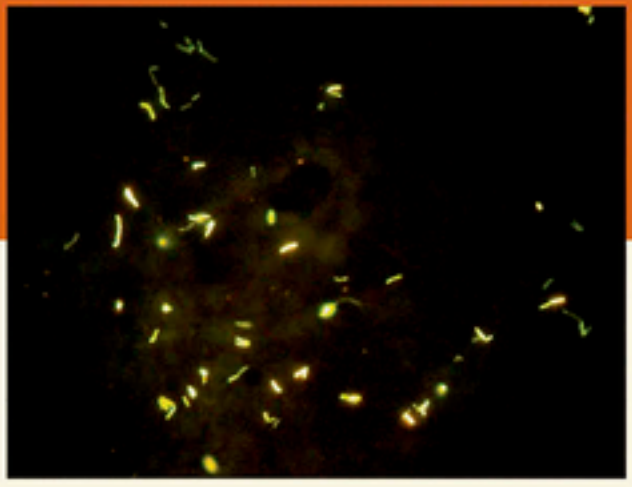
Handbook of Child & Adolescent Tuberculosis



edited by

JEFFREY R. STARKE

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OXFORD

HANDBOOK OF CHILD AND ADOLESCENT TUBERCULOSIS

There are many contributions which the pediatrician can make to the tuberculosis control program. First the negativism about the tuberculosis so prevalent in pediatrics must be overcome. . . . Wherever there are tuberculous adults, there are infected children. No one is immune.

—Edith M. Lincoln, "Eradication of Tuberculosis in Children," *Archives of Environmental Health*, 1961

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This book is dedicated to Katherine H.K. Hsu, M.D., Dr. Starke's mentor and one of the many unsung pioneers of the modern study of childhood tuberculosis who dedicated their careers to understanding, treating and preventing this disease.

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PREFACE

TUBERCULOSIS (TB) in childhood arises almost exclusively as result of infection from an adult suffering from pulmonary tuberculosis. Consequently, the occurrence of tuberculosis in a child reflects the presence of infectious cases of tuberculosis in adults within a community. Once a child is infected, the development of disease is to a large extent determined by the age of the child. During the first two to three years of life, disease will more frequently follow infection, and the consequences of infection are much more likely to be serious, in the form of tuberculous meningitis and disseminated tuberculosis and marked by a high morbidity and mortality. Between three years of age and puberty, children enjoy a period of relative protection from disease following infection. With puberty, the picture again changes, and a higher risk of disease is experienced, which is manifested by typical "adult" tuberculosis, characterized by involvement of the upper lobes of the lungs and cavitation that is likely to contribute to the further spread of tuberculosis.

It can thus be easily understood that in economically developed communities, with fewer children and fewer adults to infect children, the proportion of tuberculosis disease occurring in children will be relatively low, and it will tend to be less serious than in a developing community. It is, perhaps, a lack of appreciation of these facts, and the fact that children will usually not contribute to the spread of tuberculosis, that created the perception that tuberculosis in children was not a serious problem and that efforts towards the treatment and control of tuberculosis must be concentrated solely on sputum microscopy smear-positive adults. With luck, the problem of childhood tuberculosis would just go away in due course! The events of the last two decades and the spread of human immunodeficiency virus (HIV) infection have finally dispelled these misconceptions, and the burden that tuberculosis infection and disease among children imposes on a community, particularly a developing community and its health services, is becoming clearer. The unique features of childhood tuberculosis—that young children

can become seriously ill in a very short time, that childhood tuberculosis is often associated with malnutrition, that children absorb and metabolize many drugs differently from adults, that many cases can be prevented—require a different approach to case-finding and management than that used for adults. It also is apparent from recent epidemiological studies that childhood tuberculosis is far more common than recognized previously.

As a consequence of the above developments, there is now a greater interest in childhood tuberculosis than has existed since the days of the preventorium movement in the late nineteenth and early twentieth centuries. With many health service practitioners entering the field of tuberculosis for the first time, there is now, more than ever before, a great need for practical advice, guidance, and knowledge relating to childhood tuberculosis. It is our hope that this book, written by clinicians and researchers with great experience in childhood tuberculosis, will contribute to a greater understanding of the facts related to childhood tuberculosis and thus to the better management of the disease.

We hope that readers will also notice in this book the great spectrum of disease encompassed by childhood tuberculosis: from disseminated manifestations such as miliary tuberculosis and tuberculous meningitis, to pulmonary tuberculosis indistinguishable from that occurring in adults; it could be argued that the understanding of childhood tuberculosis contains many more scientific questions that are yet to be answered than does adult tuberculosis. These questions are of fundamental importance to our understanding of tuberculosis and its treatment, management, and prevention in both adults and children. The response of the body to tuberculosis undergoes continual change, if the changes in manifestations are any measure to go by, and these imply underlying changes in the immune system. In constructing new vaccines for tuberculosis, we are therefore aiming at a continually moving target, and the consequences of this continual change have yet to be fully understood.

An issue we have attempted to address in the book is the confusing language of tuberculosis. We have used the term "tuberculosis infection" rather than "latent tuberculosis infection (LTBI)" because the word "latent" is unnecessary and actually confusing when applied to child patients, many of whom were infected recently. As a result, we use the phrase "treatment of tuberculosis infection" rather than "preventive therapy" or "chemoprophylaxis" because the latter terms do not properly emphasize the importance of treating an established infection. We think this is important, both for accuracy and to better motivate physicians and families to make treatment of tuberculosis infection a priority. Equally confusing is the language associated with tuberculosis disease. We have not used the phrase "active tuberculosis" because the meaning of "active" is both unclear and redundant. As a result, we have simplified the language to "tuberculosis infection" and "tuberculosis disease."

Finally, we would like to thank the authors who generously gave of their time and experience to write the chapters in this book. We also want to thank the army of health care workers who care for the children afflicted with tuberculosis: who find them, get them through treatment, and support their many needs. Most importantly, we want to thank the children we have cared for and the families who have allowed us to learn from them so we know how to better prevent this disease and care for the children afflicted with it in the future.

—Jeffrey R. Starke and Peter R. Donald

ABOUT THE EDITORS

JEFFREY R. STARKE has been the director of the Children's Tuberculosis Clinic at Ben Taub General Hospital and Texas Children's Hospital for 30 years. He has published over 110 articles and 40 chapters on childhood tuberculosis. He has served on numerous advisory and guideline writing groups for the American Academy of Pediatrics, American Thoracic Society, Infectious Disease Society of America and the World Health Organization. He is a former chairman and current member of the U.S. Centers for Disease Control and Prevention (CDC) Advisory Council for the Elimination of Tuberculosis. His wife, Joan Shook, is also a pediatrician, and all three of their children are in various stages of medical training.

PETER R. DONALD is emeritus professor in the Department of Pediatrics and Child Health of the Faculty of Health Sciences, Stellenbosch University, South Africa. Professor Donald has served as a mentor to many of the authors in this book. He has been actively involved in the assessment of antituberculosis agents in children and adults for more than twenty years and was principal investigator in 12 studies of the early bactericidal activity (EBA) of antituberculosis agents that assisted in establishing the EBA technique as a reliable, objective manner of assessing an antituberculosis agent. He retired from a full-time university appointment in 2004, but remains active in various tuberculosis research activities focused on antituberculosis drug assessment in adults and children, and various aspects of the management and treatment of childhood tuberculosis. He was awarded the gold medal of the International Union Against Tuberculosis and Lung Disease for his contribution to child lung health, and obtained an A2 rating from the National Research Foundation of South Africa in 2008 that was renewed in 2013.

ABBREVIATIONS

ANTITUBERCULOSIS DRUGS

Amikacin AMK
Bedaquiline BDQ
Capreomycin CAP
Ciprofloxacin CIP
Clofazamine CFZ
Cycloserine CLS
Delamanid DLM
Ethambutol EMB
Ethionamide ETH
Isoniazid INH
Kanamycin KM
Levofloxacin LEV
Linezolid LNZ
Moxifloxacin MOX
Para-aminosalicylic acid PAS
Pretonamid PTO
Ofloxacin OFL
Pyrazinamide PZA
Rifabutin RIF
Rifampin [rifampicin] RMP
Rifapentine RPT
Streptomycin SM
Terizidone TZD
Thiacetazone THI

OTHER TERMS

Antiretroviral therapy ARV
Bacille Calmette Guerin BCG
Computerized tomography CT
Deoxyribonucleic acid DNA
Directly observed therapy short course DOT
Disseminated BCG dBCG
Drug susceptibility test[ing] DST
Extensively drug-resistant tuberculosis XDR
Human immunodeficiency virus HIV
Immune reconstitution inflammatory syndrome IRIS
Interferon-¥ release assay IGRA
International Union Against Tuberculosis and Lung Disease IUATLD

Magnetic resonance imaging MRI
Multidrug-resistant tuberculosis MDR-TB
Nontuberculous mycobacteria NTM
Polymerase chain reaction PCR
Pulmonary tuberculosis PTB
Recombinant BCG rBCG
Tuberculin skin test TST
Tuberculous meningitis TBM
World Health Organization WHO

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1

A BRIEF HISTORY OF CHILDHOOD TUBERCULOSIS

Peter R. Donald

TUBERCULOSIS IN childhood is an inevitable consequence of the presence of tuberculosis in any community, and archeological evidence of the occurrence of tuberculosis in children in early societies of Europe, Africa, and the Americas is provided by the skeletons of children that show signs of osteo-articular tuberculosis.[1] In some instances, molecular biology has detected the presence of specific DNA in these skeletal remains, confirming the role of *Mycobacterium tuberculosis*.[2,3] Despite many references in ancient texts to probable tuberculosis occurring in different populations throughout the world,[4] it was only relatively recently that tuberculosis in children was comprehensively studied and its clinical features and epidemiology shown to differ, in many respects, from findings in adults.

THE AGE OF POST-MORTEMS

From approximately the sixteenth century, an epidemic of tuberculosis engulfed Europe, then started to decline, as evidenced by data from European cities, from approximately the middle of the eighteenth century. Other industrializing cities in the Americas, Asia, and Australasia were similarly affected.[5] Under these conditions, primary infection nearly always occurred early in childhood, and mortality as a result of tuberculosis in the very young was higher than at any other period of life[6]; it was the conduct of post-mortem studies in these children dying of tuberculosis, coupled with the astute observations of clinicians, that first broadened our understanding of the pathogenesis of tuberculosis in children, but also in adults.

