Multidrug-resistant Tuberculosis

Edited by

Ivan Bastian

Mycobacterium Reference Laboratory,
Institute of Medical & Veterinary Science,
Adelaide, Australia

and

Françoise Portaels

Mycobacteriology Unit,
Institute of Tropical Medicine,
Antwerp, Belgium

KLUWER ACADEMIC PUBLISHERS
DORDRECHT / BOSTON / LONDON

Chapter 1

Introduction

Multidrug-resistant tuberculosis: past, present and future

Ivan Bastian and Françoise Portaels
Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium

The Lord hath created medicines out of the earth and he that is wise will not abhor them. Ecclesiasticus 38:4, quoted by Selman Waksman when accepting the 1952 Nobel Prize for Medicine that was awarded for the discovery of the first effective antituberculosis drug, streptomycin, which was derived from the soil bacterium, *Streptomyces griseus*.

1. HISTORICAL PERSPECTIVE

This book has been published at the close of the twentieth century when the medical profession and the general community are increasingly concerned about the threat of multidrug-resistant tuberculosis (MDRTB)[1,2]. However, at this epoch, it is enlightening to move back from our immediate concerns about MDRTB 'hot spots' in Asia, South America, and the former Soviet Union [3], and to place our current predicament in an historical context. If the results of the global survey of antituberculosis drug resistance conducted by the World Health Organisation (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) can be extrapolated, only 2.2% of TB cases worldwide are due to multidrug-resistant strains [3]. At the beginning of the 20th century, all TB cases were refractory to all available therapies.

Great advances had been made during the 19th century in the understanding of the epidemiology and pathogenesis of TB, and in the diagnosis of the disease (reviewed in references 4-7). Laënnec and the other unitarians, who believed that the numerous clinical manifestations of TB represented a single disease entity, had prevailed over those who considered scrofula, tubercles, and phthisis as separate diseases. Villemin had

I. Bastian and F. Portaels (eds.), Multidrug-Resistant Tuberculosis, 1-15.

demonstrated in 1865 that TB was caused by a transmissible agent. Koch's famous studies, which were reported in 1882, had found the agent, *Mycobacterium tuberculosis*, and his 'tuberculin' extract, though a therapeutic failure, proved useful for detecting infected individuals. Ehrlich, Neelsen, Rindfleisch, and Ziehl had improved Koch's original staining method into a practical diagnostic test [8], while Röntgen's discovery of X-rays in 1895 completed our diagnostic armamentarium for TB.

Unfortunately, the 19th century did not see similar advances in TB treatment. Patients in the early 1800s received antiphlogistic and counterirritant therapies such as emetics, cathartics, bleeding, and dietary manipulation. More supportive treatments became fashionable in the 1850s when Brehmer founded the first sanatorium in Göbersdorf, Germany. Similar institutions were established across Europe and in the United States (US) over the following decades. The sanatoria relied on strict regimens of enforced rest, fresh air, and good diet to increase the likelihood of self-healing. Though many in-patients responded to such treatment, the long-term results remained depressing. Over 60% of discharged patients died of TB within six years (ie. 17% of the "cured", 51% of the "arrested", and 72% of the "improved")[5]. The results were improved somewhat in the early 20th century when sanatorium treatment was supplemented by various surgical procedures (eg. pneumothorax, thoracoplasty) that aimed to collapse diseased and/or bleeding lung segments.

The chemotherapeutic breakthrough finally came in the 1940s with the discovery of streptomycin (S) by Waksman and Schatz and the production of para-aminosalicylic acid (PAS) by Lehmann and Rosdahl. However, the problem of acquired drug resistance was recognised even during the early treatment trials with these new drugs [6,9]. Drug-resistant organisms could be detected in 90% of patients after four months of monotherapy, and the 5-year survival after streptomycin monotherapy was no better than that obtained by sanatorium treatment [6]. Combined S-PAS trials were then performed in Britain and the US that proved that multidrug chemotherapy prevented the development of drug resistance and effectively treated TB.

The 1950s and 1960s saw the development of numerous antituberculosis drugs: isoniazid (H), the aminoglycosides, viomycin, capreomycin, pyrazinamide (Z), ethionamide, cycloserine, and ethambutol (E). The last major advance was the discovery of rifampicin (R), which was derived from another soil micro-organism, *Nocardia mediterranea*, and was first used in clinical trials in 1967 [10]. Regimens containing various selections of these antituberculosis drugs were evaluated and optimised in a succession of clinical trials conducted by the British Medical Research Council (BMRC) and others [11,12]. For patients with pulmonary disease caused by fully-susceptible organisms, a combination of H, R, and Z for two months

followed by H and R for four months (ie. 2 HRZ/4 HR) proved the shortest, best tolerated, and most effective regimen (producing cure rates >97%)[13,14]. WHO, the American Thoracic Society (ATS), and the Centres for Disease Control (CDC) all recommend this short-course chemotherapy (SCC) but with the addition of E or S in the initial phase pending the results of susceptibility tests [15-17]. Finally, operational studies performed by Karel Styblo and the IUATLD demonstrated that SCC given under direct observation could succeed 'in the field'.

