung Disease (IUATLD). In contrast, in operational terms, the designation “initial” resistance is preferred, because it encompasses genuine primary resistances and also patients who claim to have never used anti-TB medication in the past, but who have indeed used such drugs and hide the fact (either out of ignorance or intentionally to gain access to treatment). In many countries, patients who have received TB therapy in the past no longer have access to treatment regimens financed by the state, since the limited resources make it necessary to restrict treatment to initial disease cases only. The time limit for previously administered treatment to differentiate between primary and acquired resistance is 1 month, for it is acknowledged that although monotherapy may have been provided during this time, it would not have been sufficient to have allowed the selection of naturally resistant mutants in the initial bacterial population.

Finally, when a patient shows concomitant resistance at least to isoniazid and to rifampicin (a very serious situation), he or she is said to have multidrug-resistant (MDR) TB.

Thus, resistances in the context of TB reflect substandard treatment of the disease. Acquired resistance is a direct consequence of poor therapeutic practice, whereas primary resistance constitutes evidence of the transmission of resistant strains found within the community.

**Evolution and present status of drug resistance worldwide**

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<td>Depending on the more or less appropriate management of TB in the world during the past decades, regions can be found with high, medium, or low primary resistance rates (data for 1997-2000). The worst situation is found in the Baltic republics of the ex—Soviet Union (Latonia: 14.4%; Estonia: 10.2%), the Dominican Republic (6.6%), the Ivory Coast (5.3%), Russia (4%), Thailand (3.8%), Romania (2.8%), and Peru (2.5%). However, in terms of magnitude of the TB burden, the most worrisome situations are found in India (13.3%) and China (11.3%).</td>
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At the opposite extreme, there are countries with practically no cases of primary multidrug resistance. This situation is attributable to the existence of good National Tuberculosis Control Programmes (Kenya: 0%; Botswana: 0.2%; Benin: 0.3%; Scotland: 0.3%; France: 0.5%; New Zealand: 0.7%; Cuba: 0.7%; Lesotho: 0.9%; the Czech Republic: 1%; Nepal: 1.1%; Chile: < 0.5%; and Uruguay: < 0.2%), the infrequent use of rifampicin in some areas (Botswana and Lesotho), or the fact that rifampicin has always been used in combination with other drugs, especially in countries where the combination of drugs in the same tablet has become standard (Spain: 0.5%; Brazil: 0.9%).

In the 1960s and 1970s, the flawed management of TB led to a great increase in resistances to streptomycin and isoniazid, i.e., the drugs which, at that time, constituted the basis of TB therapy in many parts of the world where good National Tuberculosis Control Programmes (NTPs) had not been instituted. Extensive regions, particularly in Asia, even reported primary resistance rates to isoniazid of over 25% to 30%, all due to the indiscriminate use of the drug.

In 1967, rifampicin was introduced. Rifampicin, along with isoniazid, is considered the best antituberculous agent for TB treatment. From the start, rifampicin has always been combined with isoniazid, and in parts of the world that have good NTPs it has been used in combination with other drugs. As has been commented, the appearance of a single mutant resistant to rifampicin requires the existence of a large bacillary population ($10^7$-$10^9$ microorganisms), as a result of which this is the antituberculous drug requiring the greatest mutation rate for the selection of resistances. Consequently, if rifampicin is administered under conditions of real or masked monotherapy, it is the drug with the least tendency to lead to naturally resistant mutant selection. This, and the fact that its higher cost has not facilitated its use in many parts of the world until only very recently, caused resistances to the drug to take longer time in emerging. Nevertheless, in the last decade, rifampicin has been used increasingly throughout the world, and, unfortunately, in a manner that is not according to the guidelines of a good NTP. Consequently, resistance to rifampicin is beginning to emerge as a genuine public health problem, particularly because this resistance almost always appears associated to resistance to isoniazid (i.e., multidrug resistance [MDR]).

The WHO estimates that in the year 2000, there were 50 million people globally who were infected with MDR-TB. However, this is considered to be only the tip of the iceberg, with unpredictable consequences for the future, since in the coming years this important reservoir may give rise to a
potentially incurable TB epidemic in most parts of the world. The future concerns are closely tied to the question of whether MDR-TB is as transmissible and virulent as drug-sensitive bacilli.

An established fact is that the present situation of TB resistance, and particularly of MDR, is extremely varied in different parts of the world, with the phenomenon being associated with the past implementation of effective or ineffective NTPs. Thus, based on WHO data for the period 1997 to 2000, a series of so-called hot zones can be identified, with a significant presence of primary MDR (the most serious situation) found in the Baltic republics of the ex—Soviet Union (Latonia: 14.4%; Estonia: 10.2%), the Dominican Republic (6.6%), and other countries where TB drugs have been administered indiscriminately and with very poor control during the last decades (Ivory Coast: 5.3%; Russia: 4%; Thailand: 3.8%; Romania: 2.8%; Peru: 2.5%). More recent reports indicate that some regions in India and China—the two countries with the largest TB burdens in the world (in absolute numbers)—have MDR rates of 13.3% and 11.3%, respectively. These data, extended to the incidence of new cases estimated for both of these countries and considering their large populations, yield a figure of 238,806 possible new cases of MDR-TB in India and 158,813 in China. This would imply that 94% of all cases of MDR are developing in the 35 countries that correspond to the so-called MDR hot zones.

At the opposite end are countries with practically no cases of primary MDR. This situation is attributable to the following: 1) the existence of a good NTP (Kenya: 0%; Botswana: 0.2%; Benin: 0.3%; Scotland: 0.3%; France: 0.5%; New Zealand: 0.7%; Cuba: 0.7%; Lesotho: 0.9%; the Czech Republic: 1%; Nepal: 1.1%; Chile: < 0.5%; Uruguay: < 0.2%), 2) the still infrequent use of rifampicin in some areas (Botswana and Lesotho), or 3) the fact that rifampicin has always been used in combination with other drugs, especially in countries where the combination of drugs in the same tablet has become standard (Spain: 0.5%; Brazil: 0.9%), where although good NTPs have not been implemented, such combination dosing in the same tablet has afforded low primary resistance and MDR rates. Similar considerations may also apply to places such as Lesotho, New Zealand, and Scotland.
The future of multidrug-resistant tuberculosis in the world: infectivity, pathogenesis, and virulence

Section summary
There is considerable uncertainty about the potential magnitude of the epidemic of MDR-TB. The severity of the spread of resistant bacilli depends on the capacity of patients infected with MDR-TB to actually develop the disease. The transmission capacity and infectivity of these MDR strains is very similar to that of sensitive bacteria, and tuberculin skin test conversion in individuals recently exposed to MDR bacilli is associated with an equal prevalence of infection. However, it is very possible that the virulence of these bacteria is comparatively less, i.e., MDR-TB may be much less able to result in the development of active disease, as has been seen in the contacts of chronic infectious patients. There are molecular mechanisms to justify these observations.