From 1800, in the aftermath of the French revolution, Paris emerged as a center of scientific medical research underpinned by regular post-mortems, allowing clinical findings and experience to be coupled to post-mortem findings. Foremost amongst the researchers regarding tuberculosis was René Laénnec (1781–1826), who is best known for his invention of the stethoscope, which assisted him in correlating clinical observations with post-mortem findings. He described his experience in a treatise "De L'Auscultation médiate" and stated that the basic lesion of tuberculosis was the tubercle, and that this was "the

true anatomical character of consumption."[7] He demonstrated that tuberculosis could take many forms, but that all these manifestations in different organs were essentially the same disease. He was also well aware of the features of tuberculosis in children and stated, "The tuberculous matter is more often found (in children) in the bronchial glands and sometimes when there are no tubercles in the lungs or other serious involvement of these organs. This is particularly so in scrofulous children." Thus he was aware of the main features of primary tuberculosis in children, but he incorrectly attributed disease in the lungs to spread of disease from the mediastinal nodes.

In 1868, Jean-Antoine Villemin (1827–1892) in a treatise, "Etudes sur la tuberculosis," demonstrated that, whatever its cause, tuberculosis was infectious, and he described the successful production of tuberculosis lesions in rabbits with material from tuberculosis patients and cattle, and was then able to transfer the disease from rabbits to rabbits.[8] A major turning point was reached in 1882, when Robert Koch astounded the world with the announcement that he had discovered the cause of tuberculosis—a specific micro-organism, *Mycobacterium tuberculosis*.[9]

Joseph Marie-Jules Parrot (1829–1883) (Figure 1.1) was the son of a physician and qualified in medicine in Paris in 1857. He made a point of relating clinical experience to pathology findings. After gaining experience at the Hospice des Enfants-Assistés, he concentrated on the diseases of children and became one of the pioneers of pediatrics.[10] A significant step forward in the understanding of the pathogenesis of childhood tuberculosis was made on October 28, 1876, when Parrot's findings, following a series of 145 post-mortems carried out on children aged one to seven years with tuberculosis, were presented at the Societé de Biologie in Paris. Claude Bernard presided over the meeting, and the official report of Parrot's findings appeared in *Comptes Rendus de la Societé de Biologie (Paris)*[10]:

> M. Parrot communicated the results of his researches into the relationship between the pulmonary lesions and those in the tracheobronchial glands . . . whenever a bronchial gland is the site of a tuberculous lesion there is an analogous lesion in the lung. . . . The pulmonary lesion may be very difficult to find and this is the reason why it's existence has been denied; there are cases in which it is no larger than a pin's head.[11]

Parrot was later appointed professor of child health, but he never published a formal scientific report of his experience. His name is perpetuated in Parrot's Law: "The nodes are the mirror of the lungs."

Parrot's work was continued by a pupil, Victor-Henri Hutinel (1849–1933), and it was one of Hutinel's pupils, George Küss (1867–1936) who in 1898 published an extensive monograph, "De L'Hérédité parasitaire del la tuberculose humaine."[12] Küss focussed on the question of whether tuberculosis was hereditary, or acquired following infection, but his findings left little doubt about the association between the tuberculosis focus in the lungs and the lymph nodes draining the relevant lung area. The relationship between the pulmonary focus, which he identified as being usually sub-pleural, and the tracheobronchial nodes demonstrated that tuberculosis probably resulted from an aerogenous infection by *M. tuberculosis* and was not the consequence of a congenital infection that had been lurking in the mediastinal lymph nodes.

In Vienna in the early twentieth century, as in most major European cities, tuberculosis was very common, and it is not surprising that Viennese researchers provided a more complete view of the pathology of childhood tuberculosis. Heinrich Albrecht (1866–1922) worked at St. Anne's Hospital in Vienna and published his findings following a series of post-mortems carried out on 3,213 children, of whom 1,060 (33%) had active tuberculosis.[13] On the basis of his findings, he agreed that tuberculosis infection was aerogenous and found a primary focus on post-mortem in nearly every case of childhood tuberculosis. Albrecht discussed his findings and their implications with his colleague Anton Ghon (1866–1936), who became, probably, the person best known in the English-speaking world regarding the pathogenesis of childhood tuberculosis. Ghon studied medicine at the University of Graz and in 1902 became Professor Extraordinarius in Pathology in Vienna. In 1903, he began the studies that have entrenched his name in the medical literature.[14] Between July 1907 and December 1909, he participated in 747 autopsies on children dying at St. Anne's Hospital, conducting 644 (86%) of these autopsies himself.

Among these children, 184 had tuberculosis, and Ghon divided the cases into two groups:

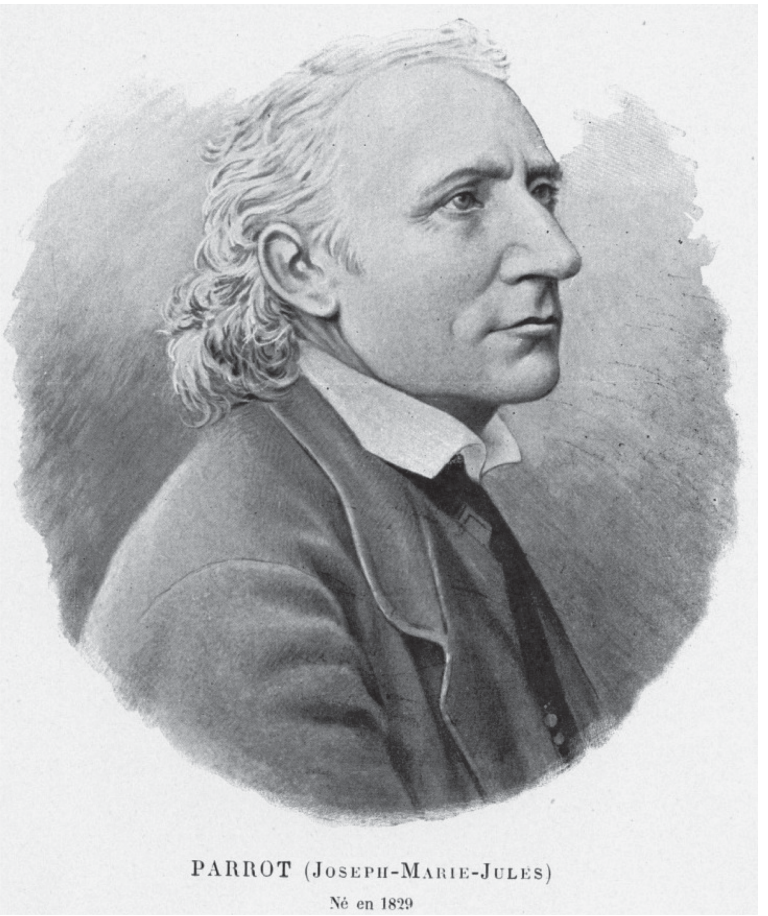

FIGURE 1.1 Joseph Marie-Jules Parrot (1829–1883). French pediatrician responsible for the first comprehensive description of the primary tuberculosis focus and complex in children.

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Group A consisted of 170 children in whom a respiratory primary focus was found, and Group B of 14 in whom no respiratory focus was evident. Three of these latter children had obvious tuberculosis of the tracheobronchial nodes but no respiratory focus; in four children, intestinal tuberculosis was found, but also involvement of the mediastinal nodes; in five children, a focus outside of the respiratory system was identified and two children were tuberculin-positive, but no focus was found. Ghon concluded: "I saw no case in which the changes [in the lungs] from a patho-anatomical point of view were in a later stage of development than those of the adjoining lymphatic glands . . . the lung focus cannot have originated in any retrograde sense from the lymphatic glands."[15]

In all of his writings, Ghon was at considerable pains to draw attention to the work of his predecessors and colleagues, and he made no claims that his findings were in any sense original. "The conception of the primary complex in tuberculosis originated with Ranke,[16] who designated by this term the 'definitely circumscribed' picture consisting of the primary focus of infection and the changes in the regional lymph nodes." Ghon added,

> The basis for the teaching of the primary tuberculous focus lies in the law of Parrot, according to which every lung infection in the virgin body of the child shows itself likewise in the regional lymph nodes. It was Küss, chiefly, who made a detailed study of the law of Parrot and established thereby the

relationship between the site of the primary lung focus and the lymph node change.[17]

Ghon's monograph was translated into English by Dr. David Barty-King and published in London in 1916. This brought Ghon's work to the attention of a large English-speaking audience and, ironically, despite Ghon's disclaimers, the primary tuberculous focus and the complex now carry Ghon's name. In 1910, Ghon became Professor of Anatomical Pathology at the German University of Prague. He retired in 1935 and died shortly afterwards, in 1936. Ghon's studies of the pathology of tuberculosis were continued in Buffalo, New York, by a pupil, Dr. K. Terplan, who published an extensive monograph, "Anatomical Studies on Human Tuberculosis," in the *American Review of Tuberculosis* in 1940.[18]

Despite the work of Parrot that culminated in the studies of Anton Ghon, there were influential researchers still of the opinion that the route of tuberculosis infection was gastro-intestinal.[19,20] Part of the confusion arose from the significant presence of *Mycobacterium bovis* in many European countries that was responsible for gastrointestinal infections in many cases, but that could also, in a minority of cases, cause pulmonary tuberculosis (PTB) and disseminated forms of tuberculosis. Thus Still (1899), working in London, recorded that in 20.5% of 259 post-mortems of children dying of tuberculosis, the portal of entry was gastrointestinal[21]; in Edinburgh, Shennan (1909) reported similar findings in 28.1% of 316 children dying of tuberculosis.[22] One of the last major post-mortem studies devoted exclusively to the pathology of childhood tuberculosis was undertaken by John Blacklock, and this assisted greatly in resolving the differing roles of *M. tuberculosis* and *M. bovis* in causing disease in children.[23]

Between 1924 and 1931, Blacklock carried out 1,800 consecutive post-mortems on children at the Royal Hospital for Sick Children, Glasgow, and tuberculosis was identified in 283 (16%) cases. One of Blacklock's main aims was to identify the causative mycobacterial strain. This had particular relevance in the west of Scotland, where bovine tuberculosis was a serious problem. Of the infecting organisms identified, 63% were *M. tuberculosis,* and 37% were *M. bovis.* In contrast to many other series, 101 of the post-mortems (36%) indicated that the primary focus was abdominal; 82% of these were associated with *M. bovis* infection. Conversely, only 3% of respiratory primary infections were due to *M. bovis,* and, although the lymph nodes were shown to be tuberculous, no primary focus could be demonstrated in the lungs. Of the respiratory infections due to *M. tuberculosis,* 86% were associated with miliary spread and 71% with cerebral tuberculosis. In the case of *M. bovis* abdominal infections, only 40% were associated with miliary tuberculosis and 46% with cerebral tuberculosis. Thus it was clear that, although *M. bovis*, entering the body via the alimentary tract, could cause respiratory tuberculosis, this was unusual; however, miliary spread accompanied by tuberculous meningitis (TBM) was a common cause of death. Conversely, there was a minority of cases where *M. tuberculosis* had undoubtedly entered the body via the alimentary tract, accompanied by involvement of the mesenteric lymph nodes, confirming the occurrence of a primary infection.