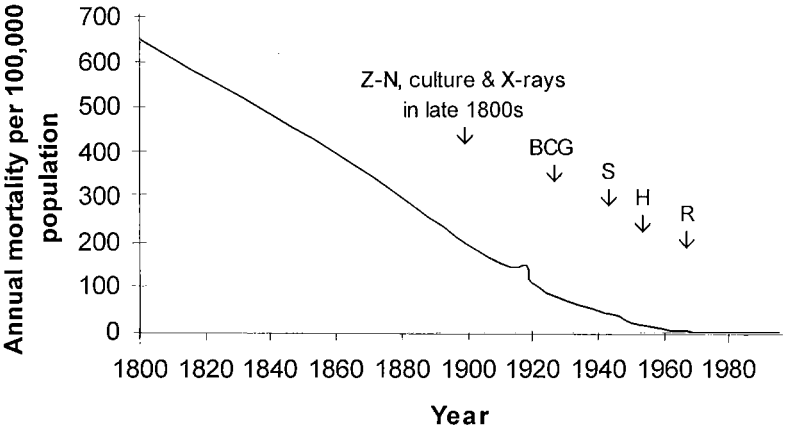

Figure 1. Mortality from tuberculosis (TB) in the United States, 1800-1995. Data were derived from the World Health Statistics Annual and from [18]. The significant decline in TB mortality prior to the introduction of various technologies is shown. Z-N, Ziehl-Neelsen stain; S, streptomycin; H, isoniazid; R, rifampicin.

Hence, in the final decade of the 20th century, WHO was able to recommend a package of diagnostic, therapeutic, and operational interventions, which was 'brand-named' "directly observed treatment, short-course" (DOTS), that could effectively control TB [19,20]. Interestingly, the burden of tuberculosis was declining sharply in industrialised countries well before the advent of these effective regimens and programs (Figure 1). This decrease has been attributed to gradual selection for innate immunity (ie. 'herd resistance') in exposed populations [21], improved nutrition and living conditions, and reduced disease transmission through the segregation of consumptives in infirmaries and sanatoria [22].

This brief historical overview has been given to highlight three points. Firstly, numerous epidemiological factors influence the progress of a TB epidemic. For example, in addition to showing the decline in TB mortality in the US during this century, figure 1 also shows the effect of the crowding and deprivations caused particularly by World War I. The incidence of TB

cases is predicted to increase from 7.5 million cases in 1990 to 11.9 million in 2005-an increase of 58% [23,24]. Of this predicted increase in TB incidence, 77% has been attributed to demographic factors (eg. population growth and changing age structures within populations) while epidemiological factors, such as MDRTB, human immunodeficiency virus (HIV) infection, and poverty, will account for only 23%. Hence, while emphasising that drug resistance presents a significant risk to the success of TB control efforts, a book on MDRTB must recognise that drug resistance is only one (minor) factor contributing to the current global TB problem.

The second point to highlight from this historical overview is that numerous disciplines have contributed to TB control (Figure 1). Microbiologists and radiologists had provided accurate means of TB diagnosis by the end of the 19th century; immunologists had provided the (imperfect) vaccine, BCG, by 1928; scientists and clinicians had produced and evaluated various antituberculosis treatments during the 1940s-1980s; and the greatest contribution to TB control may actually have come from the on-going efforts of public health specialists throughout the 19th and 20th centuries. In short, TB control has been a multi-disciplinary undertaking and must continue to be so. This book has therefore gathered contributions from authorities in multiple disciplines (eg. epidemiologists, clinicians, pharmacologists, molecular biologists, and public health specialists) to address all aspects of MDRTB.

Thirdly, this historical overview demonstrates that mankind has had at least two opportunities to effectively control TB but has failed on both occasions. The availability of various chemotherapeutics in the 1940s-1960s suggested that the battle against TB was won [4]. Strains of *M. tuberculosis* resistant to H, S and/or PAS soon appeared representing the failure of our first attempt. The discovery of R, and the success of SCC in treating strains with H and/or S resistance [25], allowed the medical community to continue to ignore the underlying factors that promote drug resistance (eg. limited healthcare funding, ineffective TB control programs, physician mismanagement, and patient non-adherence). The MDRTB of the 1980s-1990s (which now represents *M. tuberculosis* strains resistant to at least H and R) embodies our second failure to address the underlying causes of drug resistance, which also happen to be the problems bedevilling TB control in general.

2. THE DEFINITION OF MDRTB

The historical overview also shows that MDRTB has meant different things to different people during this century. Until the late 1940s, all TB

was equivalent to MDRTB (ie. effectively untreatable). In the 1950s-1970s, MDRTB came to represent *M. tuberculosis* strains resistant to H, S and/or PAS. Then in the 1980s-1990s, after the introduction of R, MDRTB was strictly classified as *M. tuberculosis* strains resistant to at least H and R [26-29].