In order to define the future state of MDR-TB in the world, it is necessary to study the transmissibility and infectivity of MDR bacteria. If it is assumed that these resistant microorganisms have the same capacity to produce contagion and cause disease, then the situation will truly become critical, since of the 50 million people infected with MDR bacilli, an estimated 10% (5 million) could develop active TB. However, as early as the 1950s, Middlebrook and colleagues demonstrated that guinea pigs inoculated with bacilli resistant to isoniazid produced much fewer lesions and had lower mortality than did animals infected with sensitive bacilli. These observations, which have been corroborated by subsequent investigators, were attributed to a lack of catalase and peroxidase production capacity on the part of the resistant bacteria—indicating that the synthesis of these bacterial enzymes was linked to virulence. It was thus assumed that bacteria resistant to isoniazid effectively lose their capacity to produce catalase and peroxidase, and are therefore less virulent than sensitive bacteria and are practically non-pathogenic.

This theory had good epidemiological support, as seen, for example, by the lack of increase in the primary resistance rates among American soldiers who were infected in Vietnam or Korea. However, this hypothesis generated controversy in the 1980s, when attempts were made to show that the resistant bacilli were as pathogenic as the rest. As a result, the alarm was raised in the United States during the late 1980s and early 1990s, when there were reported cases of important foci of nosocomial MDR-TB transmission, which led to a true panic situation that justified the implementation of a national plan to combat this so-called third TB epidemic (the first being the original epidemic and the second being the TB epidemic linked with the emergence of HIV).
Although over 80% of the cases of disease developing as a result of MDR-TB transmission were in severely immunocompromised patients (particularly HIV-infected subjects), it was the observation that a substantial percentage of health care personnel (22-50%) who were caring for these patients also showed tuberculin conversion which caused concern in the medical profession. This suggested that a reservoir of persons infected with MDR bacteria was being created, with the potential capacity of causing refractory TB disease in the future, when, theoretically, the chemoprophylactic regimens involving isoniazid would be ineffective. This concern led several prestigious scientific societies to recommend chemoprophylaxis (in many cases of a truly questionable nature) involving second-line drugs for which no preventive efficacy had been demonstrated.

The above considerations are a consequence of frequent confusion among the terms “infectiveness”, “pathogenesis”, and “virulence”. Infectiveness refers to the capacity of a microorganism to infect or colonise a given host. Pathogenesis is the capacity of that microorganism to cause clinically manifest disease, and depends on the conflict between the microorganism and the host defence mechanisms. Virulence refers to the aggressiveness of the microorganism and its capacity to cause damage or death. Concepts such as infectiveness and transmissibility are indeed intimately related, although the possibility of infection is also dependent on the initial response capacity of the non-activated alveolar macrophage population. Infectivity can be measured by the proportion of people showing tuberculin skin test conversion from negative to positive after exposure to an infectious case. Pathogenesis and virulence are intimately related terms.

For many years there has been discussion about the different transmissibility of bacilli resistant to isoniazid, even though it would seem logical for both resistant and sensitive bacteria to be equally transmissible, since transmission is dependent on physical factors such as proximity and the duration of contact, and on the capacity of the contagious individual to cough and produce aerosol droplets loaded with bacteria. Theoretically, both individuals with sensitive bacilli and those with MDR-TB have the same capacity to cough and generate aerosols, as a result of which transmissibility should be similar in both cases. Indeed, this has been shown to be the case in some studies. If a close contact inhales bacteria from a patient, the microorganisms will trigger a macrophage- and T lymphocyte—mediated immune response on reaching the alveoli. This reaction should be very similar, regardless of whether the penetrating organism is resistant or sensitive, unless the less virulent bacteria can be destroyed more easily by the non-specific host defences.

This reaction occurs in the context of tuberculin intradermoeaction conversion, i.e., the production of a cellular immune reaction to the aggres-
sion of these mycobacteria. For this reason, the fact that a given susceptible population is seen to undergo tuberculin conversion upon exposure to MDR-TB only suggests that transmission and consequent infection have taken place. Whether or not actual clinical disease subsequently develops will depend not only on the host immune system but also on the virulence of the bacteria involved. This is why the observation of new tuberculobacterial infections among health care personnel caring for MDR-TB patients does not indicate that they will have the same probability of suffering TB disease as if they had been infected with sensitive mycobacteria.

Professionals caring for chronic TB patients have observed that the great majority of the relatives of infected patients yield positive tuberculin test results, but very few of these relatives go on to develop the disease (clearly less than the estimated 10% to be expected). Similarly, a great number of publications during the 1980s and early 1990s reported a substantial number of tuberculin conversions among health care personnel caring for patients with MDR-TB, but there has been no evidence to date of these individuals developing active TB (when in theory up to 10% should have developed the disease). These epidemiological observations suggest that patients with MDR-TB are as transmissible and infective as individuals with sensitive strains, although patients infected with resistant mycobacteria are much less likely to develop active disease—probably because the resistant bacteria present attenuated virulence, as demonstrated by Middlebrook and Canetti during the period from 1950 to 1970.

**The contribution of molecular biology in predicting the future of multidrug resistance**

**Section summary**

Fortunately, *M. tuberculosis* only acquires resistance through mutation. The mutation that most frequently phenotypically expresses resistance to isoniazid is linked to a gene (*katG*) that also encodes for enzyme activities (catalase and peroxidase), which in turn are essential for the survival and virulence of the microorganism. For this reason, the transmission of MDR strains in the community probably will only have clinical importance in the future for severely immunosuppressed individuals, particularly those infected with HIV. In contrast, in immunocompetent individuals, only isolated cases of such resistance will continue to appear, probably linked to other genomic alterations that also affect resistance to isoniazid, as has occurred in past decades with the contacts of patients with chronic TB.
At present, with molecular biological techniques already incorporated into the diagnosis of TB, it has been shown that the gene altered in 90% to 98% of all cases of TB resistant to rifampicin is the \textit{rpoB} gene, which does not encode for any activity vital to bacterial virulence or survival. Therefore, resistance to rifampicin does not imply a reduction in bacterial virulence, and these resistant microorganisms will not only be as transmissible and infective as sensitive bacteria, but will also cause disease in the infected individual. The fact that this particular gene is altered in the great majority of cases of TB resistant to rifampicin, and that such resistance is frequently also associated with resistance to isoniazid, has led to the development of innovative techniques to detect this altered gene in clinical samples. Thus, if this mutation is identified, MDR status can be presumed at the time of actual TB diagnosis, and the appropriate measures can be taken.