In the context of post-mortem studies, mention must also be made of the Lübeck tragedy, as a result of which an interesting perspective was provided on the consequences of infection in infants. In Lübeck in 1930, live, virulent *M. tuberculosis* was inadvertently given to 251 newborn infants instead of the vaccine Bacillus Calmette-Guérin (BCG). The bacilli were administered orally on three separate occasions, and on each occasion, the children received a very large dose of virulent bacilli. Within the next four years, 72 infants (27%) died, but 175 were alive with arrested lesions. Primary lesions in the gastrointestinal tract were found in all of those who died. These were present in the small intestine in 98% of children, but nodal enlargement was also present in the cervical area in 78% of children. In only 15% of children were primary lesions present in the lungs, and in each of these cases, it was thought that bacilli had been aspirated from lesions in the mouth or pharynx.[24,25] Arnold Rich observed that these findings indicate that, although usually considered very susceptible to tuberculosis disease following infection, the infant does possess a considerable degree of native resistance.[26] It is also noteworthy that the mortality of these heavily infected children was similar to that reported by Miriam Brailey for a group of very young, infected household contacts with parenchymal lung lesions during her studies conducted at the Harriet Lane Home in Baltimore; these infants were presumably infected by very low-dose droplet infection within their homes.[27]

TRANSMISSION OF TUBERCULOSIS INFECTION

Following his demonstration that *M. tuberculosis* caused tuberculosis, Koch speculated in 1884 regarding the manner of infection transmission and stated that, when a previously healthy individual was briefly in close contact with a tuberculosis patient, expectorated sputum might be inhaled, leading to infection. He noted, however, that it was likely that such infections did not occur easily, as the sputum drops were not small and so did not remain suspended in the air for long.[28] A number of researchers then took up the challenge of establishing exactly how infection occurred. Foremost among these early researchers were the German bacteriologist and hygienist Carl Flügge (1847–1923)[29,30] and the French veterinary surgeon P. Chaussé.[31,32] Very early it was shown that the largest sputum drops containing the most bacilli fell rapidly to the ground, but that the smallest droplets containing as few as one to three bacilli dried rapidly and could remain suspended in room air for up to five hours. These droplets became known as "Flügge droplets." When guinea pigs were placed in close proximity to coughing tuberculosis patients, they could become infected, even after only a short period of exposure, and typical primary foci were found on post-mortem.[31,32] It was also considered likely that, to negotiate the multiple twists and turns of the bronchi and bronchioli lined with a damp mucosa, only the smallest droplets would be able to penetrate as far as the alveoli to establish an infection.

In 1931, Bruno Lange, working in Berlin, published in a series of papers, the results of investigations in different animal models, including guinea pigs, mice, rabbits, and sheep, regarding their susceptibility to aerogenous infection with *M. tuberculosis.*[33,34,35] Infections were established by tracheal intubation with very low numbers of bacilli, and on later post-mortem, only a single primary focus was found in infected animals, which accorded with the findings of many post-mortem studies in children. He stated emphatically that the airways constitute a complicated filter, and that only very small expectorated drops could be inhaled to the very ends of the airways; it was thus unlikely that infectious particles could contain more than one to three bacilli. Arnold Rich, in his classic tome *The Pathogenesis of Tuberculosis*, discusses this subject at length[36]; he conceded that reference to "massive infection" is inappropriate, but had little doubt that droplets containing as many as 300 bacilli could readily pass into the terminal bronchioles, thus disputing Bruno Lange's claim that only the finest droplets containing no more than three bacilli are responsible for aerogenous tuberculosis infection.[34] As Rich points out, this is a very relevant matter regarding pathogenesis as, if only one bacillus is inhaled, four cell divisions will produce a population of 16 bacilli, whereas an infecting dose of 100 bacilli would produce a population of 1,600 bacilli, constituting a much stronger challenge to the innate immune system. A long series of subsequent studies has supported Lange's claims that only very small particles containing no more than three bacilli are capable of reaching the alveoli.[37,38,39] More recent animal studies have again replicated the process of low-dose infection and the establishment of typical primary infections followed by hematogenous spread of mycobacteria.[40,41]

THE DISCOVERY OF TUBERCULIN AND X-RAYS

Following his demonstration in 1882 that *M. tuberculosis* was the cause of tuberculosis,[9] Robert Koch attempted to develop a cure for tuberculosis. This led him to the evaluation of tuberculin, which he extracted from heat-concentrated cultures of *M. tuberculosis.*[42] Although tuberculin failed to cure tuberculosis, Clemens von Pirquet recognized its potential as a diagnostic test and described the cutaneous scratch test.[43] In 1908, Charles Mantoux refined the tuberculin test further by administering the tuberculin at first subcutaneously, and later by intradermal injection.[44] With the aid of the tuberculin test, it now became possible to detect not only tuberculosis disease, but also tuberculosis infection.

In 1895, Röntgen announced his discovery of X-rays,[45] and by 1898, Theodore Escherich, Professor Extraordinaire and director of St. Anne's Hospital in Graz, had gathered sufficient funds to buy one of the new radiology machines and gained sufficient experience with this revolutionary technique and its use in children to write a monograph on the subject.[46] In this he drew attention to the difficulty of interpreting the mediastinal shadows in young children, something that still troubles even the most experienced clinicians and radiologists. In 1902, Escherich was appointed director of St. Anne Children's Hospital in Vienna and established the use of radiology in pediatrics. One of Escherich's pupils in Vienna was Clemens von Pirquet, who in

1907 published the results of an evaluation of tuberculin sensitivity in children admitted to Escherich's clinic in Vienna.[47] At that time socioeconomic conditions in Vienna were particularly poor, and by the age of 10 years, close to 80% of children were already tuberculosis-infected.

Testing all children admitted to the clinic, von Pirquet showed that in very young hospitalized children, a positive tuberculin test was nearly always associated with disease, but that among the older age groups, there were increasing numbers of children who were tuberculosis-infected, but only a minority were diseased (Figure 1.2). He used the term *latent tuberculosis* to describe the children infected, but disease-free. The availability of chest radiographs and findings similar to those of von Pirquet made a dramatic impact on perceptions of childhood tuberculosis. Whereas the prognosis of childhood tuberculosis was previously regarded as dismal, it was now apparent that the components of the primary complex and its complications were radiologically visible in the great majority of children following primary infection, and that these children had minimal symptoms and clinical signs, if any. Nonetheless, it was also evident that very young children and adolescents were subject to a considerable risk of disease following infection. Radiology and tuberculin testing also enabled a number of observations documenting the finer details of the pathogenesis and natural history of tuberculosis.

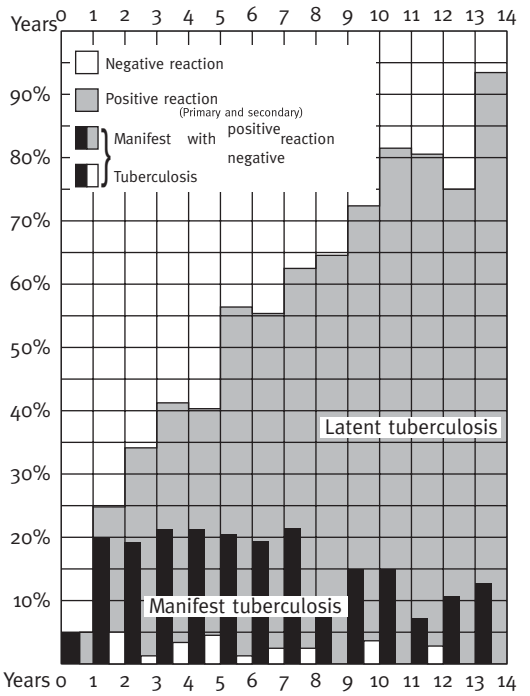

FIGURE 1.2 The results of cutaneous tuberculin testing by Clemens von Pirquet in 1,407 children admitted to the clinic of Theodore Escherich in Vienna, 1907–1908.

Reproduced from the *Journal of the American Medical Association*, 1907;52:675–678.

Herbert Assman (1882–1950), a clinician working in Leipzig, was frequently consulted by adolescents and young adults complaining of an upper respiratory infection, cough, sweating, and loss of appetite with, sometimes, acid-fast bacilli in their sputum.[48] On chest radiology they had a round, well defined, sub-apical shadow (The Assman Focus), while the apices where free of any visible lesion. The great majority of these lesions were transitory and followed by full recovery with a generally good prognosis; the lesion became known as "Fruhinfiltrat" in the German literature. Very often the infiltrates disappeared completely, but they might leave a residue of radiologically visible markings or progress rapidly to cavitation followed by extensive lung involvement. Arnold Rich, discussing this entity, concedes the undoubted existence of these lesions as a radiological entity, but added that pathology suggests several options for their origins, and after experience with post-mortem findings concluded, "infection of the sub-apical region of the lung in adult PTB may arise (a) by continuous, downward extension of an apical lesion (not visible on chest radiology); (b) by aspiration of bacilli from a small apical lesion; or (c) by direct exogenous or hematogenous infection of the subapical region in the absence of apical involvement."[49] From the perspective of childhood tuberculosis, these lesions would be seen in adolescents as a form of "adult-type" tuberculosis.