2.1 Rationale for the current definition of MDRTB

Isoniazid and R are the key drugs in SCC [15-17,28,29]. Isoniazid is potently bactericidal, inexpensive, orally active, and has few adverse reactions [16]. Isoniazid is therefore used for the duration of any treatment regimen unless contraindicated or resistance is documented. Rifampicin is also bactericidal, has excellent sterilising activity, prevents emergence of resistance to other drugs, is rapidly absorbed from the gastrointestinal tract, and is relatively non-toxic [16,30].

Drug-susceptible pulmonary TB can be effectively treated with just H and R for 9 months (ie. 9 HR) [16,31]. The addition of Z during the first two months shortens the duration of treatment to six months [32-34]. Short-course regimens containing four or five drugs (eg. 2 HRZE/ 4 HR) are still effective in the presence of H (and/or S) resistance [16,25,35]. For example, a review of 12 BMRC trials found only four (2.6%) failures among 154 patients infected with strains resistant to H and/or S who were treated with regimens containing four or five drugs [25]. However, to avoid the few failures and relapses who may acquire additional R resistance, Z should be continued for the entire six months of a four-drug short-course regimen in patients who have confirmed H-resistance [16]. Alternatively, R and E can be given for 12 months [16]. In patients with R mono-resistant TB, nine months treatment with H, S, and Z will achieve sputum conversion in 95%-100% of patients and only 5%-6% will relapse after 30 months [36].

In sharp contrast to H- or R- mono-resistance that can be treated easily and effectively with first-line drugs, combined H and R resistance (ie. MDRTB) requires treatment with at least four agents, including a quinolone and an injectable agent (ie. an aminoglycoside or capreomycin)[27,37,38]. These regimens must last for 18-24 months, have multiple adverse effects, cost US$1,850-US$9,190 [39], and are less effective. For example, Goble et al reported that, despite prolonged intensive and optimal treatment, only 56% of 171 HIV-negative MDRTB patients were cured. These patients had chronic disease and were resistant to a median of six drugs [40]. Fortunately, later series of patients with less severe disease (eg. primary MDRTB, or acquired MDRTB with previous exposure to fewer antituberculosis drugs) have produced better results (eg. cure rates of 82.5%-96%)[41,42]. Nonetheless, combined resistance to H and R still has an enormous impact

on the duration, ease and cost of antituberculosis chemotherapy, thereby justifying the definition of MDRTB as resistance to at least these two drugs [26-29]. This strict definition will be used throughout this book.

2.2 Importance of a strict definition for MDRTB

A recent debate demonstrates the importance of accurately sub-classifying "drug-resistant" TB into MDRTB and other-drug-resistant tuberculosis (ODRTB, being mono- or poly-resistance not including both H and R) when reviewing treatment outcomes and programmatic interventions. Rifampicin-containing SCC regimens employed within effective DOTS programs have been shown to reduce the prevalence of "drug resistance" in several different countries [43-45]. However, Farmer and Kim have recently asserted that SCC/DOTS has reduced the prevalence of ODRTB but not MDRTB, and has done so only in settings without pre-existing high levels of MDRTB [46].

One example used in this debate is the Beijing Tuberculosis Programme, which introduced fully supervised chemotherapy in 1978 and which has used R extensively since 1988 [43]. Random surveys were conducted biannually between 1978-79 and 1991-92. During this time period, the number (and rate) of cases with initial resistance to H, S, and PAS declined, but R resistance had become established at a rate of 1.7%. Similar experience has been reported from Korea and Algeria [44,45]. Hence, in settings where MDRTB is not established, SCC/DOTS appears to lower the prevalence of "drug resistance" by reducing the absolute number and prevalence of patients with ODRTB but may leave a small (perhaps increasing) 'residue' of MDRTB cases.

There is little experience with using SCC/DOTS in areas with established high levels of MDRTB, when MDRTB is strictly defined as resistance to at least H and R. Establishment of a DOTS program in New York City was associated with a reduction in MDRTB levels [47,48]. However, numerous other interventions were also instituted: expedited laboratory diagnoses, individualised treatment of prevalent MDRTB cases, extensive use of chemoprophylaxis, and optimised infection control procedures [47]. Hence, the individual contribution of the DOTS program to the reduction of MDRTB levels cannot be determined.

DOTS programs have also been trialed in two MDRTB-endemic prison populations in the former Soviet Union and have produced dismal results [49-51]. The rates of MDRTB among prisoners commencing treatment in Baku, Azerbaijan, and Mariinsk, Siberia, were 23% and 22.6%, respectively. After fully-supervised treatment with WHO-recommended regimens, the respective cure rates were only 54% and 46%; the mortality rates were 11%

and 4%, but the default rates were also high (ie. 13% and 6%, respectively), mainly due to inter-prison transfers, release or re-trial. Nonetheless, the cure rate of Category I (ie. 2 HRZE/ 4 HR) and II (2 HRZES/1 HRZE/5 HRE) regimens in these MDRTB-endemic populations was well short of the 85% target set for TB control programs [17]. Treatment regimens may therefore need to be adjusted in populations where a significant proportion of the "drug-resistant" TB is MDRTB.