Unlike in the case of resistance to rifampicin, which involves a single mutation, resistance to isoniazid is governed by several genetic mutations. The most frequently identified alteration affects the \textit{katG} gene. This alteration is present in 22% to 64% of all cases in which isoniazid resistance is phenotypically identified. It is the \textit{katG} gene that affects bacterial catalase and peroxidase activity. Therefore, when this gene is either mutated or missing, the resulting bacilli not only show resistance to high doses of isoniazid, but also are also unable to produce these two enzymes that are essential for the life of the microorganisms, and particularly for maintaining the intracellular status in infected individuals. Thus, in MDR-TB, where isoniazid resistance is conditioned by an altered \textit{katG} gene, the theories of Middlebrook and Canetti that pertain to a clearly lesser virulence of these bacteria would be applicable. In fact, this is the situation that is most often found in patients with acquired resistance. A different situation is presented in HIV-infected patients, in whom the existing immune deficiency makes the host vulnerable to disease produced even by microorganisms as mildly pathogenic as \textit{Pneumocystis carinii} or the \textit{M. avium} complex. Thus, in such individuals infected by much less virulent bacilli, the lack of an adequate immune response makes it much more likely for them to develop MDR-TB.

Only in those cases where resistance to isoniazid is conditioned by other genes (\textit{inhA}, \textit{ahpC}, \textit{kasA}) are the resulting \textit{M. tuberculosis} strains equally transmissible, infective, and pathogenic. This is what was probably found in the 1980s, when the theory of lesser virulence was questioned. However, in almost all cases of \textit{ahpC} gene mutation, and in 50% of \textit{kasA} gene alterations, a \textit{katG} mutation is also present. The same occurs in a substantial proportion of patients in whom mutation affects the \textit{inhA} gene.
In short, the future of MDR in the world will indeed constitute a very important problem for seriously immunosuppressed HIV-infected individuals. In contrast, the impact will be much less in the immunocompetent population, in which reports of MDR will almost always be linked to alterations of a gene other than katG producing resistance to isoniazid or, in exceptional cases, to over-expression of the ahpC gene, which could restore full virulence to these bacteria with deficient katG activities.

Basic measures for successfully combating multidrug-resistant tuberculosis

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| The treatment of MDR-TB is associated with high morbidity and mortality, and is extremely costly and difficult to treat in the great majority of poor countries. Therefore, the best first option is to work with simple and specific measures that have been shown to reduce the emergence of MDR-TB. These measures, which should be implemented under the conditions of an NTP, can be summarised as follows:
| 1. Implementation of a functioning NTP for the entire country.  
2. DOTS for all initial TB cases.  
3. Recommendation of directly observed treatment for all patients.  
4. Administration of antituberculous drugs combined in the same tablet.  
5. Minimisation of the influence of the private health sector in the management of TB.  
6. Treatment without any financial cost to the patient.  
Only by strictly implementing these measures can it become possible to prevent the emergence of drug resistance, since “even in countries with unlimited resources, it takes less time to generate 10, 50 or 100 cases of MDR-TB than to cure a single one of them.” |

Based on the above considerations, it is likely that the bleak future consequences of MDR-TB have been overestimated. Nevertheless, it remains true that treating MDR-TB is extremely difficult and very expensive (possibly costing more than US$10,000)—a situation that greatly limits the use of second-line drugs in low- and middle-income countries, which are most affected by this problem. Two directions should be taken in an attempt to reduce this problem worldwide. First, measures should be implemented under NTP conditions to prevent new cases of MDR-TB from developing. Second, efforts should be made to establish a basic second-line drug bank
to offer treatment to the existing cases with MDR-TB. The best measures are those aimed at reducing the number of future MDR cases, since “even in countries with unlimited resources, it takes less time to generate 10, 50 or 100 cases of MDR-TB than to cure a single one of them.” A series of basic measures have therefore been designed, to be implemented under NTP conditions. If implemented in their entirety, these measures will offer the best solution to the emergence of drug-resistant cases:

1. **Implementation of a good NTP for the entire country**, with demonstration of its efficacy over the years. A good control plan should include a series of actions, including the five measures indicated below, to gradually minimise the problem of MDR-TB in the world. A clear example of the importance of this measure is afforded by the experiences of countries such as the Czech Republic, Algeria, some parts of the United States, Lesotho, Botswana, Zimbabwe, Nepal, Korea, New Zealand, Scotland, and some Latin American countries (e.g., Nicaragua, Uruguay, Venezuela, Chile, and Cuba).

2. **DOTS for all initial TB cases**. The efficacy of this measure has been demonstrated in many countries and cities. Improvisation in a field as thoroughly studied as TB treatment only encourages the appearance of drug resistance.

3. **Recommendation of directly observed treatment for all patients**. When this measure is implemented under NTP conditions, it has been shown to reduce the resistance rates in both countries with limited resources (e.g., Cuba, Botswana) and those with middle-income levels (e.g., Czech Republic, Chile, Uruguay), as well as in some wealthy areas such as the United States (Baltimore, Maryland; New York; Texas).

4. **Administration of the antituberculous drugs combined in the same tablet**. This measure ensures that in the event that the patient abandons treatment, he or she does so completely, thereby avoiding the risk of selecting naturally resistant mutants. This is the measure recommended by the WHO and the IUATLD since 1988, and its efficacy in reducing the appearance of resistances in the community has been demonstrated in different studies. The low rates of resistance in Spain, Brazil, Lesotho, New Zealand, and Scotland clearly illustrate the efficacy of this measure. However, this measure does not facilitate adherence to treatment, as a result of which these drug combinations must be subjected to good quality control of both the associated drugs and their doses. The combinations of isoniazid + rifampicin, or isoniazid + rifampicin + pyrazinamide, are particularly recommended.
5. **Minimisation of the influence of the private health sector on the management of TB.** This is one of the major points of concern in highly endemic regions, such as Southeast Asia and the Western Pacific. Many of the countries in these areas present the most serious TB problems in the world, with physicians in private practice usually employing a therapeutic course that does not meet the specifications of the respective NTPs. As a rule, physicians in private health care tend not to follow NTP guidelines and do not participate in the training plans designed by these programmes. Moreover, private practice often does not recommend treatments in a standardised fashion, and these treatments also tend to be longer, expensive, and inadequate. The detrimental management effects by this influential sector in countries such as India has had much influence on the high resistance rates found in these regions. This impact is difficult to quantify, however, since the cases treated in private care often are not reported to the respective NTPs. The best way to counter this negative influence is to promote the NTPs, and to generate patient confidence and encourage the public to actively seek medical care for TB in the public sector. Another option would be to educate physicians in the private sector, as well as to offer incentives to encourage patient referral to public health care.

