THE EPIDEMIOLOGY AND MANAGEMENT

OF DRUG-RESISTANT TUBERCULOSIS IN

CHILDHOOD

Dissertation presented by

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for the degree of

Doctor of Medicine (MD) (Paediatrics and Child Health)

at

Stellenbosch University

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June 2002

Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: Date:

SUMMARY

Resistance to antituberculosis agents became evident soon after antituberculosis treatment was introduced for the first time. Combined drug therapy seemed to resolve this problem. Animal experimental studies, which showed that isoniazid (INH)-resistant strains of *Mycobacterium tuberculosis* were less infectious and pathogenic than drug-susceptible strains, gave further reassurance that drug resistance was not a major issue.

Transmission of INH- and multiple-drug-resistant strains did, however, occur. Studies in children, who develop mainly primary drug resistant tuberculosis (TB), showed that drug resistance in adults was followed by a similar rise in drug-resistant (TB) in children, and that tuberculous infection rates in childhood contacts of INH-resistant and drug-susceptible adult TB cases were the same.

It was however, only after the significant rise in the incidence of TB and large outbreaks of multidrug-resistant (MDR) TB cases in developed countries (mainly because of the human immunodeficiency virus epidemic) in the early nineties that sufficient attention was again focussed on the problem of drug-resistant TB. Drug-resistant tuberculosis, and more in particular MDR TB, posed a serious threat to global TB control programmes.

Despite this renewed interest, childhood drug-resistant TB remained neglected. The incidence of drug-resistant TB among children, which could give a good indication of currently circulating strains in a community, is hardly known. The management of childhood contacts of adults with infectious MDR TB or children with MDR TB has also not been studied prospectively.

All confirmed childhood TB cases from a specific geographic drainage area over a 3.5-year period were prospectively included in a drug resistance surveillance study. The incidence of drug resistance in children was comparable to the incidence of initial (primary plus undisclosed previous treatment) drug resistance documented in adults in the same area. The findings show that the incidence of drug-resistant TB in children in the Western Cape province is low, and probably reflects the level of primary drug resistance amongst organisms currently circulating in this community.

The short- and long-term outcome of children <5 years of age in contact with infectious adult MDR TB cases was determined by prospective follow-up for 30 months. The initial evaluation showed an infection rate significantly higher in MDR TB contacts compared with contacts of drug susceptible cases, but the disease rate was lower. On follow-up, many more children became infected or developed disease. The finding that 90% of those who developed disease did so within the first 12 months, indicates that follow-up beyond 12 months is probably not cost-effective in resource poor countries. The results demonstrate that MDR TB is not less infectious than drug susceptible TB. Despite the fact that some children received chemoprophylaxis, 24% of the children eventually developed disease. This is not different from the expected prevalence of disease in childhood contacts <5 years of age of infectious drug-susceptible adult pulmonary TB cases.

Restriction fragment length polymorphism analysis confirmed transmission from an adult source case to a child contact in 5 of 6 adult-child pairs in whom both isolates were available. If therefore an isolate of *M. tuberculosis* for susceptibility testing cannot be obtained from a child in close contact with an infectious MDR TB case, the child should

therefore be treated according to the drug susceptibility pattern of the source case's strain.

Treatment of children with confirmed and probable MDR TB included 2 or 3 drugs to which the adult source case's isolate was susceptible in addition to pyrazinamide and high-dose INH. Duration of treatment ranged from 6 to 12 months depending on the severity of the disease. INH was included in the treatment regimen because low-level resistance to INH was present in about half the cases of primary INH resistance. The pharmacokinetics of INH in children confirmed that an adequate concentration and exposure time could be achieved for this purpose. Ethionamide often caused gastrointestinal adverse events, but these could be overcome in most cases by temporary dose adjustments. The fluoroquinolones, which are not generally recommended for use in children, possibly caused arthralgia in 1 of the 17 children treated for ≥6 months. This is in accordance with previous reports of the safety of these drugs in children for short- and medium-term treatment.

TB disease occurred significantly less often in children who received appropriate chemoprophylaxis (according to the drug susceptibility pattern of the adult source case's isolate). Although this was not a randomised controlled trial, the group that received chemoprophylaxis was at higher risk for developing disease. This implies that prevention of TB in MDR contacts is possible. A prospective, randomised controlled study is necessary to evaluate the best drug combinations and the optimal duration of such chemoprophylactic regimens.

OPSOMMING

Middelweerstandigheid het na vore gekom kort nadat antituberkulose behandeling vir die eerste keer in gebruik geneem is. Die gekombineerde gebruik van middels het klaarblyklik die probleem oorkom. Diere eksperimente wat getoon het dat isoniasied (INH)-weerstandige stamme van *Mycobacterium tuberculosis* minder infektief en patogenies is as vatbare stamme, het verdere gerustelling gegee dat middelweerstandigheid nie 'n groot probleem is nie.

Die oordrag van INH- en multi-middelweerstandige stamme het egter wel plaasgevind. Studies in kinders, wat hoofsaaklik primêre middelweerstandige tuberkulose (TB) ontwikkel, het getoon dat middelweerstandigheid in volwassenes gevolg is deur 'n soortgelyke toename in middelweerstandige TB in kinders en dat die voorkoms van tuberkuleuse infeksie in kinderkontakte van INH-weerstandige en middelvatbare volwasse TB gevalle dieselfde is.

Dis egter eers toe daar 'n beduidende toename in die insidensie van TB en groot uitbrake van multimiddelweerstandige (MDR) TB gevalle in die ontwikkelde lande (hoofsaaklik as gevolg van die menslike immuungebrek virus epidemie) in die vroeë negentigerjare was dat daar opnuut aandag aan die probleem van weerstandige TB geskenk is. Middelweerstandige TB, en in besonder MDR TB, hou 'n ernstige bedreiging vir globale TB beheerprogramme in.

Tenspyte van die nuwe belangstelling in middelweerstandige TB is die probleem in kinders steeds afgeskeep. Die insidensie van weerstandige TB in kinders is onbekend alhoewel dit 'n goeie weergawe van die huidig sirkulerende stamme in 'n gemeenskap

sou gee. Die hantering van kinderkontakte van volwassenes met infektiewe MDR TB of kinders met MDR TB is ook nog nie prospektief bestudeer nie.

Alle bevestigde kinder-TB gevalle van 'n spesifieke geografiese gebied is oor 'n 3.5 jaar tydperk prospektief in 'n middelweerstandige waarnemingstudie ingesluit. Die insidensie van middelweerstandigheid in kinders was vergelykbaar met die insidensie van inisiële (primêre weerstandigheid plus onbekende vorige behandeling) middelweerstandigheid in volwassenes van dieselfde gebied. Die bevindinge toon dat die insidensie van middelweerstandige TB in kinders in die Weskaap provinsie laag is. Dit weerspieël waarskynlik die vlak van primêre middelweerstandigheid in organismes wat tans in hierdie gemeenskap sirkuleer.

Die kort- en langtermyn uitkoms van kinders <5 jaar oud wat in kontak met infektiewe volwasse MDR TB gevalle was, is prospektief tydens 'n 30-maande opvolg bepaal. Die aanvanklike evaluasie het 'n beduidend hoër infeksiekoers in die MDR TB kontakte in vergelyking met kontakte van middelvatbare gevalle getoon, maar die siektekoers was laer. Tydens die opvolgperiode het baie meer kinders infeksie of siekte ontwikkel. Aangesien 90% van dié wat siekte ontwikkel het, dit gekry het binne die eerste 12 maande, is opvolg ná 12 maande waarskynlik nie koste-effektief in hulpbron-beperkte lande nie. Die bevindinge toon dat MDR TB nie minder infektief is as middelvatbare TB nie. Tenspyte daarvan dat sommige kinders chemoprofilakse ontvang het, het 24% van die kinders uiteindelik siekte ontwikkel. Dit verskil nie van die verwagte siekte-insidensie van kinderkontakte <5 jaar oud wat in kontak met infektiewe volwasse middelvatbare pulmonale TB was nie.

Restriksie fragment lengte polimorfisme analise het oordrag van volwasse brongeval na kinderkontak in 5 uit 6 volwasse-kind pare, van wie beide isolate beskikbaar was, bevestig. Indien daar dus nie 'n isolaat van *M. tuberculosis* vir vatbaarheidstoetse van 'n kind met nabye kontak met 'n infektiewe MDR TB geval beskikbaar is nie, behoort die kind volgens die middelvatbaarheidspatroon van die brongeval se stam behandel te word.

Behandeling van kinders met bevestigde of waarskynlike MDR TB het 2 tot 3 middels waarvoor die volwasse brongeval se isolaat vatbaar was, ingesluit, tesame met pirasinamied en hoë-dosis INH. Die duur van behandeling het gewissel van 6 tot 12 maande op grond van die omvang van die siekte. INH is in die behandeling ingesluit omdat dit getoon is dat ongeveer die helfte van die gevalle met primêre INH-weerstandigheid lae-vlak weerstandigheid het. Die farmakokinetika van INH in kinders het bevestig dat genoegsame vlakke en blootstellingstyd aan INH vir hierdie doel bereik kan word. Etionamied het dikwels gastrointestinale newe-effekte veroorsaak, maar dit kon in die meeste gevalle oorkom word. Die fluorokwinolone, wat nie oor die algemeen in kinders aanbeveel word nie, het moontlik artralgie veroorsaak in 1 uit 17 kinders wat vir ≥ 6 maande behandel is, wat vorige verslae oor die veiligheid van hierdie middels in kort- en medium-termyn behandeling bevestig.

TB-siekte het beduidend minder dikwels voorgekom in kinders wat toepaslike chemoprofilakse (volgens die middelvatbaarheidspatroon van die volwasse brongeval se isolaat) ontvang het. Alhoewel dit nie 'n ewekansig gekontroleerde studie was nie, het die groep wat chemoprofilakse ontvang het die hoogste risiko vir die ontwikkeling van siekte gehad. Dit dui daarop dat voorkoming van TB in MDR TB kontakte moonlik is. 'n

Prospektiewe, ewekansig gekontrolleerde studie is nodig om die beste middel kombinasies en die optimale duur van so 'n chemoprofilaktiese behandeling te bepaal.