Closely associated with the concept of the Assman Focus were foci in the lung apices described by George Simon that became known as Simon Foci.[50] Simon considered that these lesions, single, or in many cases multiple, were probably of hematogenous origin as they often appeared shortly after primary infection, but unlike primary foci were nearly always localized in the apices. These could, of course, reactivate at any time, and could constitute one source of adult-type disease arising in adolescence.

French clinicians were probably the first to describe the spontaneous resolution of lung

consolidation in patients with tuberculosis.[51] In 1919, Kleinschmidt described the resolution of extensive "exudates" seen on chest radiography in children with tuberculosis.[52] Shortly afterwards, Helène Eliasberg and Willy Neuland, working in Berlin, reported their finding of extensive lung shadowing in several tuberculin-positive children.[53] More important, and mystifying, was the apparent good health of these children and the fact that these extensive lesions, which often involved the upper or middle lobes, tended to regress spontaneously. They coined the term *epituberculosis* for this entity, being unwilling to accept that there was a real tuberculous process present in these children. A vigorous controversy followed as to the true nature of these exudates, some favoring atelectasis as their basis, others advocating a non-tuberculous pyogenic infection. It soon became apparent that, although atelectasis might have a role in certain cases, atelectasis alone could not explain all of the features of this condition. Following an extensive review of the then-existing literature, Reichle (1933) came to the conclusion that these lesions were "probably tuberculous pneumonias which have stopped short of caseation."[54] Pathologically, the common features of epituberculosis were summarized as (1) the presence of "cells of types characteristic of a tuberculous lesion (granuloma), that is to say, epithelioid cells and typical Langhans giant cells and (2) tubercle bacilli [that] were either absent or very few in number in the affected tissue.". Arnold Rich stated in this respect, "The tubercle may resolve and be completely absorbed leaving no trace. This can only occur before connective tissue appears, but that it can and does occur is unquestionable. We have frequently observed this remarkable phenomenon. This resolution is apparently neither preceded by, nor accompanied by necrosis."[55] Experimental work summarized by Fish and Pagel (1938) threw more light on the pathogenesis of this condition.[56] When tuberculin-sensitive rabbits were inoculated intratracheally, a radiological appearance similar to epituberculosis resulted. When the organisms used were alive, a fatal caseous pneumonia followed; when dead organisms were used, the infiltration still developed, but resolved spontaneously. They concluded: "Epituberculosis might therefore be regarded as a tuberculin reaction of the allergic lung tissue." The present-day importance of an understanding of "epituberculosis" lies, perhaps, in the field of the therapeutic trial where, even more so than for adult tuberculosis, one should be reluctant to ascribe the resolution of tuberculosis lesions in children to a particular therapy, without the use of adequate controls.

THE "PRE-TUBERCULOUS" CHILD AND THE PREVENTION OF CHILDHOOD TUBERCULOSIS

Against the background of the developments described above, considerable concern developed towards the end of the nineteenth century and the early twentieth century among pediatricians and those concerned with child health regarding the fate of children infected with *M. tuberculosis,* exposed to infection or at risk of tuberculous infection and disease by virtue of poor home circumstances and malnutrition. In the introduction to a 1908 comprehensive review of tuberculosis in infancy and childhood, the editor Theophilus Kelynack drew attention to the toll exacted by childhood tuberculosis and the lack of attempts to arrest and control tuberculosis among children.[57] Evidence of tuberculosis was present in as many as 40% of children coming to post-mortem in various European countries and the United States of America. In England and Wales in 1902, deaths as result of tuberculosis in children under five years of age were 3.06/1,000; deaths as result of TBM numbered 5,961, and 68% of these occurred in children less than age five. While the importance of the "tuberculosis seed" was acknowledged, the relevance of predisposing factors was also highlighted, in particular, defective "hygienic" conditions and the proximity of open tuberculosis cases in a child's home. From these considerations, it was a short step to the development of a variety of interventions in the form of "preventoria," open-air schools and holiday camps or convalescent homes for "pretuberculous" children living in poor home circumstances or malnourished and for children already suffering from tuberculosis. In a manner similar to the alpine sanatoria developed for the management of adult tuberculosis, precise details of the structure of these institutions for the prevention and management of tuberculosis in children are provided in the compendium of Kelynack by representatives of many countries in Europe and elsewhere. Open-air classes were advised for those of school age, regular exercise and a location on the coast was to be preferred, and recommendations were even provided for where trees should be

planted to keep too much wind at bay. Connoly provides graphic details of the involvement of nurses in these many and varied interventions.[58]

As time passed, skepticism emerged about the value of these measures for the treatment of "resolving parenchymal tuberculosis of first infection" in infants and children.[59] It was also pointed out that, if not properly controlled, conditions in some institutions could promote the epidemic spread of other respiratory forms of viral or bacterial infections, including tuberculosis, leading to a considerable mortality, and that the "collective care of infants is hazardous.". The boarding out of eligible children in foster homes was still considered potentially advantageous and this was exemplified by the work of Prof. Jacques-Joseph Grancher (1843–1907) and his successors in France.[60] Later, Myers reported that children whose parents refused institutional care for the management of uncomplicated primary tuberculosis fared no worse in the long term than children receiving such care.[61] Despite these reservations, there can be little doubt that the emphasis on nutrition and health education of many of these interventions must have played some role in improving child health.

THE PLACE OF CHILDREN IN THE EPIDEMIOLOGY OF TUBERCULOSIS

Although it is possible to trace the presence of tuberculosis in many prehistoric and historic communities from skeletal remains and from ancient texts, these give no clues to the extent to which the disease might have influenced the relevant peoples. From the late seventeenth century, fragmentary documentation from European cities makes possible an estimation of the numbers of people dying from what was probably tuberculosis.[5] However, these estimates seldom provide an indication of the effects of tuberculosis regarding children. Towards the end of the nineteenth century, official notification of infectious diseases such as tuberculosis became policy in many countries, and for the first time, a more detailed picture began to emerge of the toll exacted by tuberculosis, the different forms of tuberculosis that affect young children as opposed to adults, and the influence of gender.

It was immediately obvious that age had a significant effect upon mortality, and this is, perhaps, best illustrated by the work of Wade Hampton Frost. In a classic paper, Frost explored the epidemiology of tuberculosis making use of the tuberculosis mortality rates for Massachusetts for 1880 through 1930.[6] In the introduction, he refers to the well-established age-related curve of tuberculosis mortality and points out that, for every shift in the mortality rate, there is probably a shift in balance between host resistance and "the destructive forces of the invading tubercle bacillus.". In his conclusion, he highlights the consistency of the picture of relative mortality that emerges, of high susceptibility in the very young declining to low levels in the school-age child, but then increasing in adolescents and peaking in young adults, which suggests "rather constant physiological changes in resistance (with age) as the controlling factor."

From a pediatric viewpoint, it is significant that, in the earliest data that Frost presented, up to approximately 1900, tuberculosis mortality in the very young was higher than at any other time of life. A similar high annual mortality in the very young was reported from England and Wales for the period from 1891 to 1900, where close to 400/100,000 deaths occurred in children under five years of age as result of tuberculosis[62] (Figure 1.3). Under these epidemic conditions, there was also a noticeable predominance of males dying from tuberculosis

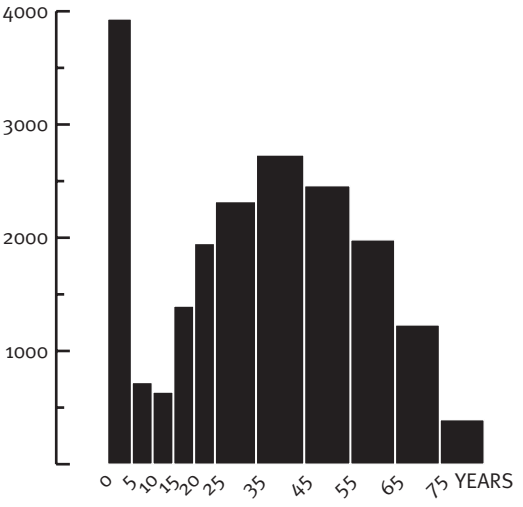

FIGURE 1.3 The average annual mortality from tuberculosis, per million living at each age period, in England and Wales, 1891–1900. (Cobbet 1910; from figures published in the "Supplement to the Sixty-fifth Annual Report of the Registrar-General, 1907," part I, cxciii.)

Reproduced from the *Journal of Pathology and Bacteriology,* 1910;14:563–604.

amongst the very young, but of females during adolescence. When these statistics are viewed from the perspective of a particular cohort, these tendencies are even more pronounced; thus, the mortality for males 0–4 years for Frost's cohort of 1880 was 760/100,000, declining to 43 for the group 5–9 years old; and then rising to 115 and 288 for the groups 10–19 and 20–29 years of age; before declining for those 30–39 and 40–49, to 253 and 175, respectively; and finally to 127 for the age group 50–59 years old. The large proportion of children in the affected populations, combined with the close household contact resulting from poor socioeconomic conditions and the exquisite susceptibility of the very young to disseminated serious forms of tuberculosis such as miliary tuberculosis and tuberculous meningitis, no doubt played a role in this distressing picture. As socioeconomic conditions improved and family sizes declined, these prominent features of the age-related incidence curve of tuberculosis changed dramatically; by 1930, the annual mortality of tuberculosis in young children in Massachusetts was lower than at any other time in life, although the peak for young adults of approximately 25 years of age remained at just below 100/100,000.