6. **Treatment without any financial cost to the patient.** In order to ensure success, this measure is one of the premises to be demanded before implementing an NTP. If the patient must personally pay for the medication, he or she will probably buy it and use it only for as long as the symptoms persist, particularly if the economic resources are limited, as is very often the case. When the symptoms improve, the natural tendency for the patient is to stop taking the medication—a behaviour that is reinforced when the patient must personally pay for the treatment and has little money to do so. At this point, the patient may decide to either completely abandon treatment, which has detrimental effects on the community but does not affect the problem of drug resistance, or selectively stop taking the most expensive drug. This second situation is understandable, especially when the patient must pay 10 times more for rifampicin and pyrazinamide than for isoniazid. However, this also represents the worst possible situation, since the patient will not only continue to be infectious to others in the community, but will also generate conditions ideal for the selection of drug-resistant mutants.
Recommended reading for the chapter

Chapter 12 - Control of tuberculosis

Chapter summary
Tuberculosis (TB) is a transmissible, preventable, and curable disease. Consequently, the cost-benefit ratios of adequate control measures are among the best known. Since no completely effective vaccine is yet available, the measures that have been shown to be most efficacious in controlling the disease have been early detection and treatment. However, the lack of resources for covering the costs of the treatments, and the fact that therapy is very prolonged, is causing these simple measures to fail in many of the poorer parts of the world.

In order to facilitate the large-scale treatment of TB cases, even under the most adverse conditions, it is necessary to implement the Directly Observed Therapy, Short Course (DOTS) strategy, which comprises five major variables: 1) political will; 2) establishment of a minimum network of laboratories capable of performing smear microscopy; 3) administration of short course and directly observed (supervised) treatment to all patients; 4) guarantee of drug and material resources; and 5) implementation of a registry and information system allowing periodic evaluation of the National Tuberculosis Control Programme.

At present, there are only three possibilities for intervention in the community in an attempt to control TB: 1) administering adequate chemotherapy to diseased patients; 2) providing preventive treatment or chemoprophylaxis for infected individuals at high risk of developing TB disease; and 3) mass vaccination of the population. Undoubtedly, the most important intervention strategy is chemotherapy, with chemoprophylaxis ranking a distant second, followed by bacille Calmette-Guérin vaccination. Efficacy, the duration of the intervention, the capacity to eliminate the sources of infection, and the possible benefits for the community are the bases of these priorities.

It is difficult to understand how tuberculosis (TB) continues to be the most important infectious disease affecting humans today, even though effective treatments have been available for over 40 years and the scientific bases for achieving disease control in the community have been known for more than three decades. This situation demonstrates the serious failure to control this old disease, despite the fact that we now have all the elements needed to combat the disease effectively.
History of the control of tuberculosis

Section summary
The lack of knowledge in the course of history concerning the aetiology of TB led to the inability to implement specific measures for controlling the disease. The means of controlling the disease first became possible when it was realised that TB was a disease transmitted through the sputum of ill patients. This led to the recommendation of isolating infected individuals to prevent further transmission. However, the sanatorium-reclusion era of TB did not likely have any epidemiological impact on the evolution of this endemic disease. Similar considerations apply to surgical treatment attempts. In truth, TB rates did not begin to decline in the developed world until the socioeconomic situation of the various populations began to improve, with a reduction in crowded living conditions and poverty. Apart from these social improvements, only the introduction of chemotherapy, with its promise of curing TB, has had a genuine impact on the prevalence of the disease. The remaining measures adopted over time have had practically no influence.

From ancient times to the discovery of the causal agent of tuberculosis

Despite the fact that TB has existed since antiquity (see Chapter 3) and that it is probably one of the illnesses most often studied and covered in the literature, there has been a surprising lack of sound knowledge about the disease throughout the course of history. This explains why humanity has been unable to defend itself from this terrible illness for the most part through history. Only very recently has it become possible to introduce measures for controlling the disease in the community. Koch was the first to mention the possibility of TB control, when he showed in 1882 that TB was an infectious disease. He not only isolated the bacterium, which was later named after him (Koch’s bacillus), from the sputum of infected patients, but he also suggested that the principal measure for controlling the disease in the community would be to isolate affected patients. This was the definitive step that paved the way for the “sanatorium era of TB”, which alleged that the prolonged reclusion of affected patients in sanatoriums was the only effective way to try to cure TB and control its spread within the community.

Improvement in socioeconomic conditions

However, as was explained in Chapters 3 and 4, even before these scientific reasonings and advances took place, the disease had begun to come under
control in richer countries, without the adoption of any specific control measures. The improvements in the socioeconomic conditions of the populations in developed countries from the mid-eighteenth century had already started to exert a slight influence over the disease, with a sustained decrease in the associated mortality and morbidity. It is now accepted that once an optimum level of development has been achieved in a given country, the resulting reduction in crowding and poverty has an important effect on the disease. By reducing the number of crowded living conditions, each source of *M. tuberculosis* transmission is no longer able to generate sufficient new cases of contagion to ensure a new diseased patient (an estimated 20 individuals would have to be infected for this to occur). In this way, the disease would tend to undergo self-elimination, with a sustained 4% to 5% annual drop in infection risk. However, these improvements in living conditions that have occurred in developed countries have still not been achieved in many of the poorer parts of the world—as a result of which spontaneous self-elimination of the disease seems unlikely in these countries.

**Isolation in sanatoriums and surgery for the treatment of tuberculosis**

It has not been possible to demonstrate whether patient isolation in sanatoriums had an impact on TB control or whether it contributed to the annual reduction in disease mortality attributed to improved living conditions. Although there were probably benefits in certain cases, the prolonged duration of contagion at diagnosis, the fact that only few patients had access to such centres, and the lack of truly effective therapy suggest that sanatoriums did not exert an important epidemiological effect on the evolution of TB. Similar considerations apply to the various surgical procedures developed to treat TB in the first half of the twentieth century. Although such treatments afforded individual benefits, patients continued to spread the disease in the community for a long time. Thus, although quantification of the impact of these control measures is difficult, it is likely that such procedures afforded little more than the benefits brought about by the improvement in socioeconomic conditions.