Dedicated to my parents, Sjoerd and Jeannette Schaaf

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ACKNOWLEDGEMENTS

I thank God for a wonderful occupation that I really enjoy. Considering different career options while still at school, it was my father, Sjoerd Schaaf, who guided me to medicine. This was confirmed through a definite calling as an answer to prayer.

During my final year at Medical School at Stellenbosch University, dedicated teachers such as dr. Esta van Schalkwyk and prof. MP (Ma) Keet instilled in me a special love for sick children and paediatrics in general. It was therefore a privilege to return to the Department of Paediatrics and Child Health at Tygerberg Hospital to specialise in paediatrics. Although I did not see myself as a researcher, prof. Budgie van der Merwe introduced me into this arena when, as a registrar, we studied the effect of liposomes as carrier substance for ampicillin in rabbits with infective endocarditis. When I qualified, prof. Peter Hesseling offered me the wonderful opportunity to do community paediatrics at this great tertiary institution.

It was not until I completed my medical studies that my mother, Jeannette Schaaf-Drost, disclosed to me that she had contracted tuberculosis in the early 1950s, before antituberculosis treatment was generally available. She was working as a general assistant in a tuberculosis hospital in The Netherlands at the time and, tuberculosis being a very stigmatised disease, never felt comfortable to talk about it. This, together with the wonderful mentorship of prof. Peter Donald, led me into the field of tuberculosis research.

Under the guidance of prof. Peter Donald, himself, prof. Nulda Beyers, prof. Robert Gie and myself started a clinical childhood tuberculosis research group in the early 1990s. Through the support of the GlaxoWellcome TB Research Initiative and the Medical Research Council we were able to initiate several new research projects, including the prospective studies on childhood drug resistant tuberculosis, which forms a big part of this thesis. The considerable assistance of many colleagues such as Helen Vermeulen, Magdalene Kennedy, Annelies van Rie, Sophia Carlini, Tommy Victor, Frik Sirgel, Etienne Nel, prof. Paul van Helden, and, of course, professors Peter Donald, Robert Gie and Nulda Beyers, is sincerely appreciated. Further studies followed, supported by the Department of Pharmacology of the Faculty of Health Sciences at Stellenbosch University and the Harry and Doris Crossley Research Foundation. With regard to the isoniazid study I would like thank dr. Don Parkin, a wonderful mentor since my student days, Heiner Seifart and Cedric Wereley in particular.

It was, however, Robert Gie with his clear scientific mind that envisioned the doctoral degree in all the work that was in progress and, with Peter Donald as promoter and Peter Hesseling as co-promoter, I was able to start putting it all together.

If it was not for many other dedicated health care workers in the laboratories and out in the field, these studies would not have been possible. Hence I want to acknowledge the support of the technologists in the tuberculosis laboratories, both at Tygerberg Hospital and at the South African Institute for Medical Research, as well as the many nursing staff that treated and followed up our patients at the local health authority clinics.

I want to thank my parents for their love and dedication to their children that made it possible for me to reach my dreams. Last, but definitely not least, I want to thank my dear wife, Francoise, and my son, Herman, for bearing with me, supporting me, and allowing me to spend many hours working on this thesis during the last 3 years, times that should have been spent with them.

ABBREVIATIONS

AAP	American Academy of Pediatrics
AFB	acid-fast bacilli
AIDS	acquired immunodeficiency syndrome
AUC	area under the concentration versus time curve
AUIC	area under the inhibitory titre
BCG	bacillus Calmette-Guérin
C_0	concentration at zero time post dose
C_{max}	concentration maximum
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CXR	chest X-ray or chest radiograph
d	day
D	dose
DNA	desoxyribonucleic acids
DOTS	directly observed therapy, short-course
E	ethambutol (in tables)
EBA	early bactericidal activity
EDTA	ethylenediaminotetraacetate
ELISA	enzyme-linked immunosorbent assay
EMB	ethambutol
ESR	erythrocyte sedimentation rate
ETH (Eth)	ethionamide (in tables)
H	isoniazid (in tables)
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
I_3	Inactivation index (3-hour)
INH	isoniazid
IUATLD	International Union Against Tuberculosis and Lung Disease
k	first order elimination rate constant
LJ	Löwenstein-Jensen
M. bovis	Mycobacterium bovis
MDR	multidrug-resistant
MIC	minimal inhibitory concentration
MRC	Medical Research Council
M. tuberculosis	Mycobacterium tuberculosis
NCHS	National Center for Health Statistics
NTP	National Tuberculosis Programme
O	ofloxacin (in tables)
PAE	post-antibiotic effect
PAS	para-aminosalisylic acid
PCR	polymerase chain reaction
PZA	pyrazinamide
R	rifampicin (in tables)
RFLP	restriction fragment length polymorphism
RMP	rifampicin
S	streptomycin (in tables)

SA	South Africa
SCC	short-course chemotherapy
SD	standard deviation
SM	streptomycin
t	time
T	thiacetazone (in tables)
TB	tuberculosis
TBM	tuberculous meningitis
USA	United States of America
Vd	volume of distribution
WHO	World Health Organization
χ^2	Chi-square

CHAPTER 1

LITERATURE REVIEW

The history of drug-resistance in tuberculosis

Literature Review: The history of drug-resistance in tuberculosis

1.1   The discovery of streptomycin

Soon after the discovery of streptomycin (SM) in 1944[1] and the introduction of this anti-tuberculosis agent in the treatment of tuberculosis (TB), the problem of drug resistance became evident.[2] Although not the first agent to be used in the treatment of TB in man, SM was the one with the most promise, showing the best clinical response. In 1946 Buggs [3] and colleagues showed that other bacteria rapidly acquire resistance to SM. In the case of *Mycobacterium tuberculosis (M. tuberculosis)* resistance emerged soon after in vivo and in vitro exposure to SM.[2]

A small number of tubercle bacilli with relative resistance to SM were thereafter demonstrated to be present in the sputa of patients who had tuberculosis but had had no previous chemotherapy, and had probably not been in contact with other patients who previously had chemotherapy with SM. Levels of resistance were low (no growth on a medium containing $\geq$ 25 µg/ml SM) and relative resistant organisms occurred infrequently i.e one in several thousand organisms. The conclusion of this early study was that "any large number of tubercle bacilli may be expected to contain organisms which are relatively resistant to SM without having been exposed to the drug".[4]

The presence of naturally resistant variants in stock cultures of the control *M. tuberculosis* strain H37RV was also demonstrated.[5, 6]

Resistance gradually increased (number of resistant organisms and degree of resistance) in 6 of 8 patients within 1 to 4 weeks of starting SM chemotherapy but only became predominantly resistant at 6 to 13 weeks.[4] It was speculated that SM did not

cause resistance, but was responsible for selective propagation of the relatively resistant organisms. Drug resistance to SM had been shown to persist after subculture and after passage through animals.[7, 8] This led the investigators to conclude already in 1947 that SM resistance was an inherited genetic change and the importance of understanding the mechanism of resistance was evident.[4]

The initial reports on results of trials with SM in pulmonary TB were "encouraging but inconclusive".[9] The first case control study of SM in the treatment of pulmonary TB was undertaken by the British Medical Research Council (MRC) from 1946 and the first report was published at the end of the initial 6 months.[9] Treatment with SM was shown to be of some benefit in about half the patients but no clinical cures were effected and only 15% became bacteriologically negative. The observation that therapeutic results were related to the degree of drug resistance that developed was probably a more important finding. In 35 of 41 patients in whom data was available, resistance was >10 times (up to 2000 times in 13 cases) that of the original strain of the patients or control (H37RV) strain. Some essential points were raised in the discussion that would have an effect on future antituberculosis management: 1.) Emergence of SM-resistant strains may be prevented by adding another drug to SM treatment or by a special rhythm of treatment; 2.) SM resistance develops within 2 to 3 months of SM treatment in a patient with open (cavitary) TB and further treatment or a repeat course later is unlikely to be effective (The time it took to determine resistance was too long to establish whether to continue SM or not); 3.) The danger to public health of dissemination of SM-resistant strains was realised.[9] The far greater frequency of emergence of resistance during treatment of cavitary TB compared with noncavitary TB was confirmed as early as 1949.[10, 11]

The first reports of transmission of SM-resistant *M. tuberculosis* in man came in 1949. Bogen was quoted with regards to the case of a nurse working with SM treated patients who contracted TB and the *M. tuberculosis* strain recovered from her sputum was resistant to 1000 µg/ml on original isolation.[12] A further two individual cases of "primary" (i.e. no previous treatment with antituberculosis drugs) SM-resistant pulmonary TB in healthcare workers were described during the same year.[13, 14] Both patients had direct contact with source cases who had pulmonary TB and had received SM treatment. None of the cases themselves had received previous chemotherapy, although in one patient a calcified nodule was present prior to the confirmation of TB. High level SM resistance was present before initiation of any treatment. These ominous cases confirmed the potential public health problem of drug-resistant TB.