3. VIRULENCE AND OTHER QUESTIONS ABOUT MDRTB

The above section not only highlights the importance of accurately defining "drug-resistant" TB but also shows how little is known about MDRTB. There are many unanswered questions. What is the natural history of MDRTB? What is the true global extent of the MDRTB problem? Do TB control programs need to adjust their strategies and treatment regimens to address MDRTB? At what prevalence of MDRTB are these adjustments warranted? Can TB control programs in low- and middle-income countries treat MDRTB patients? If so, should these patients receive a standardised MDRTB treatment regimen or should their therapy be individually tailored? What laboratory facilities do MDRTB treatment programs require? What chemoprophylaxis should contacts of MDRTB patients receive? What is the place of BCG? What practical cost-effective measures can hospitals, laboratories and other institutions adopt to limit the transmission of MDRTB?

One other basic question about "drug-resistant" *M. tuberculosis* remains unresolved and the answer has implications for our response to MDRTB — are "drug-resistant" strains as virulent as drug-susceptible strains? This issue arose in the 1950s and 1960s when strains of *M. tuberculosis* resistant to H and/or S were reported to grow poorly *in vitro*, and to have attenuated infectivity and pathogenicity in animal models [52-54]. The subsequent evidence has been contradictory. Some recent cellular and molecular studies support the assertion that H-resistant strains are less virulent while others do not. KatG, the mycobacterial catalase-peroxidase protein that protects *M. tuberculosis* from intracellular killing by hydrogen peroxide (H_2O_2) and other reactive oxygen intermediates, also activates H [55,56]. The majority of H-resistant strains have point mutations in the gene encoding KatG and some of these mutants entirely lose KatG expression and catalase activity. Isoniazid-resistant strains with reduced/absent catalase and peroxidase activity have shown reduced *in vitro* resistance to H_2O_2 in liquid medium and human monocyte cultures [56]. Similarly, an H-resistant strain of *M.*

bovis lacking catalase activity was shown to be significantly less virulent in guinea pigs than the parent H-sensitive strain [57]. Introduction of a functional *katG* into the resistant avirulent strain restored virulence and H-susceptibility. Van Soolingen et al have also argued that H-resistant strains are less transmissible or virulent based on molecular epidemiological evidence. They have studied the 'molecular fingerprints' of 4,266 TB cases in the Netherlands between 1993-1997 (ie. 78% of culture-positive cases caused by *M. tuberculosis* in the study period)[58]. Isoniazid-resistant strains were less likely to be in a cluster than H-sensitive strains (OR, 0.7; 95% CI, 0.5-0.9), suggesting that some H-resistant strains are less likely to produce secondary cases.

Unpublished experience from our own laboratory has shown that some MDRTB strains are extremely difficult to cultivate *in vitro* (eg. 10^6 bacilli from fresh sub-cultures are required to obtain one colony forming unit on Löwenstein-Jensen media). Similarly, detection of R-resistant *M. tuberculosis* in 139 sputa from Bangladesh using a molecular method [59] has demonstrated that sputa containing R-resistant strains were less-frequently positive in primary cultures than sputa containing R-susceptible strains (χ^2=5.89; p=0.015).

Nonetheless, ample evidence has accumulated over the last 10-15 years that MDRTB is transmitted and is pathogenic in immunocompetent, as well as immunocompromised, populations [49,50,60-63]. Epidemiological studies and animal models have found that drug-sensitive and drug-resistant strains of *M. tuberculosis* demonstrate a range of infectivity and pathogenicity [60,64]. In fact, the original studies of H-resistant strains in the 1950s reported the same findings. While Wolinsky et al found that H resistance correlated with loss of catalase activity and avirulence, they also found that four of 20 H-resistant strains were fully virulent or only "slightly attenuated" [52]. Furthermore, the molecular epidemiological studies of van Soolingen et al suggest that particular genotypes of H-resistant *M. tuberculosis* are transmissible and virulent. Finally, Billington et al have recently found that some mutations in the *rpoB* gene that confer R resistance in *M. tuberculosis* occur at little physiological cost to the mycobacterium [65].

Much further work is required to clarify the inter-relationship of drug resistance, virulence, and the host immune response. In the meantime, while the evidence regarding the virulence of drug-resistant *M. tuberculosis* remains conflicting, there is no doubt that some MDR strains are definitely transmissible and fully virulent. Hence, until further data are available, our approach to the current MDRTB problem must assume the 'worst-case scenario' that MDRTB is as virulent as drug-susceptible *M. tuberculosis*.