Accurate data are also available from New York for the period from 1898 to 1923 and sketch a picture of the ravages of tuberculosis among the very young, unfortunately a picture that is still all too familiar to practitioners in the cities of developing countries.[63] In 1898, tuberculosis mortality among children under age 15 years was 136/100,000; the major causes of death were TBM with a rate of 78/100,000, and PTB with a rate of 31/100,000. When one looks at infants only, however, a much a higher death rate for all forms of tuberculosis of 609/100,000 is seen, with a rate for TBM of 353/100,000. In this, and in many subsequent analyses of childhood tuberculosis mortality, TBM remains responsible for more than 50% of childhood tuberculosis deaths, irrespective of socioeconomic circumstances; thus, by 1959, in the United States, Great Britain, and France, 55%, 61%, and 68% of tuberculosis deaths in children, respectively, resulted from TBM.[64]

In the Netherlands, not only was accurate tuberculosis mortality data available from 1900–1945, but regular tuberculin testing of groups of young children allowed calculation of the annual risk of infection.[65] These data were then combined with mortality data and the annual risk of infection correlated with the causes of tuberculosis mortality in different age groups; importantly, in children, TBM was the major cause of death, particularly in the very young and was closely correlated with the annual risk of infection. More recent experience produced very similar findings in the Western Cape Province of South Africa.[66]

CONCLUSION

By approximately 1920, the broad facts underlying the manner of infection and anatomical features of tuberculosis in children and its epidemiology were established, and it now remained for concerned and persistent clinicians to make use of the tools of chest radiology and tuberculin testing to establish more precisely the natural history of tuberculosis infection in children and its long-term consequences. This subject is dealt with in Chapter 5.

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2

MICROBIOLOGY AND PATHOLOGY OF TUBERCULOSIS

Anne-Marie Demers, Andrew C. Whitelaw, and Kathleen D. Eisenach

HIGHLIGHTS OF THIS CHAPTER

- Caseating granulomas are the hallmark of the histopathological response to *M. tuberculosis,* especially in lymph nodes, which are usually a prominent part of the disease complex.
- Transport and handling of clinical specimens is particularly important for children because the bacilli are usually sparse.
- Because most forms of tuberculosis in children are paucibacillary, acid-fast stains of clinical samples are usually negative, and positive stains can be caused by nontuberculous mycobacteria.
- Gene Xpert MTB/RIF (rifampicin) is more sensitive than acid-fast microscopy when performed on respiratory samples from children, but both are less sensitive than culture.
- Both phenotypic and genotypic drug susceptibility testing are available, but discordance between them may occur because less-common mutations that confer resistance are detected only by phenotypic testing.

MYCOBACTERIOLOGY/ TAXONOMY

The genus *Mycobacterium* is the sole member of the family *Mycobacteriaceae*, which in turn is part of the order *Actinomycetales*. Mycobacteria are aerobic, non-spore-forming, nonmotile, and slightly curved or straight rods (0.2 to 0.6 μm by 1.0 to 10 μm). Compared to other bacteria, mycobacteria are characterized by their complex cell wall containing mycolic acids, and a genome with a high guanine plus cytosine (G+C) content.[1] Their high-lipid cell wall structure makes them hard to stain with dyes routinely used in bacteriology, such as the Gram stain. When stained with special procedures (e.g., Ziehl-Neelsen staining), they are not easily decolorized, even by acid alcohol, hence they are referred to as acid-fast or acid/alcohol-fast.[1]

More than 120 mycobacterial species have now been recognized, and more are constantly described.[2,3] Mycobacteria are grouped into members of the *Mycobacterium tuberculosis* complex (MTBC) and the nontuberculous mycobacteria (NTM) (also previously referred to as mycobacteria other than TB [MOTT] or atypical mycobacteria). *M. leprae* and *M. ulcerans* are often considered separately because of their distinct clinical and laboratory characteristics. Members of the MTBC include *M. tuberculosis, M. africanum, M. bovis, M. bovis* BCG, *M. microti, M. caprae, M. pinnipedii*, the dassie bacillus, and more recently *M. mungi, M. orygis,* and *M. suricattae.*[3–6] *M. canettii* is closely related to the complex but grows as smooth colonies instead of the typical rough colonies of the other MTBC members. The members of the MTBC display greater than 99.95% nucleotide sequence similarity at the genome level.[3] Of the MTBC, most human disease is due to *M. tuberculosis,* and the reservoir for *M. tuberculosis* is humans. All members of the MTBC have a doubling time close to 24 hours, compared to 20–45 minutes for most bacteria, and thus take three to four weeks to form colonies on solid media. Mycobacteria are aerobic and grow best in an atmosphere enriched with 5–10% carbon dioxide (CO_2) when using conventional solid media.[1] Their growth is also enhanced by fatty acids, which may be provided in the form of egg yolk or oleic acid. Although optimal growth temperatures vary widely among different mycobacterial species, *M. tuberculosis* grows best at 35–37°C.[1]

PATHOGENESIS

M. tuberculosis is almost invariably transmitted via the respiratory tract. Bacilli become aerosolized in droplet nuclei (1–5 μm airborne particles) generated after people who have pulmonary or laryngeal disease cough, sneeze, shout, or sing,[7] and these droplet nuclei may then be inhaled by a child or adult in the vicinity of the infectious index case. Inhaled mycobacteria enter the alveoli and are ingested by alveolar macrophages. Complex immunological events follow, which may result in either (1) complete elimination of the mycobacterium; (2) containment of the primary infection in a granuloma for a prolonged period, known as tuberculosis infection (which may persist for the individual's lifetime, or until progression to disease occurs); or (3) immediate progression to disease, usually in the context of impaired immunity or in young children (under 5 years of age)[8] (Figure 2.1).

The primary infection consists of a small parenchymal focus (Ghon focus), associated with bacilli that have spread via local lymphatics to regional lymph nodes. The Ghon focus, together with affected regional lymph nodes (with/without overlying pleural reaction), is called the primary (Ghon) complex.[9] The characteristic lesion of the primary infection is a caseating granuloma, which is a localized lesion in tissue consisting of a central area of caseous necrosis bordered by epithelioid macrophages and lymphocytes.[10] Caseating granulomas may enlarge and progress, or may calcify and heal with fibrosis and scarring. While the organisms are

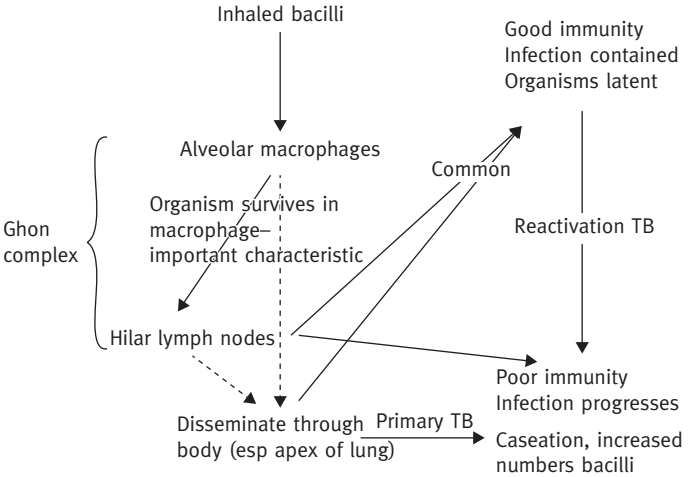

FIGURE 2.1 Pathogenesis of tuberculosis.

contained and constrained within caseating granulomas, not all are killed.

Primary infection may be associated with complications, especially in children under five years of age.[11] The parenchymal lesion may enlarge and caseate, or nodes may enlarge and compress or erode through a bronchus, causing pneumonia, atelectasis, or, more rarely, lung tissue destruction and cavity formation, invasion of the pericardium or pleural space.[12] The primary infection is usually accompanied by an occult, subclinical bacteremia that can seed distant sites, including the apices of the lungs, and cause extrapulmonary disease, particularly in the lymph nodes and the central nervous system (CNS) of children.[11] This may rapidly lead to severe forms of disease, including miliary and CNS tuberculosis. Extrapulmonary tuberculosis is more common in children than in adults.[13]

The histological aspect of caseating granulomas is similar for the primary infection in the lung as well as the secondary spread as tuberculomas in bones, brain, kidneys, lymph nodes, or other organs, in miliary tuberculosis, and in late stages of post-primary tuberculosis.[10] In most cases, the primary focus heals, and the bacteria continue to survive in a dormant state that is referred to as tuberculosis infection. Much is still unknown about the mechanisms that allow *M. tuberculosis* to survive in the host.[6,14] In a recent study using specific probes on tissue, *M. tuberculosis* was found to persist in multiple locations in the majority of persons without known tuberculosis disease. None of the organisms demonstrated was associated with granulomas, inflammatory infiltrates, or fibrosis, which challenges the traditional assumption that *M. tuberculosis* persists largely in isolated granulomatous foci.[15] In the same vein, small populations of bacilli may persist for months in a host being treated with effective drug therapy to which those bacilli are both phenotypically and genetically susceptible. Persistence seems tied to the bacilli's entering a slowly replicating or non-replicating state, but the host and bacterial conditions that enable or push a bacterium into this state are only beginning to be elucidated.[16–18]

The concentration of bacilli in the sputum depends on the type and number of tuberculous lesions from which the bacilli originate.[19,20] A 2 cm diameter cavity opening into a bronchus may contain 100 million bacilli, whereas a non-cavitated nodular lesion of the same size may contain only 100–1,000 bacilli.[21] Sputum from patients with cavitary tuberculosis have high bacillary loads, which are easier to detect in the laboratory (as detailed later). In contrast, sputum from patients with nodular or encapsulated lesions, such as those observed in childhood tuberculosis, discharge only small amounts of bacilli (hence referred to as paucibacillary) and detection of the organisms in the laboratory is much more difficult. Cavities do occur in children[12,22] but are not observed as frequently as in adults.[23]

MICROBIOLOGICAL DIAGNOSIS OF TUBERCULOSIS IN CHILDREN

Laboratory tests to diagnose tuberculosis can be grouped in two main categories[24]: (1) detection of organisms or components of organisms, which is the subject of this chapter, and (2) detection of the immune response to the organism, which is discussed in other chapters. Microbiological confirmation of tuberculosis in children is challenging for two main reasons. First, collecting specimens such as sputum from young children is typically more difficult than from adults. Second, even when sputum or other specimens are collected, they are usually paucibacillary (as described earlier), which makes detection of the organism more difficult. The high expectations that sensitive liquid culture methods and nucleic acid amplification tests (NAATs) would improve the ability to detect *M. tuberculosis* in childhood tuberculosis have not yet been met. The specimens collected are habitually of lower volumes, which can also compromise the yield: in an adult tuberculosis study, the sensitivity of an acid-fast smear from >5 ml of sputum was significantly greater than the sensitivity of a smear processed from a lower volume, as would normally be obtained from a small child.[25] Culture yield is also lower than in adults because investigations are often performed in the presence of nonspecific signs and symptoms.[26] Furthermore, a large proportion of true tuberculosis cases in children will be missed by culture.[27,28] Intrathoracic tuberculosis cases in children are rarely confirmed microbiologically,[27] defined recently for research purposes as at least one positive culture (with confirmed *M. tuberculosis* speciation) which could be sampled from expectorated sputum, induced sputum, nasopharyngeal aspirates, gastric aspirates, or string tests (or other relevant intrathoracic specimens).[26]

Notwithstanding the above concerns regarding the difficulty of confirming the diagnosis of tuberculosis in children microbiologically, there is still value in submitting specimens for microbiological testing.