In a study of the origin of resistance to penicllin and streptomycin amongst bacteria other than *M. tuberculosis*, Demerec[15] offered evidence that bacterial resistance originates from spontaneous gene mutations and that the way to prevent the development of resistance was to use two antibiotics (when such were available) that affect the same pathogen, but are independent in their actions. Although this study did not include *M. tuberculosis* organisms, it supported what previous investigators suspected.[4, 9]

By 1948 it was generally agreed that resistance to SM arises by a process of spontaneous mutation.[6] Why was it that only some patients developed SM-resistant TB while on SM-treatment? In a quantitative study with different *M. tuberculosis* strains (H37RV, and strains from two patients with pulmonary TB, one of whom developed SM-resistance on treatment) Yegian and Vanderlinde [6] noted that an increase in the population of one parent strain was accompanied by an increase in the number of variants resistant to a specific concentration of the drug but that populations in different cultures

showed a great variation in the number of resistant variants present. This was explained by chance selection. The clinical significance of their findings was that: 1) sample size can determine whether resistant forms will be observed, 2) conclusions on drug resistance from a single sample (e.g. a single sputum sample) should be interpreted with care, because all variations in resistance of the bacterial population in the diseased areas of the patient are probably never represented. This could also be influenced by uneven penetration or concentration of SM (or other drugs) to different areas which could lead to differences in selective propagation of resistant organisms. One of these highly resistant variants was found in every 10^8-10^9 bacilli in the original inoculum but proportions varied considerably with different strains and in different experiments with the same strain.[6]

1.2   Para-aminosalisylic acid (PAS)

The problem of drug resistance received much attention in early studies and some possible solutions were suggested, one of which was the use of more than one drug in the treatment of TB. In the same year that SM was introduced as treatment for TB, a second promising antituberculosis drug was being experimented with in animal models and man.[16, 17] Para-aminosalicylic acid (PAS) was discovered by Lehman in 1943 (after work done on sodium benzoate and sodium salicylate by Bernheim [18]) and showed in vitro activity against tubercle bacilli. The first patients were treated in 1946 with a favourable effect on clinical TB, one month before SM was administered for the first time.[16]

A randomised placebo controlled trial in Swedish sanatoriums showed considerable improvement in patients' condition treated with PAS compared to a placebo control group.[17] No susceptibility tests were reported in this study. In *in vitro* studies SM

resistance had no effect on the bacteriostatic effect of PAS.[19] Several studies showed an added effect of PAS and SM when given together, as long as the strain was not resistant to SM.[19-21] Combining SM with PAS in *in vitro* studies demonstrated that this combination prevented the development of SM resistance. The addition of PAS to SM treatment as a relatively non-toxic drug would therefore be advantageous to the use of SM.[22] The true value of combining PAS and SM in the treatment of pulmonary TB was confirmed in a controlled trial which showed that the association of these 2 drugs substantially reduced the risk of developing drug resistance.[23, 24] Due to the gastro-intestinal side effects of PAS, already observed in animal model,[16] a follow-up controlled trial was performed to determine whether a smaller dose of PAS would be as effective in preventing the development of SM resistance. Although the clinical results of treatment were similar in all 3 groups (5, 10, and 20g PAS daily by mouth with 1g SM intramuscularly daily), the SM susceptibility tests showed a clear difference with resistant strains remaining low only in the group on high-dose PAS.[25]

PAS resistance seemed to be much less common and took much longer to develop. Early reports determined the shortest period of single drug PAS treatment resulting in PAS resistance to be 157-184 days (22-26 weeks).[21, 26] This lack of development of PAS resistance was also observed in *in vitro* studies.[22]

1.3   Early surveillance of drug resistance

By 1951 fifteen published cases of primary (transmitted) SM-resistant TB were collected, six of whom had TB meningitis.[27, 28] The first cases of primary PAS-resistant TB were described by Thomas et al.[29] In the same report they documented the first case of primary multiple resistant TB (SM & PAS), although the source case was not

identified. The risk of treating with a single antituberculosis drug was again emphasized, since this often leads to the development of drug resistance and may be responsible for contacts being primarily infected with such strains. In their study 3 or 4 patients were most likely superinfected with a drug-resistant *M. tuberculosis* strain. This possibility was confirmed by DNA fingerprinting many years later.[30]

In 1952 isoniazid (INH) was introduced for the treatment of TB. In the series of Thomas et al.[29] two patients developed resistance to INH while on treatment with a combination of two drugs (including INH), for one of which the infecting strain was already resistant. Giving two drugs therefore did not prevent the development of INH resistance if the strain was already resistant to the second drug. Adding one drug to a failing regimen is now well recognised as a dangerous practice.

The first thorough survey of SM resistance was done during a seven-month period in 1952 in the USA.[31] Results of SM susceptibility tests of 5526 consecutive admissions to 30 hospitals were analysed. Primary SM resistance occurred in 30 of 1166 (2.6%) patients, while acquired resistance was present in 20.4% of 1360 patients with known susceptibility results of their *M. tuberculosis* strains.[31] In an editorial at the time it was concluded that, except for contact within a TB hospital (12 of the 30 patients with primary drug-resistant TB most likely contracted it in hospital), primary drug resistance did not seem to be a serious public-health hazard.[32] A survey on primary drug resistance between July 1953 and January 1955 among patients with pulmonary TB in New York yielded a prevalence of 1.6% and 2.3% for SM and INH resistance respectively, a result which gave further "reassurance" that primary drug resistance was not a major problem.[33] Beck,[34] who studied primary drug resistance in one TB hospital in New York from 1948, found an incidence of 2%. Of the 10 patients with drug resistance all had SM-resistant

tubercle bacilli and 2 of them were multiply resistant (SM & PAS).[34] In this report he also described for the first time the transmission of drug-resistant tuberculous infection at a post-mortem examination. Furthermore he concluded that the incidence of infection with drug-resistant tubercle bacilli would become more infrequent as a result of multiple-drug treatment, surgical procedures and the availability of more antituberculosis drugs.

Fox et al.[35] were much more cautious in their verdict on the public health hazard after a national survey of primary drug resistance in the United Kingdom during 1955-1956. The prevalence of primary drug resistance to one or more of the commonly used drugs (SM, PAS, and INH) was 5,1% (95% confidence interval: 4.1-6.5%) with SM resistance at 2.3%, PAS at 2.2%, and the recently introduced INH already at 0.7%.[35] Contrary to the previous investigators they found the prevalence of drug resistance high enough "to make it a problem of some importance". They suggested that patients with cavitary pulmonary TB should begin treatment with all three the common antituberculosis agents simultaneously until susceptibility results were available.[35] The adoption of this initial triple drug regimen already took place between 1956 –1959 in the United Kingdom.[36]

In an International Union against Tuberculosis survey during 1957, drug resistance was confirmed to be a worldwide problem.[37] Acquired drug resistance in hospital admissions in several countries was 42% and primary drug resistance was 6.5%. Crofton [37] called for not only the correct drug combinations to be used but specifically for enthusiastic cooperation between all branches of TB services (public health services) to be able to defeat TB.

By the end of the initial era of SM and PAS, it was clear that treating patients with either of these drugs or a combination of both agents showed results much better than those in groups treated by bed rest only.[38] Although high dose PAS (20g/day) in combination with SM decreased the development of SM resistance dramatically, even lower doses of PAS (5 & 10g/day) had considerable effect on the reduction of SM resistance.[38]

1.4 Thiacetazone

A third antituberculosis drug was developed in Germany in 1946 by Domagk et al.[39] Thiacetazone or Conteben (4-acetylaminobenzaldehyde thiosemicarbazone) was selected as the best drug for human therapeutics from a group of compounds called thiosemicarbazones. These compounds were developed from studies with sulfonamides and sulfones (the first antibiotic agents) as well as the sulfathiazole and sulfathiodiazole series.[39-41] The drug was employed to treat patients with TB in Germany from 1947 but its evaluation was hampered by many post-war changes and problems such as increased weight of returning soldiers because of improved diets, and the unavailability of adequate chest radiograph facilities.[42, 43] Thiacetazone showed good clinical promise from observations in more than 7000 patients treated with this drug but it fell into disuse because of its relative toxicity at the high dosages given at the time and the discovery of the antituberculous activity of INH in 1951.[43]

It was only when a cheap effective drug was sought as a companion drug to INH to prevent the emergence of resistance for the use in low income countries when thiacetazone was brought back into treatment regimens in the early 1960s.[44] After a number of pilot studies to determine the optimal dose of both drugs, a controlled clinical

trial using INH 200mg (100mg twice daily) plus thiacetazone 150mg as a single daily oral dose proved to be as effective as the standard combination of INH and PAS in producing sputum conversion and in preventing the emergence of INH-resistant strains.[45] (In follow-up studies the dose of INH was increased to 300mg per day.[46, 47]) An initial supplement of 8 weeks of SM improved these results, with significantly less INH resistance developing in the SM-group and a considerably higher rate of bacteriologically negative sputa at 6 and 12 months in the latter group.[46, 47]

1.5 Isoniazid (INH)

In 1952 Bernstein et al.[48] described the antituberculous activity of isoniazid (INH), or isonicotinic acid hydrazide (Nydrazid) as it was originally named, for the first time. INH and its derivatives had a great specificity for mycobacteria.[49] The drug was a highly active antituberculosis agent in mice at low oral dose and had a wide therapeutic index.[48] There was complete and rapid absorption after oral administration of INH to mice and dogs, and it was well tolerated.[50]

Investigations in man of the antituberculous activity of INH started in November 1951.[51] Pharmacokinetic studies confirmed rapid absorption and low potential for toxicity after oral administration of 3mg/kg/day.[51] High concentrations of INH were attained in cerebrospinal fluid. This first report was, however, too soon to make statements on the effectiveness of INH in tuberculosis treatment in humans or the development of drug resistance. The addition of another new antituberculosis agent was viewed with some reserve by the Executive Committee of the American Trudeau Society.[52]

The first report of INH resistance developing during treatment of patients came in April 1952.[53, 54] Steenken et al.[53], who also described in vitro INH resistance in some variants of drug susceptible cultures of tubercle bacilli together with others,[55-57] demonstrated resistance in 4 of 6 patients treated with hydrazines of isonicotinic acid (including INH). Their conclusion was that patients should receive treatment with INH in such a way as to not encourage resistant organisms to appear, i.e. combination therapy.[53]