**Modern chemotherapy**

Beginning in the late 1950s, the cure of TB cases based on chemotherapy became the main option for shortening the epidemiological chain of the disease. It finally became possible to quickly detect contagious cases and provide treatment to shorten the period of infectivity. However, such measures
to establish cure, which seemed straightforward and promised an optimistic future for the control of TB, are in fact very difficult to implement because of the high rate of default associated with the lengthy treatment required for the eradication of the disease.

**The cost-effectiveness and difficulty of controlling tuberculosis**

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<td>TB is a transmissible, preventable, and curable disease. Consequently, the cost-effectiveness of adequate control measures is among the best known. Since no completely effective vaccine is yet available, the measures that have been shown to be most effective in controlling TB have been the early detection and curing of cases. However, the lack of resources to cover the costs of these treatments, and the fact that therapy is very prolonged (which leads to a high rate of treatment default), is causing these simple measures to fail in many of the poorer parts of the world. These challenges, and the bleak situation of the disease in many parts of the world, suggest that the eradication of TB will remain an elusive goal for many decades to come.</td>
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As is well known, TB is a transmissible, preventable, and curable disease. Consequently, the cost-benefit ratios of adequate control measures are among the best known. It has been calculated that if good disease control measures (treatment and cure) were implemented, the cost per year of life saved would be less than US$10. This has led the World Bank to regard adequate antituberculous treatment—involving short duration or course, with the inclusion of rifampicin—as the most cost-effective public health intervention today, offering performance only comparable to vaccination against measles or oral hydration formulations. The problem is that while these interventions are clearly profitable, they are associated with a cost that many poor countries with high TB rates cannot afford; in fact the amount dedicated to TB management alone would exceed their total budget for all health problems. These considerations should encourage wealthier countries to dedicate more resources towards helping less fortunate nations, with the dual aims of controlling TB in these regions and ensuring that the massive migratory movements from such economically depressed areas to industrialised countries do not constitute a step backward in the struggle against the epidemic. TB not only fuels poverty but is also fuelled by poverty. Consequently, failure to control TB in the community is of great importance, not only in terms
of the resulting high morbidity and mortality, but also in terms of the economic repercussions of the increase in the number of ill patients, owing to the failure to control the infection.

The best way to eradicate an infectious disease is to use an effective vaccine against it. Since it is well known that the only available TB vaccine has failed to yield the desired efficacy—offering no epidemiological impact on the course of the disease—it is necessary to correctly implement those intervention strategies that have demonstrated efficacy in controlling the disease. However, while these measures, which are based on the early detection and healing of TB cases, are able to adequately reduce the rates of this endemic disease, the reduction is slow (10-14% annually in the best of cases) and can easily be affected. This aspect and the current bleak world situation of the disease suggest that the eradication of TB will remain an elusive goal for many decades to come. Perhaps only a truly effective vaccine will be able to change this disheartening prognosis. TB will only enter the “elimination phase” when the annual rate of new ill patients (incidence for all forms of TB) becomes less than 1 per 100,000 inhabitants. Styblo defined the “elimination threshold” as fewer than 20 cases per 100,000, and the “advanced elimination phase” as fewer than 10 cases per 100,000. Theoretically, “elimination” will be accepted when the rate of smear-positive cases drops to less than 1 per million inhabitants, or when the prevalence of infected individuals in the general population drops to less than 1%. In relation to these estimated figures, which will take 40 to 50 years to reach in countries that have been most effective in their struggle against TB, the term “low-incidence countries” is accepted in reference to nations where the annual rate of affected patients is less than 10 per 100,000 inhabitants. This status has already been achieved by some developed countries, but remains well out of reach in the great majority of regions.

The need to supervise therapy in order to achieve treatment success

Section summary
Despite the great expectations raised by the introduction of chemotherapy and its disease-curing capacity, it soon became apparent that treatment with effective drugs is not synonymous with treatment success in the case of TB, since therapy must be continued for many months, even after symptoms disappear. For this reason, starting in 1958, it became clear that the administration of treatment must
be supervised (i.e., directly observed) in order to be effective. This requires special organisation of the treatment services—a very difficult goal to achieve, particularly in the poorest countries. As a result, in most countries, especially those with fewer economic resources, the impact of chemotherapy on TB control proved limited or even negligible for more than three decades. Thus, although individual successes were recorded, an analysis of the cure rates globally showed the figure to be no better than 50% in most cases.

Despite the great expectations raised by the introduction of chemotherapy with its disease-curing potential, it soon became apparent that treatment with effective drugs is not synonymous with treatment success in the case of TB, since therapy must be continued for many months, even well after symptoms have disappeared. This necessity was realised during the 1950s, and gave rise to two major challenges vital to the future of TB control: 1) the quest for new drugs capable of shortening treatment time; and 2) the development of strategies to ensure increased patient compliance with therapy.

The introduction of rifampicin and pyrazinamide in antituberculous treatment shortened the duration of therapy to the 6 to 8 months recommended at present. Unfortunately, there have been no other significant advances in TB treatment for more than 25 years—a situation that reflects the fact that the disease fundamentally affects the less favoured sectors of the population, which have fewer economic and political influences. It is universally accepted that if a 2- to 3-week treatment regimen were available, it would be possible to heal almost all patients, and the impact on TB control in the community would indeed be substantial.

Since the mid-1950s, the possible options for ensuring successful completion of a TB treatment scheme have been evaluated, especially since compliance is a crucial factor for TB control at both the individual and collective level. Initially, it was proposed to admit TB patients to specific sanatoriums for prolonged stays. However, the studies made by Madràs in the late 1950s showed treatment efficacy to be similar between interned patients and individuals who received TB treatment on a supervised ambulatory basis. In 1958, Wallace Fox, supervisor and promoter of other studies, drew attention to the need for direct observation or supervision of the administration of medication in order to ensure efficacy—although this measure requires special organisation of treatment services, particularly in the rural setting. At that time, it was becoming apparent that the basis for TB control in most of the world would be what is now called the Directly Observed Therapy, Short Course (DOTS) strategy.
Over the following decades, it became clear that it was very difficult to organise such services in countries with low-income levels and poor health care infrastructures. In most countries, especially those with fewer economic resources, the impact of chemotherapy on TB control proved to be limited or even negligible for more than three decades. Although individual treatment successes were recorded, an analysis of the cure rates globally showed the figure to be no better than 50% in most cases. A number of studies have demonstrated that while the large-scale administration of drugs to the tuberculous population (without control or individualised follow-up) increases cure rates and reduces mortality, the percentage of patients remaining smear positive for years in the community is greater than when no chemotherapeutic intervention is carried out (i.e., when no treatment is provided and TB is allowed to continue its natural course). So while the provision of self-administered treatments, without effective control, can afford individual benefits (i.e., cure and reduced mortality) from the community perspective, this practice is in effect worse than no treatment, since in the latter case affected patients die and can no longer spread the disease in the community. These same studies also showed that the best intervention at the individual and community level involved directly observed treatments, with individualised control of each case.