- Some children with tuberculosis who have not yet been started on therapy will still be identified using laboratory tests.
- Drug susceptibility testing (DST) is becoming more important and (for the most part) requires isolation of the organism.
- Microbiological confirmation, for all its limitations, is still a vital part of research activities in tuberculosis, such as vaccine trials, treatment trials, and epidemiological surveys.

Nosocomial transmission of *M. tuberculosis* from patients or specimens is of major concern to health care workers and laboratory personnel.[1] Even if children with tuberculosis disease may be less contagious than adults, the same cannot be said for their adult caregivers if they are the source of the child's infection.[13] High-risk areas for tuberculosis transmission include spaces reserved for aerosol-generating procedures such as sputum collection areas.[7] In the laboratory, infection with *M. tuberculosis* is acquired by inhalation of *M. tuberculosis* produced by aerosols, which may be generated during processing of clinical or pathological tissue specimens, or during handling of cultured live *M. tuberculosis* for DST or other purposes.[29] A detailed discussion of infection control practices is beyond the scope of this chapter; however, infection control guidelines to minimize the risk of tuberculosis transmission are available for health-care facilities[7,30] and tuberculosis laboratories.[31]

Specimen Collection and Transport

One of the most important parameters affecting the performance of a microbiological diagnostic test is the quality of the specimen. Clinicians and pediatricians have tried to collect a broad variety of specimen types to improve the microbiological diagnosis of intrathoracic tuberculosis in children (Table 2.1). References on the most common methods of obtaining clinical specimens from children are available.[30,32,33] Specimens need to be representative of the site of infection, preferably collected aseptically, and stored and transported rapidly to the laboratory to minimize multiplication of contaminating organisms.[24] Ideally, specimens should arrive in the laboratory on the day of collection. If transport to the laboratory is delayed more than one hour, specimens should be refrigerated at 4°C as well as upon arrival in the laboratory until they are processed.[1] One study in adults showed that mycobacterial load and culture time to positivity were not significantly affected by refrigerated storage up to three days.[34] If prolonged storage or transport is unavoidable, preservatives can be added to the specimens to inhibit growth of contaminant bacteria and thus improve the yield from culture. Examples of these preservatives include sodium carbonate, cetylpyridinium chloride, and sodium borate. There are concerns that some of these compounds may not be compatible with some of the newer liquid-based culture systems such as the Bactec Mycobacteria Growth Indicator Tube (MGIT) system (Becton Dickinson Diagnostic Systems, Sparks, Md.), and they may also reduce the sensitivity of microscopy.[24] Fine-needle aspirates can be submitted in a culture medium (Middlebrook 7H9, glycerol, and Tween), which allows them to be stored for up to seven days prior to inoculation with no significant reduction in culture yield.[35] Gastric aspirates are commonly neutralized by the addition of sodium bicarbonate when collected[1,36]; however, one study has cast some doubt on this practice, finding a significant reduction in culture yield on neutralized gastric specimens.[37] More research is needed to confirm these findings. The laboratory should be contacted ahead of time for details regarding optimum collection and transport of specimens.

Processing

Processing specimens for mycobacterial culture is a complex process. Many specimens received for mycobacterial culture are viscous (particularly respiratory specimens), and some form of digestion is required, both to release mycobacteria that may be trapped in the specimen as well as to improve the decontamination process. Digestion and liquefaction of the specimen also facilitate concentration of the specimen. Since mycobacteria are usually slow growing and require long incubation times, other microorganisms (such as commensal or colonizing bacteria and fungi) can overgrow cultures of specimens obtained from non-sterile sites.[1] Specimens for microbiological investigations can be grouped according to the level of likely contamination (Table 2.2).[24] To eliminate contaminants as much as possible without affecting the viability of mycobacteria, specimens from non-sterile sites (Table 2.2, Groups 2 and 3) need to be decontaminated. This processing step is a delicate procedure: if it is too harsh, the yield is affected, as mycobacteria are also killed; if too mild, specimens will be overgrown

Table 2.1. Specimens used in the diagnosis of intrathoracic tuberculosis in children for microbiological tests

TYPE OF SPECIMEN	DESCRIPTION/FINDINGS
Gastric lavage/ aspiration	Traditionally diagnostic procedure of choice in young children unable to produce sputum. During sleep, the mucociliary mechanism of the respiratory tract sweeps mucus which may contain tubercle bacilli into the mouth. These secretions are swallowed and can be recovered in the gastric content, especially if the stomach has not emptied. Children are often hospitalized for the procedure, but it has also been successfully performed in outpatients.[36]
Expectorated sputum	Usually collected in older children who can cooperate.
Induced sputum	Another option to collect respiratory specimens in children unable to produce sputum. Sputum is collected after nebulization with hypertonic saline followed by nasopharyngeal suction. The technique has been safely performed in infants as young as 1 month of age.[93] Both ultrasonic and jet nebulizers have been used. Chest physiotherapy can also be performed to induce coughing and sputum production.[33]
Broncho-alveolar lavage (BAL) with bronchoscopy	Rarely performed in resource-poor countries. The diagnostic yield from bronchoscopy is no higher than that of gastric aspirates or induced sputum but may be useful to detect possible tracheobronchial obstruction or alternative diagnoses.[94]
Laryngeal swabs	Specimen used in older studies[95,96] but rarely if ever used in children anymore.
Nasopharyngeal aspirate	Suctioning of the nasopharynx collects upper respiratory tract secretions; stimulation of cough reflex may include lower respiratory secretions.[49]
String test	A weighted gel capsule containing a coiled nylon string is swallowed, with the trailing string held at the mouth and then taped to the cheek. Peristalsis carries the weighted capsule, which later dissolves, into the duodenum while the string unravels, extending from the mouth to stomach/duodenum. The string is left in situ for 4 hours during which it traps sputum along its length as lower respiratory tract secretions are carried up by the mucociliary escalator and spontaneously swallowed. Trapped secretions are retrieved upon withdrawal of the string and processed similar to a sputum specimen for AFB smear and culture.[97]
Fine-needle aspiration biopsy of peripheral lymphadenopathy	Children with pulmonary tuberculosis may have extrapulmonary disease manifestations in 10–30% of cases. Tuberculous lymphadenitis is the most common form of extrapulmonary disease in endemic areas, where up to 50% of extrapulmonary cases manifest as peripheral lymphadenopathy.[98] Fine-needle aspiration is a very useful adjunct to culture of respiratory specimens. The procedure may be performed safely on an outpatient basis by appropriately trained staff in a resource-limited setting.[49]
Stool	Collected on the rationale that young children tend to swallow rather than expectorate sputum, *M. tuberculosis* has been recovered in stool cultures after surviving the transit through the gastrointestinal tract. Stool cultures have been insensitive in general, but molecular techniques are promising.[99–102]

(continued)

Table 2.1. Continued

TYPE OF SPECIMEN	DESCRIPTION/FINDINGS
Urine	*M. tuberculosis* can be found in the urine of patients with active pulmonary tuberculosis, but the sensitivity of smear and culture is low. New diagnostic methodologies such as urine mycobacterial lipoarabinomannan (LAM) and urine mycobacterial DNA are being evaluated.[103] *M. tuberculosis* bacilli from infective foci in the lungs are destroyed by the immune response, releasing cell-free nucleic acids in plasma. The smaller sized cell-free nucleic acids pass through the kidney during filtration to produce transrenal DNA, which can be measured in urine by nucleic acid amplification techniques.[104]
Blood culture	Mycobacteremia due to *M. tuberculosis* is a common cause of bloodstream infections among HIV-infected adults in sub-Saharan Africa. However, the yield was low in among an ill, HIV-infected pediatric patient population in an area with a high tuberculosis burden, possibly due to the volume of blood collected being insufficient to recover mycobacteria.[105]
Bone marrow aspiration/biopsy	Not recommended routinely. It may assist in confirming an uncertain diagnosis of disseminated mycobacterial disease, establish an alternative diagnosis, or help rule out underlying malignancy.[106]
Cerebrospinal fluid (CSF)	A lumbar puncture should be performed in cases of suspected congenital or neonatal tuberculosis and in infants with disseminated disease.[107]

by other microorganisms, making the recovery of *M. tuberculosis* difficult. Of the various methods, the most frequently used is the NALC (N-acetyl L-cysteine)-NaOH (sodium hydroxide) method,[24] where NALC acts to digest the specimen, and NaOH decontaminates it. Briefly, an equal volume of NALC-NaOH is added to each specimen, then mixed by vortexing and left to stand for 15 minutes. Specimens are then neutralized by the addition of distilled water or phosphate buffer to reduce the continued action of the NaOH and lower the viscosity of the mixture.[38] Specimens are concentrated by centrifugation for another 15 minutes; the supernatant is discarded, and the remaining sediment (pellet) is resuspended in phosphate buffer. This resuspended pellet is then used for culture inoculation, smear microscopy, and NAATs. It has been estimated that up to one-third of the mycobacteria present in a specimen may be killed by this process, and it is possible that the NaOH concentrations used for processing specimens from adults may be inappropriate for paucibacillary specimens, such as those from children.[39] As a general rule, contamination rates of between 3% and 5% are accepted (up to 8% for broth-based culture).[40,41] Lower rates imply over-decontamination, and higher contamination rates indicate that the decontamination process is suboptimal.