An additive (synergistic) effect of SM and INH was documented in vitro.[49, 58] It was further shown that tubercle bacilli resistant to SM were still susceptible to INH and vice versa.[49] Middlebrook[55] isolated INH resistant variants (mutants) of *M. tuberculosis* with 4 different in vitro methods from INH-susceptible cultures. Untreated cultures of tubercle bacilli contained a higher proportion of INH-resistant mutants than of SM-resistant mutants.[55, 57, 59] Middlebrook[55] could not, however, get any organisms to grow from these same cultures in mediums containing both SM and INH at low concentrations. He suggested that combination therapy would probably be essential for the public health control of the disease.[55]

1.6   Combination therapy *vs.* single drug treatment

After a series of randomised controlled studies comparing different options of TB treatment, the British MRC compared INH treatment with SM and PAS, the best antituberculosis treatment at the time.[59] It was now established that INH resistance does develop in patients, the rate at which this occurred was, however, still unclear.[60] Clinical and radiological improvement was similar in both groups after 3 months of treatment although weight gain was better with INH alone. Resistance to INH developed early in the INH alone treatment group. Within a month, two months and three months after

initiating the latter treatment, 10%, 50% and 70%, respectively, of cultures obtained were resistant to INH.[59] The second report of the British MRC [61] on INH treatment confirmed this development of resistance to INH but also clearly showed that a combination of SM and INH, if the initial culture was susceptible to both, prevented the development of drug resistance to either of these drugs in the majority of cases. A combination of INH and SM was also shown to be the best treatment for TB.[61] Some important lessons on the development of drug resistance were now evident: 1.) No antituberculosis drug should be used alone in the treatment of pulmonary TB; 2.) Treatment regimens should contain 2 drugs to which the organism is susceptible, therefore drug susceptibility tests should be done before therapy is started. If not, resistance to one drug in an unsuitable combination may lead to resistance developing to the second drug, and; 3.) Taking a history of previous use of antituberculosis drugs before initiating treatment could be of great value in considering which drugs to use in a combination, since culture/susceptibility results are not always immediately available. A history of previous treatment with any (single) drug or contact with a person who received previous TB treatment and the results of this source case's susceptibility tests may be a guide in making the correct choice of treatment.[61]

The fourth report of the British MRC [62] on the treatment of pulmonary TB with INH, SM and PAS addressed the specific issue of emergence of drug resistance up to six months of treatment. In the group treated with INH alone, 58% of the total number of patients were INH-resistant at the end of the six month period (that is 100% of all cultures obtained at six months). Combinations of SM plus INH and SM plus PAS, however, restricted the development of resistance to SM and/or INH to between 0 to 5% of the total number of patients in each group confirming the value of these combinations

in preventing drug resistance.[62] In vitro evidence suggested that strains resistant to INH may revert to susceptibility when contact with INH was stopped.[49, 63] Although its occurrence was considered in some patients after INH treatment was stopped,[64] the British MRC study did not show a general reversion to susceptibility over a 3 month period after discontinuing INH which suggested that resistance to INH is not usually transient.[62]

Despite a recommendation by members of the Twelfth Chemotherapy Conference held in 1953 at Atlanta, Georgia, that studies with INH alone should be abandoned because of emergence of resistance with single drug therapy, Phillips [65] compared treatment with INH or INH plus PAS in groups of patients with noncavitary pulmonary TB. He found both groups to do equally well with little INH-resistance developing. This was probably because of the low number of bacilli in these lesions and therefore the risk of drug-resistant mutants being present was small (see section 1.12). The same results were obtained with INH alone by Lauckner [66] in primary TB in Nigeria.

Due to cost constraints in developing countries, INH alone was again compared to combination treatment in patients with extensive pulmonary TB in these countries and was shown to be definitely inferior to combination therapy even despite high dose INH.[66, 67] After 8 years of trials it was finally recommended that INH should not be used alone in these patients and that combination therapy should be preferred even in low-income countries.[67] This was where thiacetazone, one of the first antituberculosis agents, re-entered the scene. (see section 1.4)

1.7 Pyrazinamide (PZA)

At about the same time when INH was introduced as an effective antituberculosis agent, McKenzie and her co-workers reported the effect of nicotinamide on experimental TB of mice.[68] Kushner and associates then developed pyrazinamide (Aldinamide) and evaluated its effect on experimental TB in mice over the next three years.[69] The antituberculous activity of pyrazinamide (PZA) in mice and guinea pigs was demonstrated to lie between that of SM and PAS, being superior to the latter.[69,70] PZA had no effect on *M. bovis*.[69]

Treatment with PZA was started in the first patients in 1949.[71] It appeared that PZA had a moderate degree of antituberculous activity with initial clinical improvement in most patients. Resistance emerged rapidly, in some cases within 15 days of initiating treatment. It was again noted that resistant tubercle bacilli appeared more rapidly in those patients who had large pulmonary cavities and expectorated large quantities of sputum with high bacillary contents.[71] The method for determining resistance to PZA was, however, complex and development of other methods was desirable.[72] The determination of resistance to PZA has remained a serious technical problem.

Combinations of PZA and INH were more effective than either drug used alone.[73-75] More important was that this combination was the only regimen capable of sterilizing the organs of mice after a 12-week treatment period, an observation at first ignored by the TB community but which later contributed to the development of short course chemotherapy.[76-78]

1.8   Other antituberculosis drugs developed in the 1950s

Several other antituberculosis drugs were developed during the period 1950 to 1960. Many were derived from different *Streptomyces* species, e.g. viomycin (1950; *Streptomyces puniceus*),[79] cycloserine (1955; *Streptomyces orchidaceus*),[80] kanamycin (1958; *Streptomyces kanamyceticus*),[81] and capreomycin (1960; *Streptomyces capreolus*).[82] They were all less effective, had to be used in combination with existing antituberculosis drugs, and caused more pronounced toxic effects.[83]

Ethionamide (alpha-ethyl-thioisonicotinic acid), another second line antituberculous drug, was derived from isonicotinic acid in 1958. It exerted a bactericidal effect on growing cells and was reported to be more effective than SM but less effective than INH.[84] As with all previous antituberculosis drugs, clinical trials indicated that it should not be used alone. Toxic effects, which were more common in adults than in children and restricted its use, were gastric intolerance and reversible liver damage.[83]

1.9   Ethambutol (EMB)

In 1961 a new synthetic compound, ethambutol (EMB), was described. It was chemically unrelated to any of the known antituberculosis drugs and shown to be active *in vivo* and *in vitro* against mycobacteria.[85, 86] It acts only on actively proliferating cells, and is specifically antimycobacterial.[85-87] Development of resistance in vitro was slower and smaller in magnitude than with SM or INH, and EMB was fully active against SM- and INH-resistant mycobacteria.[85, 86] Studies in monkeys with experimental TB showed that although EMB had substantial antituberculous activity, it was inferior to INH but that the therapeutic outcome of a low dose combination of the two drugs was strikingly better than either compound given alone.[88] Studies in humans began in 1961.[89] It was

initially used with good effect as a single oral drug in retreatment cases, but resistance developed in about 35% of cases.[90] The only apparent side effect was visual disturbance. This was dose related.[89, 91] When EMB was used in combination with other antituberculosis drugs to which the infecting strain was susceptible, clinical responses were good with bacteriological relapses and bacterial resistance being mostly prevented.[90, 92, 93] Specifically in combination with INH with or without SM, EMB has shown to be superior to PAS, mainly because of less side-effects and better acceptance.[90, 94, 95]

1.10 Rifampicin (RMP)

Rifampicin (RMP) was the last of the current "first line" antituberculosis drugs to be discovered. It remains the most important component of short course chemotherapeutic regimens. It was developed by Maggi et al.[96] in 1965 as a semisynthetic derivative of the rifamycins, a group of antibiotics isolated from *Streptomyces mediterranei* in 1957.[97] In the first clinical studies reported in 1967 and 1968, 120 adults who had chronic cavitary pulmonary TB and were excreting bacilli resistant to primary and most secondary drugs or were intolerant to these, were treated with RMP, either alone or in conjunction with other drugs.[98] In the pooled results summarized in the review, 72 patients showed bacteriologic evidence of sputum conversion after 2 to 6 months, but in 11 cases RMP resistance appeared after 6 weeks to 4 months.[98] Virchow and Flemming[99] noted that when RMP was given alone, it soon lost its efficacy and selectively induced resistant mutants. Although not mentioned as such in an extensive review on rifampicin in Drugs,[98] some of the many chronic pulmonary TB patients with drug resistance who subsequently received RMP alone, must have

developed multidrug-resistant (MDR) TB (i.e. resistance to INH and RMP with or without resistance to other drugs). In a randomized controlled trial of new TB cases, treatment with RMP in combination with INH or EMB compared with RMP alone was very effective.[100] RMP resistance emerged in only one of 30 patients in the RMP-alone group.

After all the lessons learned from previous experience, and a generally accepted rule that at least two drugs to which the infecting strain is susceptible should be used in the treatment of TB, RMP was again often initially used alone or as a single drug added to an already failing regimen, with the result that resistance often emerged. Canetti et al.[101] confirmed that, although the rate of resistant mutants against RMP was low in most wild strains (in the order of 10^7 or less), this did not justify treatment of cavitary TB with RMP alone. However, when RMP was used in combination with EMB as a second new drug in a failing regimen, the results were promising.[102]

It soon became clear that the efficacy of RMP was comparable to that of INH.[101] Even more important was its sterilizing effect when given in conjunction with INH.[103-105] Sterilisation in this context referred to not being able to culture any bacilli from infected mice after treatment, a result that had not been obtained in mice after 4 months of treatment with any other combination of antituberculosis drugs.[104] This was of fundamental importance in the subsequent development of six-month short-course chemotherapy for patients with primary TB.[106]

1.11 A call for revival of active interest in drug-resistant tuberculosis

About 20 years after the discovery of the first antituberculosis drugs, Canetti called for a revival of active interest in drug resistance in TB.[107] The main biological facts of

TB drug resistance were all discovered in the first 10 years of chemotherapy and in the following 10 years a decline in the general interest in research in this area was apparent. The main reason for this lack of interest was that optimal chemotherapy reduced the emergence of resistance so drastically that it was thought to be of only historical importance. Canetti [107] warned that although the problem might be small for advanced countries, although primary drug resistance was relatively high in France at the time,[108] developing countries did not have the resources to apply the new methods of preventing drug resistance and would therefore still produce a considerable amount of drug resistance.