4. MDRTB IN THE PRESENT AND FUTURE

The chapters in this book address many of the questions currently surrounding MDRTB. Chapters 2 and 3 describe the epidemiology of MDRTB in industrialised countries and in low- and middle-income countries. The true extent of MDRTB is better described now than even a few years ago [66], largely due to the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance [3]. However, Espinal highlights in chapter 3 that the prevalence of MDRTB remains undefined in much of Asia and many parts of Eastern Europe.

MDRTB has been linked with HIV infection in some outbreaks in high-income countries. The interaction of HIV and MDRTB is discussed by McCray and Onorato in chapter 4 and they conclude that nosocomial outbreaks largely account for the apparent association. However, they note the unusual incidence of rifampicin mono-resistance among HIV-positive patients and suggest that further research is required into the propensity of HIV/AIDS patients to develop drug-resistance. The practical factors contributing to the development of MDRTB are reviewed by Pablos-Méndez and Lessnau in chapter 5. These causes can be classified under two headings: clinical mismanagement (eg. delayed diagnosis, inadequate initial treatment, failure to recognise pre-existing drug resistance, addition of a single drug to a failing regimen, failure to promote adherence) and programmatic factors (eg. weak political commitment, irregular drug supplies).

Controversy has surrounded the appropriateness and effectiveness of the DOTS strategy in MDRTB-endemic areas [46,49,50]. In chapter 7, Raviglione presents evidence that DOTS prevents the emergence of drug resistance but concludes that standard SCC within a routine DOTS program may not adequately address high pre-existing levels of MDRTB. A later chapter by Dye and Williams on the mathematical modelling of MDRTB comes to a similar conclusion. Both chapters suggest that operational studies must determine the appropriate mix of additional interventions (eg. earlier detection of drug resistance, judicious use of second-line drugs) required in these MDRTB-endemic areas.

MDRTB can be effectively controlled in high-income countries, as shown by the experience in New York City [47,48]. The current methods for detecting and treating MDRTB are reviewed in chapters 8, 10, and 11, by experts from the National Jewish Medical and Research Centre in Denver, who have extensive experience in managing MDRTB patients. In a subsequent chapter, Telzak shows that with prompt institution of this specialised care, which is currently only possible in industrialised countries, the outcome of MDRTB patients can be greatly improved and their mortality significantly reduced, even if they are HIV-positive. The many difficulties

(and possibilities) of adapting such treatment programs for use in developing countries are discussed in chapter 12. However, there is also some cause for optimism because Crofton and Van Deun do present some encouraging results from an MDRTB treatment program in Bangladesh that used a standardised treatment regimen and achieved culture conversion rates of 96.1% after 3 months.

Chapters 14 and 15 discuss the appropriate management of health care workers and other individuals exposed to MDRTB patients. Both chapters weigh the advantages and disadvantages of BCG in these circumstances. They also highlight the difficulties of using an unproven prophylactic regimen (eg. Z and ofloxacin) that has appreciable adverse effects. In chapter 18, Richards and Jarvis present recommendations on the administrative measures, engineering controls, and personal respiratory protective devices required to control the spread of *M. tuberculosis*, including MDRTB. The authors also consider the costs of these measures and their applicability and adaptation for low-resource countries.

All of the above chapters show that the diagnostics and therapeutics for managing MDRTB are currently available in high-income countries (but not elsewhere). Three other chapters by Takiff, Palomino, and Barry demonstrate that the research technology also exists to develop cheap rapid simple diagnostics and new effective chemotherapeutics that may be used in low- and middle-income countries.

The final chapter by Farmer and other proponents of "DOTS-Plus for MDRTB" provides a framework for delivering these resources and expertise to MDRTB-endemic populations in low- and middle-income countries. However, their chapter epitomises the current predicament of all MDRTB intervention programs. There is a lack of data on several aspects of MDRTB epidemiology and treatment. For example, Farmer et al discuss the risk of producing resistance to additional drugs if patients with ODRTB or MDRTB receive standard SCC (ie. the 'amplifier effect'). They present evidence that confirms the existence of the 'amplifier effect' but the frequency of this phenomenon remains largely undefined. Extensive studies are in progress comparing pre- and post-treatment isolates from patients with initial drug resistance who fail standard SCC. These studies are employing DNA 'finger-print' analyses to differentiate the 'amplifier effect' from other explanations for finding post-treatment isolates with resistance to additional agents (ie. super-infection, mixed infection, and mis-labelling of specimens).

There are also other unanswered questions about MDRTB. What prevalence of MDRTB justifies institution of a "DOTS-Plus" program? What format should "DOTS-Plus" programs take? Nonetheless, MDRTB cannot be ignored in the 'hot spots' until we know all of the answers. Farmer et al correctly emphasise that on-going operational studies are required to

answer these questions. In fact, WHO has established a Working Group to co-ordinate pilot "DOTS-Plus" projects that will answer some of the above questions while assessing the feasibility of MDRTB management within TB control programs [67].