The DOTS strategy

Section summary
In the late 1970s, the International Union Against Tuberculosis and Lung Disease (IUATLD) considered it a priority to ensure the adequate establishment of National Tuberculosis Control Programmes (NTPs), particularly with the purpose of facilitating the treatment success of large numbers of cases even under the most adverse conditions. The IUATLD designed a practical intervention model that included collaboration between the clinical services and laboratories, the performance of smear microscopy on a routine basis, the development of an effective and practical information system, and multiple variables for administering treatment on a directly observed (supervised) basis. The model showed that it was possible to achieve high cure rates. These studies constituted the definitive stimulus for elaborating the currently acknowledged basic strategies for the control of TB in the community, which reflect the fundamentals of the DOTS strategy, designed in 1994 by the World Health Organization (WHO) with the purpose of implementing the strategy in the largest number of countries possible.
The DOTS strategy comprises five major variables: 1) political will; 2) the establishment of a minimum network of laboratories capable of performing smear microscopy; 3) administration of short course and directly observed (supervised) treatment in all patients; 4) the guarantee of drug and material reserves; and 5) elaboration and implementation of a registry and information system that allows periodic evaluation of the corresponding NTP.

In the late 1970s, the IUATLD considered it a priority to ensure the adequate implementation of NTPs, particularly with the purpose of facilitating the large-scale treatment success of cases even under the most adverse conditions. The IUATLD designed a practical intervention model that included collaboration between the clinical services and laboratories, the performance of smear microscopy on a routine basis, the development of an effective and practical information system, and multiple variables for administering treatment on a directly observed (supervised) basis. The model was tested in five countries (Tanzania, Malawi, Mozambique, Benin, and Nicaragua), with the support of international bodies and the dedication of much effort, and showed that it was possible to achieve high patient cure rates, even under the worst circumstances.

These studies constituted the definitive stimulus for elaborating the currently acknowledged basic strategies for the control of TB in the community. However, there have been two major limitations. First, the extent of the HIV epidemic throughout most of sub-Saharan Africa and the extreme poverty in these countries have led to such a tremendous increase in the number of TB cases that the success of these strategies is being compromised. Tragically, the number of new cases is increasing yearly in sub-Saharan Africa, even with good NTPs in operation—clear evidence that HIV infection and extreme poverty are exacerbating the problem of TB. Second, NTPs have not been as effectively established in other parts of the world, as a result of which global success has proved elusive and the TB endemic has remained out of control.

In view of the above, and considering the global epidemiological situation, the WHO in 1994 declared the disease a global health emergency and established the DOTS strategy as the basis for intervention in as many countries as possible. This strategy, based on the work of the IUATLD, comprises five major components (and not only directly observed treatment):

1. The political will of governments to solve the problem of TB in their respective countries. This is the first requirement, since without the necessary political commitment, the rest of efforts will be of little help. It is
very common to find countries where the authorities speak of their commitment to the struggle against TB, when in fact they fail even to secure the funds required for minimal maintenance of the personnel in charge of implementing management, or to guarantee the provision of drugs.

2. The establishment of a *minimum laboratory network* for performing smear microscopy. Smear microscopy is used to establish diagnosis among symptomatic cases. Culture and radiology cannot serve this function, although they may be useful in specific cases. Attempts must be made to ensure that the entire population has access to smear microscopy, with adequate quality control of the studies made. Until this is achieved, it is not advisable to introduce other diagnostic methods, such as culture, identification, or sensitivity testing. A laboratory network should ultimately be established in each country, structured in the following way: level 1 (capacity to perform smear microscopy); level 2 (smear microscopy, quality control for level 1 laboratories, culture and identification of *M. tuberculosis*); and level 3 (central level, with all the diagnostic capacities needed for the country in question). It is also advisable to have an extensive distribution of centres at the most peripheral level for the collection of samples.

3. The administration of *DOTS* to all smear-positive cases, at least during the intensive management phase (2 months). The importance of direct observation will be discussed further.

4. The establishment and maintenance of a system of *regular supply of the necessary drugs and materials* for functioning of the programme. This is one of the main inconveniences in the poorest countries. If this supply cannot be guaranteed, it is best to postpone initiation of the NTP until the required drugs can be obtained. The irregularity of medicine supplies is an important cause of NTP failure. In some cases, in order to begin operating such a programme, contributions may have to be sought to ensure the availability of the needed drugs. However, in subsequent years, the cost must be assumed by the governmental authorities of the country, since this is the only way to ensure that the programme will be sustainable.

5. The implementation of an adequate registry and information system to allow periodic evaluation of the NTP. This system will also serve as the basis for requesting material and drugs. It should include at least the following instruments:
   - An individualised case declaration form.
   - A report of the results of treatment (studying the patients in 3-month, 6-month, or annual cohorts).
– A General Case Registry.
– The following items, depending on the organisation and resources available in each country:
  • Microbiological study request form
  • Laboratory registry
  • Registry of symptomatic respiratory and suspected TB cases

In the past 5 years, major resources have been dedicated and significant efforts have been made to implement this strategy in as many countries as possible, with the aim (for the year 2000) of healing at least 85% of the cases diagnosed on the grounds of positive smear microscopy, and of detecting 70% of the existing smear-positive cases. Although it is now clear that these objectives have not been reached, it is also true that there has been considerable progress in the last 3 to 4 years in the implementation of the DOTS strategy. Indeed, if things continue in the same direction, the currently bleak world situation may begin to improve in the coming years.