1.12 Bacillary populations and drug resistance

Resistance is a phenomenon linked to large bacillary populations. In human TB the greatest populations, in the order of 10^7 to 10^9 bacilli, are found in cavities, whereas those found in hard caseous foci, such as found in extrapulmonary TB (and primary TB in children), usually do not exceed 10^2 to 10^4 bacilli.[107] Wild strains of *M. tuberculosis* do contain a few resistant mutants for almost all drugs among 10^6 bacilli.[107] Hugo David [109] calculated that the highest proportion of mutants that can be expected in unselected populations of the tubercle bacilli was observed to be as follows:

3.5×10^{-6} and 3.1×10^{-6} to 0.2 and $1.0\mu g$ of INH/ml, respectively;

3.8×10^{-6} to $2.0\mu g$ of SM/ml;

3.1×10^{-8} to $1.0\mu g$ of RMP/ml;

0.5×10^{-4} to $5\mu g$ of EMB/ml.

The mutation rates to resistance to the drugs were determined to be:

2.56×10^{-8} mutations per bacterium per generation for INH;

2.95×10^{-8} mutations per bacterium per generation for SM;

2.25×10^{-10} mutations per bacterium per generation for RMP;

1.0×10^{-7} mutations per bacterium per generation for EMB. [109]

These naturally occurring drug resistance mutation rates were later established to be as follows:

1 in 10^8 for RMP;

1 in 10^6 for INH, EMB, SM and PAS;

1 in 10^3 for ethionamide, capreomycin, cycloserine and thiacetazone.[110]

Both the number of organisms present and the possibility of resistant mutants being present have important implications for treatment of patients. In latent TB infection, with low numbers of bacilli, there is an almost negligible chance of eliciting resistance by using INH as a single chemoprophylaxis agent.[107] An even more important question was whether an initial phase of treatment with multiple antituberculosis drugs and a continuation phase with fewer drugs for a longer period would be sufficient treatment and still prevent emergence of resistance.[107, 111] The rationale behind this was that the bulk of the bacilli are killed with the initial treatment, and the low number of remaining bacilli held a low risk for the development of resistance during the continuation phase. This had been experimentally confirmed in mouse studies [112, 113] and in humans in the first International Union against TB trial.[114] This principle is now generally accepted.

1.13 Cross-resistance

Cross-resistance is rare among the unrelated antituberculosis drugs. Some cross-resistance occurs between related agents. Cross-resistance is a rare cause of the emergence of resistance.[115] The therapeutic consequence of the latter observation and the number of organisms present in cavitary lesions, makes the presence of mutants which are double-resistant to any two drugs unlikely in any previously untreated patient.[107] A doubly resistant mutant should only occur once if the total bacillary population were about 10^{12}, an occurrence rate higher than expected, even in extensive pulmonary TB.[115] This reasoning confirms the well known fundamental prerequisite for antituberculosis treatment of at least two drugs to which the strain is susceptible.

Knowledge of cross-resistance such as that between ethionamide and thiacetazone and some of the aminoglycosides is important for selecting acceptable drug-regimens for specifically multidrug-resistant tuberculosis (MDR TB).[116]

1.14 Virulence of drug-resistant *M. tuberculosis* strains

The virulence of INH-resistant strains of *M. tuberculosis* has been a subject for debate since the early 1950s. Many researchers clearly demonstrated that INH-resistant tubercle bacilli lost no virulence for mice or guinea pigs.[60, 117, 118] Subsequently, however, reports from reputable investigative laboratories claimed a decrease or loss of virulence of these strains in experimental models.[119-123] Although a loss of virulence in catalase-negative strains is expected to have its counterpart in human TB, it is also known that this must be limited, as cases of primary drug resistant TB with highly INH-resistant catalase-

negative strains do exist.[107] This loss of virulence was not found in *M. tuberculosis* strains resistant to SM.[7, 8]

Canetti [107] and Tripathy et al.[124] showed that INH-resistant strains obtained from patients with primary INH-resistant TB had considerable lower levels of INH resistance than those obtained from patients with acquired INH-resistant TB. Two possible explanations were given: 1.) the resistance may have dropped in the newly infected host through prolonged multiplication of the resistant strain in the absence of INH, or 2.) higher experimental virulence of the low INH-resistant, catalase-positive strains may have caused the infection rather than the highly INH-resistant, catalase-negative strains with lower virulence.[107, 125] Both of these mechanisms may be relevant. This observation of low-level INH resistance in primary INH-resistant TB may have the implication that INH could still be of value in the treatment of these patients, including all children with INH-resistant TB.[124, 126-128]

1.15 A change in the focus of TB treatment from drugs to management

Once effective antituberculosis drugs were available, and drug resistance could be prevented, the focus of the management of TB started to change.

The number of TB cases sent for treatment increased to such an extent that sanatoria could no longer cope and such institutions were in any event unaffordable and impracticable in developing countries.[66] Available oral antituberculosis drugs made outpatient treatment possible. This option was usually not considered, since it was believed that patients needed to rest, should be free of emotional stress, receive a nutritious diet, and not be allowed to infect their household contacts. In a randomized controlled trial in Madras, India, home treatment and treatment in a sanatorium was

compared, both groups receiving INH plus PAS for one year.[129] Efficacy of chemotherapy was not increased by giving treatment in a sanatorium nor was the risk of infecting household contacts decreased.[129, 130] For effective ambulatory treatment and to suppress the emergence of drug resistance, good patient compliance with drug-taking was essential. This could be improved by fully supervised drug-taking,[131] more recently renamed directly observed treatment (DOT).[132]

Not all drugs are equally good in suppressing the emergence of resistance to another drug. A highly potent drug is able to prevent the growth of, and usually kills, all the organisms in the lesion, and continues to do so despite slight irregularities in drug-taking by the patient. Examples of potent drugs are INH and RMP, and less potent drugs are SM, PZA and EMB. In clinical studies thiacetazone and PAS were much less effective.[115] In the 1970s it became normal practice to start treatment with three drugs because even the most effective pair (INH plus RMP) does not always prevent the emergence of resistance.[115] Furthermore initial INH resistance may lead in effect to monotherapy.

Developments to decrease the frequency and duration of drug-taking followed to make supervised therapy more practicable. These were; 1.) intermittent therapy and; 2.) short-course chemotherapy (SCC). Intermittent (2 or 3 times weekly) treatment was first tried in 1955 as part of a British MRC controlled trial.[133] SM was administered twice weekly together with daily INH but in comparison with daily treatment of both drugs permitted an increase in emergence of drug resistance probably because dosages were not adjusted.[134] A randomized controlled trial in India comparing SM plus INH twice weekly to PAS plus INH showed both regimens to be equally effective as treatment and in preventing drug resistance.[135] Growth of tubercle bacilli was inhibited for several days

after varying periods of exposure to different antituberculosis drugs such as INH, SM and RMP with in vitro studies.[134, 136] These drugs are therefore suitable for use in intermittent therapy regimens and several randomized controlled studies have confirmed this.[135, 137]

New hope came for shorter treatment regimens when RMP was discovered. In 1970 the sterilizing effect of both PZA and RMP in combination with INH in animal experiments eventually led to the first controlled clinical trials of 6-month short course regimens of chemotherapy.[138-141] A comparison was made between four 6-month daily regimens, all containing SM plus INH, with a third drug added in 3 of the regimens – RMP, PZA, or thiacetazone (T) – and a standard 18-month regimen (3SM,INH,T/15INH,T) in the treatment of newly diagnosed smear-positive extensive pulmonary TB. The SCC regimens containing RMP or PZA were highly effective and comparable to the standard 18-month regimen. The few patients that did relapse, nearly all still had drug susceptible organisms.[139-140] SCC regimens of 6 to 9 months are now used worldwide for nearly all new TB cases, at least in developed countries. Intermittent SCC has also been introduced reducing the number of observed doses to be administered to as little as 62 without increasing the risk of drug resistance.[137]

1.16 Resurgence of TB and renewed interest in drug resistance, mainly MDR tuberculosis

Although therapeutic TB studies continued after the early 1970s they were on a much smaller scale than before, as it was believed that the available drugs and effective combinations of these drugs had overcome this disease. Despite the warnings from experts such as Canetti [107] and Horne [142] that research and surveillance of drug resistant TB should continue, this was to a large extent ignored. TB case numbers were steadily

decreasing mainly in developed countries, where effective regimens could be implemented, and the United States was even contemplating the elimination of the disease.[143] In many developing countries, chemotherapy had, however, failed to have a significant impact on TB morbidity during these years, mainly because the commonly used treatment regimen of 12-18 months often failed due to patient non-adherence.[144]

In 1989 Rieder et al.[143] focused the attention on the rising incidence of TB in the USA from 1985. This trend was also observed throughout the world and in 1993 the WHO reported that, more than 40 years after the introduction of chemotherapy for TB, there were more new cases (8 million per year) than ever.[144] The main reason for the increasing number of TB cases throughout the world was the growing epidemic of human immunodeficiency virus (HIV) infection, which is now known to be the most potent facilitator of TB infection and disease.[144, 145]

Outbreaks of MDR-TB, originally described in HIV-infected persons, were in large measure responsible for focusing the attention of the industrialized countries on TB for the first time in decades.[146] The global resurgence in TB, the association of HIV and TB, and MDR-TB was described as the three epidemics of TB.[147] Drug-resistant TB was always a global problem and to start with developing countries had higher rates of primary and acquired resistance than developed countries.[142] MDR-TB was, however, mainly a problem in countries making use of the more expensive RMP-containing SCC regimens. This was reflected in data from several global reviews on drug resistance surveillance.[145, 148, 149]