5. "THE PERFECT EXPRESSION OF OUR IMPERFECT CIVILIZATION"

In conclusion, this book affirms that the tools for controlling TB (and MDRTB) are available in industrialised countries but health care professionals, national governments, and international organisations lack the will to make these facilities available in low- and middle-income countries. The current situation regarding TB and MDRTB is encapsulated in a quotation from a book on the history of TB, "Tuberculosis has been called the perfect expression of our imperfect civilization" [68].

The excessive disease burden affecting the world's poor is now worrying economists as well as health professionals [69,70]. Only 18% of the world's population live in high-income countries but they consume >60% of the global non-renewable resources (eg. oil)[69]. WHO estimates that US$56 billion is spent annually on health research but less than 10% of that sum is used to study diseases that afflict 90% of the world's population [71]. The industrialised countries must address this disparity for several reasons [69]:

1. international travel, trade and migration can easily spread emerging and re-emerging infectious diseases (including TB and MDRTB) to industrialised countries;
2. continued neglect of the health needs of the world's poor will result in social dislocation and unrest; and, most importantly,
3. there is a moral imperative to do so.

Novel approaches are urgently required to finance health programs in developing countries, to fund drug and vaccine development, and to recognise intellectual property rights while still providing the new drugs and vaccines where they are needed [70].

TB and MDRTB exemplify the disparity between rich and poor. TB was the seventh leading cause of death in 1990 [72]. This ranking will be unchanged in 2020. Eighty percent of all incident TB cases in 1997 occurred in just 22 low- and middle-income countries, with more than 50% occurring in 5 SE Asian countries [73]. As chapters 2 and 3 in this book show, the MDRTB 'hot spots' also cluster in low- and middle-income countries.

This publication is therefore presented not only as a textbook for health professionals and scientists interested in MDRTB, but also as a prod to governments and international organisations to support and fund an effective

response to the problem of drug-sensitive as well as "drug-resistant" TB. We have the technology. Let's 'just do it'!

REFERENCES

1. Heymann SJ, Brewer TF, Wilson ME, Fineberg HV. The need for global action against multidrug-resistant tuberculosis. JAMA 1999; 281: 2138-40.
2. Garrett L. TB surge in former East Bloc. Newsday, March 25, 1998, pA 21.
3. Pablos-Méndez A, Raviglione MC, Laszlo A, et al. Global surveillance for antituberculosis drug resistance, 1994-1997. N Engl J Med 1998; 338: 1641-9.
4. Ryan F. The forgotten plague: how the battle against tuberculosis was won – and lost. Little, Brown & Co., Boston 1992.
5. Ayvazian LF. History of tuberculosis, Chapter 1, 1-20. In: Reichman LB, Hershfield ES (eds.), Tuberculosis: a comprehensive international approach. Marcel Dekker Inc., New York 1993.
6. Rossman MD, MacGregor RR. Introduction and brief history, xvii-xxiii. In: Rossman MD, MacGregor RR (eds.), Tuberculosis: clinical management and new challenges. McGraw Hill Inc., New York 1995.
7. Herzog H. History of tuberculosis. Respiration 1999; 65: 5-15.
8. Bishop PJ, Neumann G. The history of the Ziehl-Neelsen stain. Tubercle 1970; 51: 196-206.
9. McDermott W, Muschenheim C, Hadley SJ, et al. Streptomycin in the treatment of tuberculosis in humans. Ann Intern Med 1947; 27: 769-822.
10. Sensi P. History of development of rifampin. Rev Infect Dis 1983; 5(suppl 3): S402-6.
11. Schluger NW, Harkin TJ, Rom WN. Principles of therapy of tuberculosis in the modern era, Chapter 60. In: Rom WN, Garay SM (eds.), Tuberculosis. Little Brown & Company, Boston, 1996.
12. Iseman M, Sbarbaro JA. Short-course chemotherapy of tuberculosis. Hail Britannia (and friends). Am J Respir Dis 1991; 143: 697-698.
13. Hong Kong Chest Service/British Medical Research Council. Controlled trials of 2, 4, and 6 months of pyrazinamide in 6-month, three-times-weekly regimens for smear-positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampicin, and pyrazinamide, results at 30 months. Am Rev Respir Dis 1991; 143: 700-706.
14. Combs DL, O'Brien PJ, Geiter LJ. USPHS tuberculosis short-course chemotherapy trial 21: effectiveness, toxicity, and acceptability. The report of final results. Ann Intern Med 1990; 112: 397-406.
15. Centers for Disease Control. Initial therapy for tuberculosis in the era of multidrug resistance: recommendations of the Advisory Council for the Elimination of Tuberculosis. MMWR Morb Mortal Wkly Rep 1993 (RR-7): 1-8.
16. American Thoracic Society/Centers for Disease Control. Treatment of tuberculosis and tuberculosis infection in adults and children. Am J Respir Crit Care Med 1994; 149: 1359-1374.
17. Global Tuberculosis Programme. Treatment of tuberculosis: guidelines for national programmes. 2nd ed (WHO/TB/97.220). World Heath Organization, Geneva, 1997.
18. Grigg ERN. The arcana of tuberculosis: with a brief epidemiologic history of the disease in the U.S.A. Am Rev Tuberc Pulm Dis 1958; 78: 151-172.