On the other hand, many developed countries have been experiencing a different situation. While TB endemic rates in these parts of the world began to decrease more than a century ago as a result of improved socioeconomic conditions, in the past 40 to 50 years good NTPs have been instituted, leading to early detection and high cure rates. For this reason, the risk of infection in these countries has further declined throughout the last century, particularly with the introduction of chemotherapy. At present, these nations not only have low disease rates, but the remaining infected subjects tend to be adults or elderly individuals—thus paving the way for possible complete elimination of the disease within 30 to 40 years. Still, it should be emphasised that TB will not be completely eliminated in any country until it is brought under control globally.

Interventional strategies

<table>
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<th>Section summary</th>
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<td>At present, there are only three possible interventions for controlling TB in the community: 1) administering adequate chemotherapy to ill patients; 2) providing preventive treatment or chemoprophylaxis to infected individuals at high risk of developing the disease; and 3) large-scale vaccination of the population. Undoubtedly, the intervention strategy on which all efforts should focus is chemotherapy, with chemoprophylaxis ranking a distant second, followed by bacille Calmette-Guérin (BCG) vaccination. Reasons such as efficacy, action, rapidity, duration of the intervention effects, the capacity to eliminate the sources of infection, and the possible benefits for the community all justify these priorities.</td>
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There are presently only three possible interventions for controlling TB in the community: 1) administering adequate chemotherapy to ill patients; 2) providing preventive treatment or chemoprophylaxis to infected individuals at high risk of developing the disease; and 3) large-scale vaccination of the population. Each of these three possibilities have been, or will be, analysed in detail elsewhere in this Guide, according to their level of importance, with emphasis on the conditions affecting the significance of each possibility in TB control.

Undoubtedly, the intervention strategy that should earn most attention is chemotherapy, with chemoprophylaxis ranking a distant second, followed by BCG vaccination. Different reasons account for this order of priorities:

1. **Efficacy.** While the efficacy of an adequate treatment regimen approaches 100%, the best studies on chemoprophylaxis report rates of no more than 75% to 90% (in terms of capacity to prevent disease). In turn, and despite considerable controversy, recent meta-analyses indicate that the BCG vaccine affords a mean efficacy of only 50%.

2. **Action.** Chemotherapy exerts very rapid action, starting from the first drug dose, thus decreasing the risk of death and contagion in the community. Chemoprophylaxis also exerts rapid action, since from the first moment of administration it also reduces the risk of TB. In contrast, the BCG vaccine is very slow acting, and months or even years are needed to evaluate its effects.

3. **Duration.** Chemotherapy protects the cured patient for life (except in the infrequent cases of relapse or exogenous reinfection). The duration of protection afforded by chemoprophylaxis is not known. However, in immunocompetent individuals, chemoprophylaxis has been shown to protect for at least 20 years, although little is known about its protective effects in immunocompromised subjects. In contrast, the effect of BCG vaccination is both transient and inconstant.

4. **Infection sources.** Chemotherapy eliminates sources of infection (this being the best option), whereas chemoprophylaxis merely avoids such sources (only a small proportion of treated subjects go on to become sources of infection), and BCG vaccination has no effect on infection sources.

5. **Benefit for the community.** Chemotherapy benefits both patients (because they get cured and do not die) and the rest of the population (since the risk of contagion is eliminated). With chemoprophylaxis, only some infected subjects stand to possibly benefit (i.e., the few who could go on to develop the disease), and not the rest of the community (because only
patients with active disease transmit the bacillus). BCG vaccination only benefits those few cases who, at very young ages, may have become ill with serious forms of the disease.

**Basic measures for controlling tuberculosis**

**Section summary**

The first aim of all NTPs must be to maximise the cure rates among patients subjected to treatment—for which it is essential not only to use short-course treatment regimens (6-8 months), but also to ensure patient compliance. In countries with low- or middle-income levels, all patients should undergo directly observed treatments, the aim being to achieve cure rates of over 85% among smear-positive cases as well as a treatment default rate of less than 6%. In order to evaluate performance, systematic cohort studies must be conducted of the treatment outcomes.

A second aim is to maximise passive case detection, i.e., localisation of TB cases among the population that is using the health care system. In order to improve case detection and shorten the time to diagnosis, the education of physicians, health care professionals, patients, and public is important, particularly to make them aware that any respiratory symptom (cough and expectoration for more than 2-3 weeks) can constitute a case requiring the evaluation of possible TB. It is also essential to facilitate access to health care.

Only when the process of ensuring effective detection and treatment of cases has been achieved can active detection of cases and infected individuals among high-risk groups in the population (i.e., sectors with disease rates of over 100 cases per 100,000 inhabitants) be undertaken. This action is most effective in individuals who have contact or live with TB cases and who are smear positive, as well as in HIV-infected subjects—these individuals should receive treatment (if they constitute disease cases) or chemoprophylaxis (if infected). Still, even adequate implementation of chemoprophylaxis has not been shown to have an epidemiological impact, since the action is carried out on a population that is not diseased. The epidemiological impact of BCG vaccination on the community has been demonstrated to be practically zero.

The implementation of other control measures is only warranted in the industrialised world, especially with regards to immigrants from highly endemic regions, the homeless, and subgroups in which the incidence of TB is high.

**Cure of cases**

The first aim of all NTPs must be to maximise the cure rates among patients undergoing treatment. This is the most effective means of disrupting the
chain of transmission of *M. tuberculosis*, since it eliminates the sources of infection in the community. In order to achieve this aim, two equally important points must be taken into account: 1) the selection of a good chemotherapeutic regimen (with preference going to short-course regimens lasting 6–8 months); and 2) strict confirmation that the patient takes the medicine correctly until the end of treatment. If the patient is correctly treated but follow-up is deficient (an operational problem), then antituberculous action in the community can fail and the personal efforts of many health care workers can be rendered ineffective.

TB is a disease that can be cured in practically all cases; however, it requires strict adherence to prolonged treatment lasting at least 6 months. Incorrect treatment, or treatment lasting less than that required, will give rise to treatment failure and relapses—this in turn ensures the persistence of sources of contagion in the community and the risk of transmission of drug-resistant bacilli. It has been estimated that approximately half of all patients do not use the prescribed medication correctly, with the rates of poor compliance ranging from 20% to 80%, depending on the country, socioeconomic and cultural levels, and many other factors. This is why for the past 40 years, direct supervision of antituberculous treatment has been emphasised as the only sure way to ensure cure. In countries with low- or middle-income levels, all patients should receive treatment with direct supervision by health care personnel. It is debated whether these same measures should also apply to all TB patients in developed countries. In any case, directly supervised treatment means that the patient is directly seen to take the medication, or that trained personnel administer the dose.