The outcome of MDR-TB patients has been notoriously bad. The mortality of HIV-infected individuals with MDR-TB during nosocomial outbreaks in the USA was 72-89%

within six months.[150] Even in HIV-negative patients with MDR-TB 48% died within 5 years and only 33% were cured, an outcome not dissimilar to TB patients before the development of antituberculosis treatment [151, 152] or patients not receiving these drugs.[153] Of particular concern is the prolonged duration of infectiousness, since the diagnosis of drug resistance is often delayed and up to 20% of patients' sputa remain smear positive for as long as 5 years or more. MDR-TB is transmissible and transmission of MDR-TB was responsible for outbreaks among institutionalized HIV-infected patients,[150] and has also been confirmed in HIV-negative patients.[154, 155]

The persistent problem of drug-resistant TB has been attributed to several factors of which the most important was the failure to adhere to effective drug regimens.[145, 148, 156] This failure is because of inappropriate regimens being prescribed, irregular drug supply, or poor compliance with treatment by the patients.[148, 157] A second important problem was the failure of governments to supply and maintain community based health care services in order to permit directly observed therapy.[145, 158-160] In developed countries the problem of drug resistance was exacerbated by an influx of immigrants from countries with a poor TB control programme.[145]

1.17 HIV and drug-resistant TB

Although HIV infection was associated with an increase in drug resistance in studies, mainly from the USA,[145, 161] this was not the case in several other studies, specifically from Africa,[162-165] where the incidence of HIV is extremely high. Mono-resistance to RMP is generally rare.[145, 166] Recently it was noted that mono-resistance to RMP was on the increase and restriction fragment length polymorphism (RFLP) fingerprinting found the strains to be of independent origin.[166] Most of these strains were

obtained from HIV-infected patients, and one of the possible explanations was selective malabsorption of RMP in some HIV-positive patients. Malabsorption of other antituberculosis drugs was also suggested by a number of studies,[167-169] but reports to the contrary have been published.[170] This has important implications for the management of HIV-infected patients with TB as well as for TB control programmes, since malabsorption of one or more drugs could in effect lead to monotherapy and therefore to the emergence of drug resistance. This aspect regarding TB treatment in HIV-infected patients needs further clarification.[171, 172]

1.18 Treatment strategies in the global era

The global resurgence of TB, its close association with HIV/AIDS and especially the epidemic of MDR-TB has been largely responsible for an influx of resources into TB research and public health care. A further impetus was given when the WHO in 1993 declared TB a global emergency.[173] Directly observed treatment, short-course (DOTS), was strongly advocated in the management of TB.[132, 174] Where DOTS was practiced it resulted in a dramatic decline in the prevalence of drug-resistant TB.[175-178] Good National TB Programmes that included the implementation of DOTS did not, however, succeed in lowering TB incidence rates in countries with a very high prevalence of HIV infection.[177, 178]

Regimens with an initial intensive phase of four drugs were introduced in the treatment of new TB cases, because of high rates of primary drug resistance.[179] Previous studies found that four-drug SCC regimens containing INH, RMP, PZA, and SM or EMB, whether given 5 times weekly or three times weekly, achieved excellent results even if patients were initially resistant to INH and/or SM.[180] In 1993 the Advisory

Council for the Elimination of Tuberculosis in the United States recommended that a 4-drug regimen (INH, RMP, PZA, and SM or EMB) should be introduced in any area if the prevalence of INH resistance is ≥4%, and that only PZA should be discontinued at 8 weeks, thus continuing with three drugs for a total of 6 months.[179] The WHO guidelines adopt a similar approach except that the continuation phase of four months includes only INH and RMP.[181]

1.19 Treatment of MDR TB cases and their contacts

New antituberculosis drugs have not been readily forthcoming since the development of RMP in 1965. Amikacin, an aminoglycoside with a structure closely related to kanamycin, was added in 1982, and another aminoglycoside, paromomycin, has been shown to have antituberculous action. The fluoroquinolones, the only new bactericidal compounds, were introduced in 1983.[182-185] The development of further drugs is urgently needed to enlarge the armamentarium of drugs for the treatment of MDR TB and to further shorten the duration of treatment.

Principles for the management of MDR TB treatment have been formulated. Most important is the prevention of drug resistance by prescribing and supplying correct drug regimens and ensuring that directly observed treatment takes place in every new patient. Patients who are MDR should be managed in specialized units, since the reserve drugs are more expensive, less effective and have more side effects.[116] These units must have access to reliable susceptibility testing and reliable drug supplies. Principles that need to be observed in the treatment of these patients are: 1) employ at least 3 drugs with proven in vitro susceptibility, including one injectable drug (or 2 bactericidal drugs); 2) when possible, use drugs that are active in vitro and have not been employed previously, and 3)

try to put together a regimen with tolerable side effects.[116, 186] Treatment should continue until 12-18 months after the last positive culture.

In developed countries the individualized treatment regimens for MDR TB patients are used as described, but this is not always possible for developing countries. The WHO has recently launched the "DOTS Plus" strategy, making use of standardized regimens for patients with retreatment or treatment-failure TB, to address the MDR TB problem in these countries, since it became clear that all patients with active TB, including MDR patients, should be treated.[187, 188]

Until a decade ago INH was the only medication proved to be efficacious and recommended for the prevention of TB in latent TB infection.[154] Recently several combinations of INH, RMP and PZA have been compared to INH alone for 6 to 12 months for their efficacy to prevent TB in mainly tuberculin skin test positive HIV-infected adults. In a prospective randomized trial Gordin et al.[189] demonstrated a 2-month regimen of daily RMP plus PZA to be similar in efficacy and safety to a 12-month regimen of INH. Halsey et al.[190] compared a 2-month intermittent RMP plus PZA regimen to a 6-month course of INH and showed the same outcome. Other studies compared a 3-month regimen of RMP alone and 3 months of INH plus RMP to INH alone for 6 months in mainly HIV-infected adults and showed them to be as efficacious, although the numbers in these studies were too small to come to a definite conclusion.[190, 191] RMP plus PZA for two months or RMP alone for 4 months is now recommended as preventive treatment in latent TB infection in HIV-infected adults and in contacts of INH-resistant pulmonary TB cases.[192, 193]

Persons exposed to MDR TB pose a special problem. There has been no controlled trial to evaluate any treatment regimen for chemoprophylaxis of contacts of MDR TB cases.[154, 194] The American CDC has developed a set of guidelines for the management of such exposed persons.[194] The probability of infection with MDR *M. tuberculosis* among contacts thought to be newly infected has to be estimated and thereafter the likelihood that such an infected person will develop TB disease should be determined before starting any prophylactic regimen. If the risk of infection is intermediate to high and risk of disease is relatively low, two options are available: a.) administer no preventive therapy and provide careful clinical follow-up, or b.) consider multidrug preventive therapy with drugs other than INH and RMP. In the latter group as well as those with high risk of developing disease preventive therapy with at least two antituberculosis drugs to which the possible infecting strain is susceptible for a period of 6-12 months should be considered. A Delphi-technique survey to determine which drugs experts would use for preventive treatment for high-risk contacts of patients with MDR-TB resulted in support for PZA and ofloxacin or ciprofloxacin.[195]

1.20 New technology in the battle against drug-resistant TB

In the 1980s phage typing and drug susceptibility testing were used to trace the epidemiological route of transmission of drug-resistant bacilli to contacts.[196] More recently powerful molecular strategies have been developed that permit rapid and accurate bacterial strain characterization for use in molecular epidemiology.[197, 198] One such method is DNA fingerprinting. The presence of repetitive genetic insertional elements in *M. tuberculosis* permits the identification of individual strains by DNA fingerprinting with restriction-fragment-length polymorphism (RFLP) analysis.[199] The

technique was developed in the early 1990s and a standardized method for the IS6110 band was described by van Embden et al.[200] Additional probes were developed to enhance the accuracy of strain typing especially in cases of low IS6110 copy numbers.[201] This DNA fingerprinting technique has been used to demonstrate the transmission of particular *M. tuberculosis* strains in outbreaks of disease and, together with conventional epidemiologic investigations, has improved our knowledge of the epidemiology of tuberculosis in communities,[198, 199, 202] and our knowledge of the dynamics of the disease.

The genetic origin of drug resistance in tubercle bacilli was already suspected shortly after the emergence of drug resistance to the first antituberculosis drugs. Only recently was the molecular genetics of resistance determined for several antituberculosis drugs.[203, 204] Mutations in, or deletions of specific genes cause drug resistance, for example: mutations in the *katG* gene or *inhA* gene cause INH resistance, mutations in the *rpoB* gene confers resistance to RMP, and mutations affecting the *strA* and 16S ribosomal RNA have been identified in SM-resistant isolates of *M. tuberculosis*.[203] The identification of these genes and their mutations are valuable tools in molecular epidemiology which assist the rapid identification of drug resistance using a polymerase chain reaction-based (PCR-based) technique, single-stranded conformation polymorphism (SSCP) to enable the detection of single-base substitutions, as well as small deletions and/or insertions.[204] These techniques are, however, often unavailable in those countries that most need them in their fight against drug-resistant TB. It is therefore necessary that countries should work together to be able to turn the tide against TB. Developed countries will not be safe from the threat of tuberculosis and drug resistance until the disease has been controlled in developing countries.

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CHAPTER 2

LITERATURE REVIEW

AND AIMS OF STUDY

Drug-resistant tuberculosis in children

Literature Review: Drug-resistant tuberculosis in children.