19. WHO Tuberculosis Programme: framework for effective tuberculosis control (WHO/TB/94.179). World Health Organization, Geneva, 1994.
20. Kochi A. Tuberculosis control - is DOTS the health breakthrough of the 1990s? World Health Forum 1997; 18: 225-247.
21. Stead WW. The origin and erratic global spread of tuberculosis: how the past explains the present and is the key to the future. Clin Chest Med 1997; 18: 65-77.
22. Wilson LG. The historical decline of tuberculosis in Europe and America: its causes and significance. J Hist Med Allied Sci 1990; 45: 366-396.
23. Centers for Disease Control. Estimates of future global tuberculosis morbidity and mortality. MMWR Morb Mortal Wkly Rep 1993; 42: 961-964.
24. Dolin PJ, Raviglione MC, Kochi A. Global tuberculosis incidence and mortality during 1990-2000. Bull World Health Org 1994; 72: 213-220.
25. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. Am Rev Respir Dis 1986; 133: 423-430.
26. Kochi A, Vareldzis B, Styblo K. Multidrug-resistant tuberculosis and its control. Res Microbiol 1994; 144: 104-110.
27. Iseman MD. Treatment of multidrug-resistant tuberculosis. N Engl J Med 1993; 329: 784-791.
28. Pablos-Méndez A, Raviglione MC, Laszlo A, et al. Global surveillance for antituberculosis drug resistance, 1994-1997. N Engl J Med 1998; 338:1641-1649.
29. Antituberculosis Drug Resistance in the World: The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 1994-1997 (WHO/TB/97.229). World Health Organization, Geneva, 1997.
30. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. Tubercle 1985; 66: 219-225.
31. Dutt AK, Moers D, Stead WW. Short-course chemotherapy for tuberculosis with mainly twice-weekly isoniazid and rifampin. Am J Med 1984; 77: 233-242.
32. Hong Kong Chest Service/British Medical Research Council. Five-year follow-up of a controlled trial of five 6-month regimens of chemotherapy for pulmonary tuberculosis. Am Rev Respir Dis 1987; 136: 1339-1342.
33. Hong Kong Chest Service/British Medical Research Council. Controlled trial of 2, 4, and 6 months of pyrazinamide in 6-month, three-times-weekly regimens for smear-positive tuberculosis, including an assessment of a combination preparation of isoniazid.rifampin, and pyrazinamide: results at 30 months. Am Rev Respir Dis 1991; 143: 700-706.
34. Combs DL, O'Brien RJ, Geiter LJ. USPHS tuberculosis short-course chemotherapy trial 21: effectiveness, toxicity, and acceptability. The report of final results. Ann Intern Med 1990; 112: 397-406.
35. Singapore Tuberculosis Service/British Medical Research Council. Clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. Am Rev Respir Dis 1979; 119: 579-585.
36. Hong Kong Chest Service/British Medical Research Council. Controlled trial of 6-month and 9-month regimens of daily and intermittent streptomycin plus isoniazid plus pyrazinamide for pulmonary tuberculosis in Hong Kong. The results up to 30 months. Am Rev Respir Dis 1977; 115: 727–735.
37. Crofton J, Chaulet P, Maher D, et al. Guidelines for the management of drug resistant tuberculosis (WHO/TB/96.210). World Health Organization, Geneva, 1997.
38. Bastian I, Colebunders R. Treatment and prevention of multidrug-resistant tuberculosis. Drugs 1999; 58: 633-661.