Direct Tests: Smear Microscopy

Although used for more than 100 years, acid-fast bacilli (AFB) smear microscopy still plays an important role in the diagnosis of tuberculosis: (1) It can detect mycobacteria rapidly compared to culture results, which become available only after weeks of incubation; (2) it can identify the patients who are most likely to transmit *M. tuberculosis* to others; (3) it is still used in the identification work-up of positive cultures; and (4) it is often the only available diagnostic method in many developing countries. Smear microscopy is rapid, simple, inexpensive, and can be performed directly on clinical specimens. Two techniques are mainly used: carbol fuchsin stains (Ziehl-Neelsen or Kinyoun) for bright-field microscopy, and the fluorochrome stains (e.g., auramine O or auramine-rhodamine) for fluorescence microscopy (Figure 2.2).[42] Smear microscopy can detect a minimum of 5,000 to 10,000 bacilli per milliliter of sputum specimen. Given the paucibacillary nature

Table 2.2. Specimens according to level of contamination (adapted from[24])

GROUP	DESCRIPTION/EXAMPLES	DECONTAMINATION PROCEDURE
Group 1: Specimens collected aseptically from a site without commensal organisms	Specimens obtained by aspiration, biopsy, or surgical excision, and include cerebrospinal fluid, lymph node aspirates, aspirates from abscesses, bone marrow and joint aspirates, as well as tissue biopsies	These specimens do not require a decontamination procedure: they are inoculated directly onto the culture media (after centrifugation if necessary)
Group 2: Specimens are secretions from parts of the body with no or minimal commensal organisms, but connected to the body's surface by means of an open connection that harbors commensal organisms. These commensal organisms will be mixed with the secretions from the infected site as they pass through the opening to the surface	Specimens include those from the respiratory tract, gastric aspirates, urine, as well as uterine specimens	These specimens can usually be effectively decontaminated before culture is performed
Group 3: Specimens from parts of the body colonized with commensal and/or environmental organisms. They include normally colonized areas like the skin, oropharyngeal cavity, colon, and vagina, and secondarily colonized areas like ulcers and open wounds	Specimens like stool, skin specimens, as well as draining lymph nodes or abscesses belong to this group	These specimens are more heavily contaminated, which can affect the accuracy of microscopy and increases the chances of an unsuccessful mycobacterial culture

of childhood tuberculosis, this threshold explains why microscopy is of limited value in the diagnosis of childhood tuberculosis. Concentration of the specimen in the processing procedure (as described above)[43] and fluorescent microscopy (FM) increase smear microscopy sensitivity[43–45] by approximately 10%. FM implementation is limited by its high cost due to expensive mercury vapor light sources and the need for regular maintenance and a darkroom.[46] An alternative to FM is light-emitting diode (LED) microscopy, which is less expensive, requires less power, and can run on batteries, making it practical in resource-limited settings.[45] LED microscopy is marginally more sensitive (5% [95% confidence interval (CI), 0–11%]) with specificity similar to that of conventional fluorescence microscopy.[46] The World Health Organization (WHO) recommends that conventional fluorescence microscopy be replaced by LED microscopy, and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy.[46] Reporting of smears is done using a semi-quantitative scale (Table 2.3). Due to an historical inaccuracy, the FM reporting scale for positive smears has been revised; the actual field observed is larger, and therefore more AFB are visible per field than previously calculated.[42] The American Thoracic Society and Centers for Disease Control and Prevention use a different scale, graded from negative to 4+.[47] Confirmation of FM low-positive smears by re-staining with Ziehl-Neelsen should not be done.[42] It is not possible to differentiate the various species of mycobacteria on microscopy.

Direct Tests: Nucleic Acid Amplification Tests (NAATs), Including GeneXpert

With the development of new molecular diagnostic tools, the rapid diagnosis of tuberculosis has made significant progress in recent years. These assays are based

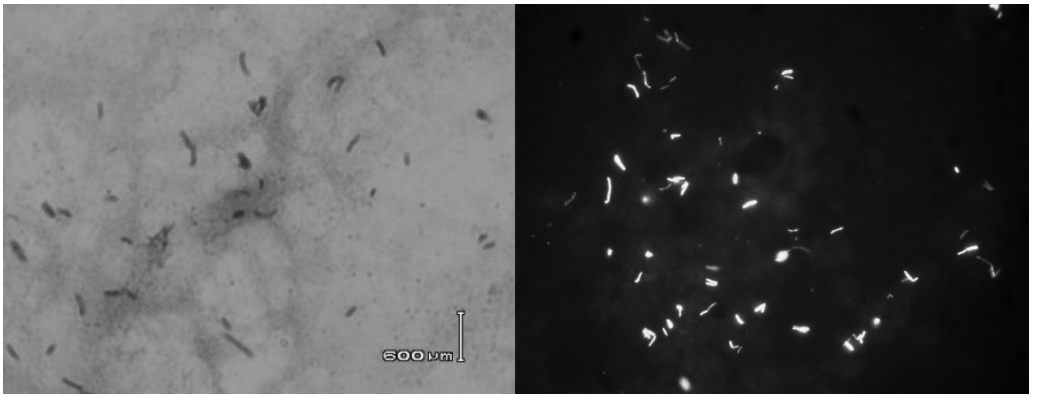

FIGURE 2.2 Smear microscopy: Ziehl-Neelsen staining at 1000X magnification (left side) and auramine staining at 200X magnification (right side).

on the detection of specific nucleotide sequences (DNA or RNA) and/or mutations in the *M. tuberculosis* genome, indicative of the presence of *M. tuberculosis* and/or associated drug-resistance mutations.[48] While detection of the nucleic acids is probably most often accomplished using the polymerase chain reaction (PCR), other nucleic acid amplification technologies also exist, such as loop mediated isothermal amplification (LAMP), ligase chain reaction (LCR), transcription mediated amplification (TNA), and strand displacement amplification (SDA), and all have been used in various assays to detect *M. tuberculosis*. In addition, after amplification of the nucleic acid, different methods can be used to detect the presence of specific mutations or polymorphisms in the amplified DNA—either to further speciate the isolate, or to detect mutations responsible for drug resistance. These techniques included hybridization to immobilized probes (as used in the Genotype MTBDR*plus* [Hain Lifescience, Nehren, Germany] line probe assays), or molecular beacons (as used in the GeneXpert MTB/RIF, Cepheid, Sunnyvale, Calif.).

Many of the NAATs can be performed directly on clinical specimens or on the resuspended sediments (pellets) after processing. Advantages of NAATs are numerous: (1) They are theoretically highly sensitive and able to detect very low copy numbers of nucleic acid; (2) they have a rapid turnaround time (<24 hrs. usually); (3) they may not require biosafety level 3 facilities; and (4) they are relatively easy to automate.[49] Disadvantages are:

(1) For many tests, NAATs require sophisticated laboratory infrastructure and highly skilled technicians.

(2) The risk of contaminating the test site with amplified DNA requires stringent quality-control procedures and a specific infrastructure to limit contamination.

(3) Although the sensitivity of commercial NAATs to detect *M. tuberculosis* is high in sputum acid-fast smear-positive specimens, it is lower in smear-negative and in extrapulmonary specimens and not as sensitive as culture.

(4) Rapid molecular assays for identification and detection of drug resistance in primary patient specimens do not replace culture-based methods, which remain the gold standard for diagnosis and phenotypic DST.

Newer NAAT methods have been developed to overcome some of the limitations outlined above. At the present time, the best-studied is the GeneXpert MTB/RIF test, which is completely automated and self-contained, and not dependent on reference laboratories or a high degree of technical expertise.[50] Multiple NAAT platforms and technologies are now available and described in detail elsewhere.[51]

Culture

Culture remains the gold standard for the laboratory diagnosis of tuberculosis disease. It has higher sensitivity than smear microscopy but a much longer turnaround time. Mycobacterial culture can be performed on solid egg-based media (e.g., Lowenstein-Jensen [LJ]), on solid agar-based media (e.g., Middlebrook 7H11) or on liquid media (e.g., Middlebrook 7H9). Liquid media

Table 2.3 Reporting of smear microscopy (from [42]). **(A)** Using a bright field microscope, Ziehl-Neelsen smears are examined with the 100X oil objective (10X eyepiece for a total of 1000X magnification). **(B)** With a fluorescent microscope, the smear is scanned with the 20X objective (with 10X eyepiece for a total of 200X magnification), occasionally using the 40X objective to see more detailed bacterial morphology

(A) GRADING SCALE FOR CARBOL FUCHSIN STAINS FOR BRIGHT-FIELD MICROSCOPY

WHAT YOU SEE	WHAT TO REPORT
No AFB in 100 fields	No AFB observed
1–9 AFB in 100 fields	Record exact number of bacilli
10–99 AFB in 100 fields	1+
1–10 AFB per field, check 50 fields	2+
More than 10 AFB per field, check 20 fields	3+

(B) GRADING SCALE FOR FLUOROCHROME STAINS

WHAT YOU SEE (200X)	WHAT YOU SEE (400X)	WHAT TO REPORT*
No AFB in one length	No AFB in one length	No AFB observed
1–4 AFB in one length	1–2 AFB in one length	Confirmation required**
5–49 AFB in one length	3–24 AFB in one length	Scanty
3–24 AFB in one field	1–6 AFB in one field	1+
25–250 AFB in one field	7–60 AFB in one field	2+
>250 AFB in one field	>60 AFB in one field	3+

*The number of AFB indicates how infectious the patient is. It is important to record exactly what you see.
**Confirmation required by another technician; or prepare another smear, stain and read.

are more sensitive than solid media for culture,[1,52] which is an advantage for the paucibacillary disease observed in children. The WHO recommends the use of liquid medium for culture and DST, emphasizing the need for rapid diagnostic tools facilitating species identification.[53] These liquid culture systems, however, are more prone to contamination by other non-mycobacterial organisms or NTM, even in experienced laboratories.[45] Commercial liquid culture systems (such as MGIT, the most widely used of these) are also more expensive, and many resource-poor countries still depend on LJ egg-based solid medium for the detection of growth of MTBC isolates.[54] Since some strains of the MTBC will grow better or only on solid media, the CDC-recommended gold standard for the detection of *M. tuberculosis* is to inoculate at least one tube each of solid and liquid media.[54] However, whether this is a cost- and/or labor-effective approach in endemic areas is not yet clear. Antibiotics and other additives can be added to media (whether solid or liquid) to make them more selective and inhibit other bacteria that may have survived the decontamination process.