2.1 Epidemiology of drug-resistant tuberculosis in children:

2.1.1 The first case report of drug-resistant TB in a child.

An 11-week-old infant who presented with streptomycin (SM)-resistant miliary TB in 1948 was probably the first childhood case of drug-resistant TB to be documented.[1] The mother was diagnosed with TB when the infant was aged 2 weeks and the two were immediately separated. Neither of them had received previous SM-treatment. Although this was most likely primary SM resistance, no isolate of *M. tuberculosis* was obtained from the mother, and few people in the community could have had SM-treatment at the time. Nevertheless, attention was drawn to the desirability of drug-susceptibility tests on all diagnostic cultures obtained from new TB patients on entry to hospital or at the start of treatment.

2.1.2 Primary INH resistance in childhood – the first reports.

Although Debré et al.[2] gave Murdoch and Grant [3] the credit for reporting the first case of primary isoniazid (INH)-resistant TB in a child, this was not correct. The sibling to whom the INH-SM-resistant tubercle bacilli were transmitted was already more than 20 years of age at the time. The next case report by Noufflard et al.,[4] described a French child admitted in 1954 with tuberculous cervical adenitis. Culture from aspirated material, however, revealed a strain of *M. bovis* resistant to INH. No human TB source case was identified.

It seems likely, therefore, that the first case of primary INH-resistant TB due to *M. tuberculosis* in a child was that reported by Klemm and Meissner from Germany.[5] They described a child 16 months of age with tuberculous pneumonia, infected by her father who had been treated with INH. Both father and child had tubercle bacilli resistant to INH at the same concentration (MIC 50 µg/ml), which were catalase negative and of attenuated pathogenicity for guinea pigs.

2.1.3 Early monitoring of childhood drug resistance.

Wilking et al.[6] reported the first comprehensive study on childhood drug-resistant TB from New York. SM-susceptibility tests were done on *M. tuberculosis* isolates from 152 of 193 children <13 years of age between 1946 and 1951.[6] Seventeen (11%) had one or more SM-resistant isolates of whom 5 of 115 (4%) had primary drug resistance. However, as acknowledged by the authors, this study could not report on incidence, because of inherent problems with the study methods. The study did show that primary and acquired drug-resistant TB could occur in children, both of which could have a fatal outcome.

In 1959 Debré et al.[2] realised that the incidence of primary drug resistance in children is an important reflection of the presence in the community of sources of infection with bacilli resistant to SM and INH. In their laboratory, Debré and co-workers had used the same method for drug susceptibility testing from 1950 onwards, and in the survey they included only children who had received less than a week's treatment thus identifying strains that actually infected the children.[2] This report was therefore probably the first to give some evidence of incidence of primary drug resistant TB in childhood. Their first case of SM-resistant TB that responded poorly to SM-treatment was a child

seen in 1949 with TBM. Primary SM resistance continued to increase until effective multiple-drug treatment was introduced in 1952. The first primary INH-resistant *M. tuberculosis* strains obtained from children in this study were identified in 1957, and 2 of 4 strains were also resistant to SM. Primary drug resistance was usually reported 3 to 5 years after acquired resistance to a drug became evident and primary drug resistance usually remained much less frequent than acquired resistance. The incidence of primary SM- resistant TB in children was 4.6% (18/394) and primary INH-resistance was 6-9% (4-6/65). Despite encouraging earlier observations from animal experiments that INH-resistant strains were less virulent than INH-susceptible strains, Debré et al.[2] found them to be as infectious as SM-resistant strains. There was also no difference in the clinical presentation of drug-resistant and drug-susceptible TB in children in this study.

2.1.4 Primary drug-resistant TB in childhood in the United States and other countries.

After 1959 the few reported surveys of drug-resistant TB among children almost all came from the United States of America (USA). Furthermore, these surveys were mainly hospital or clinic-based, since it is difficult to obtain proper specimens for culture of *M. tuberculosis* from children. This could lead to selection bias, as children with TB are often referred to hospital because of more severe disease, poor response to treatment or other risk factors for having a drug-resistant organism.

The first report of drug-resistant TB in children from the USA was published in 1961.[7] In this retrospective survey 7 of 33 clinics did regular drug-susceptibility testing on isolates obtained from children. Of 325 strains tested before any treatment, 11 (3.4%)

were resistant to SM, and of 235 strains tested after the introduction of INH, 7 (3%) were resistant to INH. Five of the latter patients were also resistant to SM and therefore had multiple-drug resistance [i.e. resistance to two or more drugs without resistance to rifampicin (RMP)].

Steiner and Cosio [8] made the point that studies on disease due to primary resistant organisms are of great importance and that primary drug resistance could best be studied in children before the institution of any drug therapy, as primary TB in children represents recently acquired infection. This would afford a more accurate assessment of the incidence of primary drug-resistant TB infection in a community. Many years later, Rieder,[9] an epidemiologist, confirmed that cross-sectional appraisal of prevalence of drug resistance in adults provides little information on the susceptibility patterns of currently circulating strains. TB in children, however, is a sentinel event for TB transmission in the community. Therefore the frequency of drug-resistant TB in children, and especially those under 5 years of age, reflects a precise evaluation of the currently circulating strains.[9] Unfortunately such studies are rare, because specimens from children for culturing *M. tuberculosis* are difficult to obtain and the cultures are frequently negative.

Steiner and colleagues started the first of a series of 4-yearly surveys of primary drug-resistant TB in children (1 month to 13 years of age) at the Kings County Hospital in Brooklyn, New York in 1961.[8] The SM and para-aminosalicylic acid (PAS) resistance (3% each) rates were stable compared to Zitrin's findings before 1961, and the rates were similar to that of strains in adults in New York City. The incidence of primary INH-resistant TB in children (6.3%) defined as resistance at a minimal inhibitory concentration of 1.0 µg/ml, however, was unexpectedly higher than that reported in adults (2.6%).[8] The explanation offered was that the children in this study were drawn

from a socio-economic deprived area with a high TB incidence while the adults were drawn from the greater New York area.

On completing six 4-year surveys of primary drug resistance in children at Kings County Hospital from 1961 through 1984, Steiner et al.[10] showed that SM resistance was on the decrease, INH resistance was stable at 9.9% and that since the introduction of RMP in the early seventies, the resistance to RMP had increased significantly. This followed the marked rise in RMP resistance found in adults in the same area. Only a single case of childhood MDR TB was, however, found during this period. (Steiner et al. 1986)

After 1984 primary INH resistance increased to 18.2% (8/44) for 1989 through 1992 and RMP resistance rose to 15.9% (7/44).[11] However, the most disturbing finding was the rise in primary MDR TB in children from 0% in 1973 through 1976 to 13.6% (6/44) in 1989 through 1992. Resistance rates in strains isolated from adults at the same medical center for 1989 through 1991 were 20.2%, 13.0% and 11.4% respectively for INH, RMP and INH plus RMP.[11] The children's strains therefore reflected a similar increase in resistant strains isolated from the adult population. This was to be expected if INH-resistant strains were as infectious and cause as much disease as drug-susceptible strains because children contract TB from adult source cases.

An interesting observation from the surveillance of primary drug resistance in the USA was that resistance rates varied inversely with age.[12] Bloch et al.[13] confirmed this in a nationwide survey of drug-resistant TB in the USA during the first quarter of 1991. They again showed a decreasing incidence of drug resistance with increasing age, with 21.6% of children ≤14 years of age resistant to one or more drugs compared to 9.4%

adults ≥65 years of age. Although children ≤14 years of age were not included, age was however, not shown to be significantly associated with drug resistance in developing countries.[14, 15]

Except for the early studies mentioned already, only a few surveys from countries other than the USA reported on the prevalence on drug-resistant TB in children, or included children in their surveys. A report on 10 years' experience of paediatric intrathoracic TB from British Columbia in Canada showed a positive culture yield in 45 (22%) of 202 children. Eight (17.7%) of these children had drug-resistant strains, 4 of which were INH-resistant and 4 strains were multiple drug-resistant.[16]

The only recent survey on the prevalence of childhood drug-resistant TB from outside the North American continent was from Turkey. Dilber et al.[17] reported the prevalence of primary drug resistance among children at their hospital from 1975 to 1995 to be 26.7% (16 of 60 children). INH resistance was present in 4/60 (6.7%) and RMP resistance in 3/46 (6.5%), but there were no MDR cases. They report all cases in whom susceptibility tests were done, but do not say how many culture confirmed cases they had during the same period that were not tested. No studies on the prevalence of drug resistance in children from developing countries could be found.

2.2 Infectiousness (virulence) and pathogenicity of drug-resistant *M. tuberculosis* strains.

2.2.1 Infectiousness of drug-resistant strains

As discussed in Chapter 1, there is some experimental evidence of reduced virulence for laboratory animals of those strains of tubercle bacilli that are resistant to

INH.[18-20] The studies of Riley et al.[21] on the risk of infection for guinea pigs breathing air from a tuberculosis ward showed that untreated patients excreting INH-resistant organisms were infectious for guinea pigs but less infectious than untreated patients excreting susceptible organisms. This, and other data on reduced catalase production of such strains led to the assumption that INH-resistant *M. tuberculosis* organisms are much less infectious and cause less disease than INH-susceptible strains.[18] This postulate was already challenged by Debré et al.[2] and thereafter by several reports of outbreaks of TB caused by INH- and multiple drug-resistant strains even before the HIV-era. In some of these outbreaks high tuberculin skin test conversion rates were documented.[22, 23]

In a study of drug-resistant contacts compared with a control group of drug-susceptible contacts Snider et al.[24] found the infection rate to be even higher in the contacts of drug-resistant TB patients than in the contacts of drug-susceptible TB patients. Furthermore both Steiner et al.[25] and Snider et al.[24] showed no difference in the rate of disease development in children infected with INH-resistant and INH-susceptible strains.

2.2.2 Pathogenicity of drug-resistant *M. tuberculosis* strains.

Evidence in the 1950's indicated that INH-resistant organisms were attenuated in both virulence and pathogenicity for laboratory animals. It was postulated that these strains might be attenuated for humans, too, with regard to their ability to cause significant disease.