The implementation of directly observed treatment constitutes one of the major challenges of NTPs, since it requires considerable organisational effort on the part of the health authorities. However, although it may be easy to recommend such measures, implementation can be an entirely different matter. Nevertheless, the example set by countries as poor as Tanzania, Peru, and Nicaragua, with their many limitations, shows that such measures can be implemented even in the worst settings.

Although the maximisation of cure rates must be the central concern of NTPs, evaluation of the work done in this area is equally important. Cohort studies are therefore needed (covering periods of 3, 6, or 12 months) of the results of treatment of patients diagnosed with TB. In this way, all patients enrolled in the programme must enter or leave the programme based on the following definitions:

1. *Cured.* This is a patient for whom there is evidence that treatment was correctly completed, and the microbiological results were negative at the
time of treatment suspension. In developed countries, the acceptance of negative microbiological results should comprise at least a negative culture at the end of the fourth month of treatment and a smear-negative study after 6 months. In the case of longer treatment regimens, the requirement would be a negative culture 2 months before suspending therapy and a negative smear study at the end of suspension of therapy. In poorer countries, evaluation would be restricted to smear microscopy, although at the same treatment time points as described above. As has been commented in Chapter 7, follow-up of treatment should not be done radiologically.

2. Completed treatment. This is a patient for whom there is evidence that treatment was correctly completed, although no negative microbiological results were obtained at the time of treatment suspension. This definition mainly comprises patients who either do not expectorate or from whom samples cannot be obtained (as in the case of extrapulmonary TB).

3. Treatment defaulted. This is represented by a disease case in which directly observed treatment at the periodic controls indicates that the patient had abandoned or interrupted the antituberculous regimen for more than 1 month.

4. Transferred out. This is represented by a patient included in the cohort of a given centre and who in the course of treatment changes place of residency and from that point onwards is followed up at another centre. If possible, the final patient outcome at the destination centre (when known) should be recorded.

5. Died. This is a patient diagnosed with TB who dies in the course of antituberculous treatment. When possible, include either of the following classifications in this category: death due to TB or death due to other causes.

6. Failure. This is a patient who is known to have correctly adhered to treatment but who presents with positive cultures at the end of the fourth month of therapy (fifth month in the 8-month treatment scheme). In poor countries, a positive smear would suffice as treatment failure. All cases of treatment failure must be confirmed by culture.

In developed countries, the aim is to achieve cure rates of over 90% in all patients, with a treatment default rate of less than 6%. It is necessary to carefully analyse the cohort, since the desired cure rate may not be reached for many reasons, such as high mortality (common in countries with a high prevalence of HIV infection) and high transfer rates. However, in poorer countries, the goals are more modest, and a positive outcome will be consid-
ered with a cure rate of over 85% in relation to patients with smear-positive results (the only subjects to be evaluated in the cohort), with a default rate of less than 6%.

In countries with low- or middle-income levels, the follow-up of TB cases until cure is achieved should not be a specific responsibility of specialist physicians, whose main role instead should be the management of cases with diagnostic problems (cases with suspected TB but serial smear-negative results) and treatment difficulties (e.g., adverse effects, re-treatments). Action should be limited to following the treatment guidelines recommended by the NTP and referring cases to the programme, where treatment supervision will be ensured and cohort studies will be performed based on the outcomes of treated patients. Since the great majority of TB cases diagnosed by specialist physicians are ultimately referred to the peripheral levels of health care, it is essential for the specialist physician to ensure that the transferred patient reaches his or her destination centre. This way, these specialists more selectively influence the interruption or default of treatment and transfer rates.

However, this important aspect analysed in the section above is different in developed countries, which tend to have few patients but many specialist physicians, who may then be involved in patient follow-up and performing cohort studies.

**Passive case detection**

The second objective—which should not be a priority concern until cure rates of over 85% are achieved with treatment default rates of under 6%—is represented by maximisation of passive case detection, i.e., localisation of TB cases among the population consulting the health care system. An extremely important consideration here is the definition of suspected tuberculous disease and TB case (these being the minimum criteria for initiating a study).

Since pulmonary TB is the most frequent presentation of the disease and the truly infectious form, most efforts should focus on identifying those individuals suspected of having this form of TB. TB should be suspected in any person presenting with cough and/or expectoration for more than 2 to 3 weeks (or other clinical manifestations suggestive of TB). These subjects are referred to as symptomatic respiratory patients, and require a chest radiograph and sputum sampling for smear microscopy and culture. In the poorest countries, serial smear microscopy studies will suffice as a diagnostic tool to identify TB disease. Despite the fact that a patient with such respiratory manifestations is unlikely to actually have TB (1-6%, depending on the pre-
valence of TB disease in the community), the resources and organisation of the NTP must focus only on case detection among symptomatic cases by smear microscopy.

In order to evaluate this important aspect relating to TB control, all elements of the NTP registration and information system must be strictly followed. Analysis of the population over the ages of 10 to 15 years, the number of symptomatic respiratory patients detected, the number of diagnostic sputum smear microscopies performed, the number of smear-positive cases, and the total number of cases entering the NTP will all provide very useful information for the evaluation of case detection status. In developing countries, where standardised information systems are often not used, it is essential to encourage case reporting by the diagnosing physicians, with the use of active epidemiological surveillance systems. One recommendation involves the control of microbiologically confirmed results (smear microscopy, culture), the control of hospital discharges with the diagnosis of TB, and the crossing of TB and HIV registries. Pharmacy registries relating to antituberculous drug use (particularly rifampicin) and death registries can also be useful.

Another important aspect in the detection of cases is the possibility of calculating the diagnostic delay, which should be based on three parameters: 1) total delay, comprising the period between the onset of symptoms and the start of treatment; 2) delay partly attributable to the patient, which will depend on the cultural level, the accessibility of health care, and the TB information available in the community (comprising the period from symptom onset to patient consultation of the health care service; and 3) delay attributable only to the health care system or NTP, comprising the period from patient consultation to the start of treatment. This diagnostic delay, which is little studied in most NTPs, is extremely important, since it represents the time during which the patient continues to infect the community.

In order to improve case detection and shorten the time to diagnosis, a critical consideration is the knowledge and education of the physicians, health care professionals, patients, and public. In particular, they should be made aware that any respiratory symptom (cough and expectoration for more than 2-3 weeks) can constitute a case requiring the evaluation of possible TB. It is also essential to facilitate access to health care for all patients with such symptoms.

If the NTP functions properly, the specialist physician in countries with low- or middle-income levels will play a limited role in case detection, since he or she will only see those cases presenting with smear-negative results