Identification Methods from Culture

Once growth is detected in the culture medium (whether it is solid or liquid), it is important to identify the isolate, as well as to perform drug susceptibility testing. In the past, either biochemical methods or chromatography were used for this

purpose; however, they have been replaced with newer rapid molecular or antigen-based tests.

Some identification techniques will only differentiate *M. tuberculosis* (or members of the MTBC) from NTM. An example of this involves detecting the presence of a *M. tuberculosis* complex-specific antigen, MPT64.[55] If the antigen is present, the isolate is identified as *M. tuberculosis*, and if absent, it is presumed to be one of the NTMs. However, the test should be performed on a positive culture with sufficient bacterial load, as false negatives have been reported.[56,57] A number of commercial lateral flow assays are available, and are a quick, easy, and relatively inexpensive way of confirming that the isolate in culture is *M. tuberculosis.*

Other assays, usually molecular, are able to identify both *M. tuberculosis* as well as certain NTMs. Molecular assays for identification are usually based on reverse hybridization, although sequence-based assays also exist. Certain tests are also able to differentiate between members of the MTBC. This is of particular relevance in children when BCG disease is suspected. Examples of molecular kits able to speciate mycobacteria are the Accuprobe (Gen-Probe, San Diego, Calif.), Inno-LiPA Mycobacteria assay (Innogenetics, Ghent, Belgium), the GenoType MTBC assay and Genotype Mycobacteria CM/AS assays (Hain Lifesciences, Nehren, Germany), and the MicroSeq 500 system (Applied Biosystems, Calif.).[58–62]

Drug Susceptibility Testing (DST)

Multidrug-resistant tuberculosis (MDR-TB) is associated with longer, more expensive, and more toxic treatment courses, as well as with worse outcomes.[63] The more recent recognition of extensively drug-resistant tuberculosis (XDR-TB) has reemphasized the need for regimens based on drug-susceptibility testing results, and has highlighted the importance of expediting the availability of these results.[64,65]

DST of mycobacteria can be performed phenotypically and genotypically. The principle of phenotypic testing assesses whether the organism can survive and/or grow in the presence of the antibiotic. This is used to infer whether the patient infected with the strain is likely to respond to treatment. Genotypic methods involve detecting the presence of genes or mutations known to be associated with resistance; again, the inference is that a strain with the mutation will not respond to treatment with the drug. Phenotypic susceptibility testing is often regarded as the gold standard; however, it is time-consuming since it relies on growth of the organism. Molecular methods are much faster, and, for certain antibiotics, there is excellent correlation between the presence of a specific mutation/s and phenotypic resistance.

DST can also be direct or indirect. "Direct" testing refers to testing drug susceptibility directly on the clinical specimen. Typically this involves an acid-fast smear-positive specimen's being inoculated onto antibiotic-containing and antibiotic-free medium after decontamination. "Indirect" testing implies that the DST is performed once the organism has been isolated in culture.

Phenotypic DST

The three standard methods of phenotypic DST are the absolute concentration method, the resistant ratio method, and the proportion method. All three have been described in numerous reviews.[66–72] Most commonly used in routine laboratories is the proportion method, which assumes that if more than 1% of the organisms in a given population are resistant to a drug, the strain will be resistant to that drug. Agar plates containing a defined concentration (called the critical concentration) of the drug are used. After inoculation of the isolate onto both antibiotic-containing and antibiotic-free media, the plates are incubated for up to three weeks. The number of colonies on both plates is counted and compared, and the proportion of resistant colonies (i.e., those growing on the antibiotic containing medium) is calculated.

When performed in liquid culture, the isolate is inoculated into a vial with the critical concentration of antibiotic, and into an antibiotic-free vial. There are numerous commercial non-radiometric culture systems that can be used to perform DST. Although the algorithms, critical concentrations, and inoculation procedures may differ across systems, they all follow the essential principle of comparing the growth in the antibiotic-containing vial to growth in the antibiotic-free vial to determine whether the isolate is susceptible. A major advantage of using liquid culture is the faster time to generate a result, but results can still take 10 to 14 days after the initial isolation of the organism. Although not widely implemented, direct DST can be performed with re-suspended sediments using the MGIT DST method, which avoids the need

to have an isolate from the original culture and shortens the time to results.[73]

A key factor in performing the proportion method is the critical concentration, which is the lowest concentration of the agent that inhibits growth of wild-type (susceptible) strains.[71] The critical concentration varies for different culture media. Critical concentrations for many antibiotics were published by the WHO in 2008,[74] and updated in 2012.[75] Phenotypic DST results using current critical concentrations are very reliable for rifampicin and isoniazid, but less so for ethambutol and streptomycin.[76,77] The current critical concentrations for fluoroquinolones and injectable agents (amikacin, kanamycin, and capreomycin) also yield reliable results; however, the evidence for this may not be as strong as for rifampicin and isoniazid.[77–79] Critical concentrations have been suggested for testing pyrazinamide (PZA), but testing is difficult since the drug is more active at a low pH, which itself inhibits mycobacterial growth.[80] The reliability of the proposed critical concentrations and methodology for PZA has been questioned, based on studies correlating phenotypic DST to molecular methods.[81,82]

Genotypic DST

Drug resistance in *M. tuberculosis* is due to chromosomal mutations; acquisition of resistance genes through mobile genetic elements such as phages has not been described. Molecular assays in general are based either on detecting the presence of a specific mutation and/or detecting the presence or absence of a wild-type gene (or region of the gene). Molecular assays work best when resistance is associated with either a limited number of mutations, or mutations in limited regions of the genome. This is probably best exemplified by rifampicin resistance. The vast majority (>95%) of rifampicin-resistant isolates (based on phenotypic DST) have mutations in an 81bp region of the *rpoB* gene that encodes RNA polymerase B, the target of rifampicin.[83,84]

The more diverse the range of mutations responsible for resistance, the more technically challenging molecular DST becomes, although with the advent of next-generation sequencing technology this may become less of an issue. Most molecular assays use DNA probes corresponding to wild-type sequences or specific mutations and determine whether specific mutations or wild-type sequences are present in the isolate's DNA. It is likely that in the future sequence-based techniques will become more widely used.

Commercial reverse-hybridization assays are available for determining rifampicin and isoniazid resistance. The Inno-LiPA Rif TB assay (Innogenetics, Ghent, Belgium) detects resistance to rifampicin only, and the GenoType MTBDR*plus* assay tests for both rifampicin and isoniazid resistance. Both have excellent sensitivity when it comes to rifampicin resistance, ranging from 95–100% compared to phenotypic results. The sensitivity of the GenoType MTBDR*plus* system for isoniazid resistance is on the order of 73–90%, and is due to the greater variety of molecular resistance mechanisms for isoniazid compared to rifampicin's. Both assays are close to 100% specific.[74,85,86] Both systems can detect resistance directly from smear-positive clinical specimens, and while the performance may not be quite as good as when performed on cultured isolates, it is still excellent.[87] A more sensitive second version of the GenoType MTBDR*plus* assay is now available and allows its use with acid-fast smear-negative sputum specimens. The main drawback to the routine implementation of these methods for drug susceptibility testing (whether it be from culture or specimen), is expense. The Hain Genotype MTBDR*sl* line probe assay detects resistance to the fluoroquinolones, injectable agents (amikacin, kanamycin, and capreomycin), and ethambutol. The performance of this assay was recently reviewed.[88,89] In summary, the assay performs well for both the fluoroquinolones and injectable agents, but is less reliable for ethambutol. For the fluoroquinolones and injectable drugs, the specificity was higher than its sensitivity, and the assay is thus probably more suitable as a rule-in test than a rule-out test (i.e., more reliable to detect resistance than to detect susceptibility), and phenotypic testing should be performed in addition to the molecular assay. A new version of the MTBDR*sl* has been released recently, which has removed the probes for *embB* (ethambutol), and replaced them with probes for the *eis* gene (to detect additional kanamycin resistance). The use of the assay will vary, depending on the local prevalence of resistance to these agents, as well as on local distribution of specific resistance mutations. As with the GenoType MTBDR*plus*, this assay can be performed both on clinical specimens and cultured isolates.[88] This more sensitive version of the MTBDR*sl* assay will also allow the testing of smear-negative specimens.

The GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, Calif.) is an automated, semi-nested real-time PCR assay that both detects the presence of *M. tuberculosis* in clinical specimens and uses molecular beacons to determine whether wild-type *rpo*B sequences are present in the amplicons. The assay is designed for use on clinical specimens, primarily sputum, although more evidence is accumulating to describe its use in extrapulmonary specimens. It combines automated DNA extraction with amplification and detection in a cartridge format, and can be used by technicians with even with minimal formal training in molecular techniques. As with other molecular assays for detection of rifampicin resistance, it has excellent sensitivity and specificity.[90] Furthermore, the next-generation GeneXpert assays are being designed to have sensitivity more equivalent to cultures' and will detect fluoroquinolone resistance.

CONCLUSION

Although laboratory techniques described more than 100 years ago are still in use, and a perfect, quick and sensitive point-of-care tuberculosis diagnostic test is not yet available, more effort is being made to improve microbiological confirmation in children, especially as rates of drug resistance increase. However, the paucibacillary nature of childhood tuberculosis remains a major limitation of new sampling methods and laboratory techniques. It is encouraging to see children included in reviews of current and potential future technologies[51,91] as well as collaborations between end-users and product developers regarding the targets and specifications that should be met for new diagnostic methods.[92]

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