39. Bastian I, Rigouts L, Van Deun A, Portaels F. Directly observed treatment, short-course strategy and multidrug-resistant tuberculosis: are any modifications required? Bull World Health Org (in press).
40. Goble M, Iseman MD, Madsen LA, et al. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. N Engl J Med 1993; 328: 527-532.
41. Telzak EE, Sepkowitz K, Alpert P, et al. Multidrug-resistant tuberculosis in patients without HIV infection. N Engl J Med 1995; 333: 907-911.
42. Park SK, Kim CT, Song SD. Outcome of chemotherapy in 107 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. Int J Tuberc Lung Dis 1998; 2: 877-884.
43. Zhang LX, Kan GQ, Tu DH, et al. Trend of initial drug resistance of tubercle bacilli isolated from new patients with pulmonary tuberculosis and its correlation with the tuberculosis programme in Beijing. Tuberc Lung Dis 1995; 76: 100-103.
44. Kim SJ, Bai GH, Hong YP. Drug-resistant tuberculosis in Korea, 1994. Int J Tuberc Lung Dis 1997; 1: 302-308.
45. Boulahbal F, Khaled S, Tazir M. The interest of follow-up of resistance of the tubercle bacillus in the evaluation of a programme. Bull Int Union Tuberc Lung Dis 1989; 64: 23-25.
46. Farmer P, Kim JY. Community based approaches to the control of multidrug resistant tuberculosis: introducing "DOTS-plus". BMJ 1998; 317: 671-674.
47. Frieden TR, Fujiwara PI, Washko RM, Hamburg MA. Tuberculosis in New York City – turning the tide. N Engl J Med 1995; 333: 229-233.
48. Moore M, Onorato IM, McCray E, Castro KG. Trends in drug-resistant tuberculosis in the United States, 1993-1996. JAMA 1997; 278: 833-837.
49. Coninx R, Mathieu C, Debacker M, et al. First-line tuberculosis therapy and drug-resistant *Mycobacterium tuberculosis* in prisons. Lancet 1999; 353: 969-973.
50. Kimerling ME, Kluge H, Vezhnina N, et al. Inadequacy of the current WHO re-treatment regimen in a central Siberian prison: treatment failure and MDR-TB. Int J Tuberc Lung Dis 1999; 3: 451-453.
51. Portaels F, Rigouts L, Bastian I. Addressing multidrug-resistant tuberculosis in penitentiary hospitals and in the general population of the former Soviet Union. Int J Tuberc Lung Dis 1999; 3: 582-588.
52. Wolinsky E, Smith MM, Steenken W. Isoniazid susceptibility, catalase activity, and guinea pig virulence of recently isolated cultures of tubercle bacilli. Am Rev Tuberc 1956; 73: 768-772.
53. Mitchison DA, Wallace JG, Bhatia AL, et al. A comparison of the virulence in guinea pigs of South India and British tubercle bacilli. Tubercle 1960; 41: 1-22.
54. Cohn ML, Davis CL. Infectivity and pathogenicity of drug-resistant strains of tubercle bacilli studied by aerogenic infection of guinea pigs. Am Rev Respir Dis 1970; 102: 97-100.
55. Zhang Y, Heym B, Allen B, et al. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature 1992; 358: 591-593.
56. Manca C, Paul S, Barry CE III, et al. *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. Infect Immun 1999; 67: 74-79.
57. Wilson TM, de Lisle GW, Collins DM. Effect of inhA and katG on isoniazid resistance and virulence of *Mycobacterium bovis*. Mol Microbiol 1995; 15: 1009-1015.
58. van Soolingen D, Borgdorff MW, de Haas PEW, et al. Molecular epidemiology of tuberculosis in the Netherlands: a nationwide study from 1993-1997. J Infect Dis 1999; 180: 726-736.

59. De Beenhouwer H, Lhiang Z, Jannes G, et al. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tuberc Lung Dis 1995; 76: 425-430.
60. Snider DE, Kelly GD, Cauthen GM, et al. Infection and disease among contacts of tuberculosis cases with drug-resistant and drug-susceptible cases. Am Rev Respir Dis 1985; 132: 125-132.
61. Kritski AL, Ozorio Marques MJ, Rabahi MF, et al. Transmission of tuberculosis to close contacts of patients with multidrug-resistant tuberculosis. Am J Respir Crit Care Med 1996; 153: 331-335.
62. Victor TC, Warren R, Beyers N, van Helden PD. Transmission of multidrug-resistant strains of *M. tuberculosis* in a high incidence community. Eur J Clin Microbiol Infect Dis 1997; 16: 548-549.
63. Bifani PJ, Plikaytis BB, Kapur V, et al. Origin and interstate spread of a New York City multidrug-resistant *M. tuberculosis* clone family. JAMA 1996; 275: 452-457.
64. Ordway DJ, Sonnenburg MG, Donahue SA, et al. Drug-resistant strains of *Mycobacterium tuberculosis* exhibit a range of virulence for mice. Infect Immun 1995; 63: 741-743.
65. Billington OJ, McHugh TD, Gillespie SH. Physiological cost of rifampin resistance induced *in vitro* in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1999; 43: 1866-1869.
66. Cohn DL, Bustreo F, Raviglione MC. Drug-resistant tuberculosis: review of the worldwide situation and the WHO/IUATLD Global Surveillance Project. Clin Infect Dis 1997; 24(Suppl 1): S121-S130.
67. Espinal MA, Dye C, Raviglione M, Kochi A. Rational 'DOTS Plus' for the control of MDR-TB. Int J Tuberc Lung Dis 1999; 3: 561-563.
68. Dormandy T. The White Death: a history of tuberculosis. Hambledon Press, London, 1998.
69. Guerrant RL, Blackwood BL. Threats to global health and survival: the growing crises of tropical infectious diseases—our "unfinished agenda". Clin Infect Dis 1999; 28: 966-986.
70. Sachs J. Helping the world's poorest. The Economist August 14, 1999: p. 17-20.
71. Balms for the poor. The Economist August 14, 1999: p. 63-65.
72. Murray CJL, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997; 349: 1498-1504.
73. Dye C, Scheele S, Dolin P, et al. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. JAMA 1999; 282: 677-686.