The same could not, however, be said of SM-resistant strains. Already in 1949 a case of childhood tuberculous meningitis (TBM) was caused by a SM-resistant *M. tuberculosis* strain.[2] Of 19 children diagnosed with SM-resistant TB from 1949 to 1956,

no less than 10 (53%) had TBM with or without miliary TB, 8 of whom died, and 9 (47%) had primary TB.[2]

Only 4 cases of *M. tuberculosis* resistant to INH (plus SM resistance in 2) were identified in this study. One child had a pleural effusion and 3 had primary TB. No specific conclusion was drawn regarding the pathogenicity of INH-resistant strains, but they came to the conclusion that there is no clinical difference in disease caused by drug-resistant and drug-susceptible tubercle bacilli in general.[2]

In the first official childhood drug-resistant TB surveillance study from the USA, 7 cases of INH resistance with or without SM resistance were identified. Of these, 1 had TBM, 1 had progressive primary TB, 2 had bone TB and 3 had primary TB.[7] Again no comment was made regarding pathogenicity, but the severity of the disease is obvious and 1 child, resistant to both INH and SM, died.

Steiner and Cosio by implication queried the significance of drug-resistant TB disease in the first of their surveys.[8] They found no difference in response to treatment with INH and SM in the children who had INH and/or SM resistant TB compared to that of children with drug-susceptible TB. No deaths occurred although the expected death rate of children with untreated TB was as high as 20%.[8] However, in the follow-up survey 1965 through 1968, 2 of 14 children deteriorated on treatment, one of whom died of TBM and the second child responded only when treatment was changed according to the susceptibility pattern of the organism.[26]

In further studies comparing drug-resistant disease to drug-susceptible disease, both Steiner et al.[27] and Snider et al.[24] concluded that from a clinical point of view, there was no difference in the type or severity of disease caused by either isoniazid-resistant or

susceptible strains of tubercle bacilli. Furthermore there was no difference in either the rate of infection or disease found on contact investigation.[24]

In a study comparing the rates of infection and progression to disease between household contacts (both adults and children) of patients with MDR TB and drug-susceptible TB, Teixeira et al.[28] found the prevalence of infection and progression to disease to be similar in the two groups. Although this study included both adult and childhood contacts, no specific data on the outcome of the children were presented. To date, no similar studies have been done for MDR TB in children.

2.3   Epidemiologic proof of transmission of drug-resistant *M. tuberculosis.*

2.3.1 Correlation of susceptibility patterns of children and their adult source cases.

In a remarkable study of the correlation of susceptibility patterns of *M. tuberculosis* strains isolated from children with those isolated from identified adult source cases, Steiner et al.[29] showed that strains of *M. tuberculosis* highly resistant (MIC >5µg/ml) to INH can be transmitted. Furthermore these strains caused disease indistinguishable from that produced by INH-susceptible strains. In a follow-up study correlating susceptibility patterns in matched child and adult source case strains, 20 (69%) of 29 matched resistant strains had identical susceptibility patterns, and in 14 (93%) of 15 adult-child matched with pairs with INH resistance the susceptibility tests were identical.[25] None of these children had previously received antituberculosis treatment therefore transmission from the source case is likely. It further alerts the physician to the fact that INH prophylaxis might fail in these cases. Probably the most important information derived from these

studies is that the susceptibility pattern of the adult source case, when available, is a useful guide in planning the child's treatment.[25]

These early studies included only children with mono- or multiple-drug-resistant TB and no cases of MDR TB. Furthermore only epidemiologic measures and susceptibility patterns were used to compare strains of contacts and source cases. The development of new technological methods to aid epidemiology, such as phage typing and DNA fingerprinting, and the rise in drug-resistant TB called for further studies.

2.3.2 Phage typing and DNA fingerprinting as aids in the epidemiology of drug-resistant tuberculosis cases.

In the early eighties outbreaks of TB caused by multiple-drug-resistant strains were considered rare events. The only report of such an outbreak prior to the community outbreak described by Reves et al., [23] was that of Steiner et al.[22] which occurred in a family. All 23 members of the immediate household of the source case, from whom a strain of *M. tuberculosis* resistant to INH, SM plus PAS was isolated, were infected and 6 of the family members had active disease. Of those with active disease, *M. tuberculosis* was cultured from 3, 2 of whom were children. The susceptibity pattern was identical to that of the source case.[22]

Although the first community outbreak with multiple-drug-resistant *M. tuberculosis* involved high school children of >14 years of age, this was of epidemiologic importance. In 8 of 22 cases that were epidemiologically linked by case histories and the unusual (for that time) drug-susceptibility patterns, phage typing of the isolates could confirm the same strain type.[23]

In the early nineties several outbreaks of MDR TB among mainly HIV-infected patients in institutions in the USA were described.[30-32] Confirmation of transmission in these outbreaks was aided by the development of DNA fingerprinting methods for the identification of *M. tuberculosis* strains and the development of specific probes such as IS6110.[33] Ridzon et al.[34] reported an outbreak of multiple-drug-resistant (INH-SM-ETH) tuberculosis in a high school in which DNA fingerprinting by restriction fragment length polymorphism (RFLP) analysis and polymerase chain reaction (PCR) were used to confirm the epidemiologic link between cases. Eighteen TB cases were identified at the school from 1991 to 1993 and DNA fingerprinting was done on *M.tuberculosis* strains obtained from 12 of these students. Eight of these had an identical fingerprint pattern and in 7 of these the drug-susceptibility pattern was also identical. Four strains had both different susceptibility patterns and distinct DNA fingerprinting.[34]

DNA fingerprinting has become an important epidemiologic tool in the evaluation of transmission of tubercle bacilli. Studies involving the epidemiologic evaluation of children with MDR TB, or children in contact with adult pulmonary MDR TB source cases are rare. Teixeira et al.[28] identified six paired *M. tuberculosis* isolates from adult MDR TB index cases and their contacts during contact tracing follow-up. Although their study included child contacts, no indication was given whether any of the culture-confirmed cases were children. Nevertheless DNA fingerprinting showed all pairs to be identical even though one contact patient had a fully susceptible *M. tuberculosis* strain.

2.4   Multidrug-resistant tuberculosis in children.

RMP was the last of the major antituberculosis drugs to be discovered. Together with INH it forms the cornerstone of antituberculosis treatment through its unique ability

to rapidly sterilise TB lesions. The development of, and the dramatic increase in, MDR TB throughout the world over the last two decades, therefore poses a major threat to effective TB control.

The first cases of primary MDR TB in children were reported soon after the development of acquired multidrug resistance in adults,[27] and a marked increase in numbers occurred following the rapid rise of MDR TB in adults in the same areas.[10, 35] The first community outbreaks of MDR TB were reported in adults [30, 36] and outbreaks in children [31] and infants, who acquired infection in a nursery during 1992-1993, soon followed.[37] Further experience with MDR TB in children is derived mainly from case reports.[11, 38, 39] The single cases of HIV associated MDR TB in children did not differ clinically or radiologically from HIV-seronegative cases.[11]

2.5   Children at risk for drug-resistant TB.

The vast majority of children with drug-resistant TB have not been previously treated with antituberculosis drugs and therefore have primary drug-resistant TB.[11] This emphasises the importance of source case tracing in every case of childhood TB and the importance of determining the drug susceptibility pattern of the source case's strain. Source cases are, however, only identified in about 40% [11, 40] to 70% [41] of children with TB. This depends to a certain extent on the way that these children present to the health care setting, either as contacts of adult TB cases or with symptoms, and the age of the children because it is easier to trace a source case in younger children. Furthermore, because source cases are often not traceable, or children live in communities with high prevalence rates of drug-resistant TB, or there are more than one adult TB source case in

the household,[42, 43] every effort should be made to culture the organism from the child and determine the drug susceptibility of the strain.[11]

On the other hand, *M. tuberculosis* is isolated from gastric aspirates or other sources such as sputa and broncho-alveolar lavage in less than 50% of cases.[40, 44] It is therefore critically important that a clinician treating a child with TB infection or disease be familiar with the resistance patterns of the *M. tuberculosis* strains of the community in which the child resides.[45]

The type of TB lesions found in children with primary TB disease is such that they normally contain relatively low numbers ($10^3 - 10^5$) of tubercle bacilli. Drug resistance to a single drug is present in about 10^5-10^8 organisms depending on the drug. (See section 1.12) This implies that development of acquired drug resistance is less likely in children than in adults, unless the child has either extensive pulmonary TB or cavitary disease.

Except for those children from whom a drug-resistant *M. tuberculosis* strain is isolated, the following children are at high risk and the possibility of drug resistance should be considered in the management of these children:

a) Known adult source case with drug-resistant TB.

b) No known source case, but the community (or country) in which the child resides (or had resided) has a high prevalence of drug-resistant TB.

c) Adult source case is a treatment defaulter (not compliant), a treatment failure (compliant but sputum still positive at end of treatment), a retreatment case (second episode of TB) or a chronic case (TB despite 2 previous treatment courses) with unknown drug susceptibility pattern.[46]

d) Child does not respond satisfactorily or deteriorates while on TB treatment and is compliant.

e) Child with pulmonary TB relapses after incomplete or incorrect TB treatment.

2.6 Differences in childhood and adult TB: How it could influence drug management.

2.6.1 A major microbiologic determinant of the success of antituberculosis treatment is the size of the bacillary population within the host.[35] Adults and children with cavitary pulmonary TB or children with extensive pulmonary infiltrates have large bacillary populations and these may contain many single-drug-resistant bacilli. However, for children with infection only (positive tuberculin skin test) or slight changes on chest radiograph (i.e. lymphadenopathy or small infitrates) or moderate extra-pulmonary TB, bacterial populations are small to medium-sized and are not metabolically very active. This may influence both the number of drugs and the duration of treatment necessary to treat children effectively, and it is believed that children are generally overtreated.[45]

2.6.2 Extra-pulmonary TB is more common in children. Although the populations of organisms in these lesions are medium-sized, the sequestered localisation of these bacilli (e.g. osteo-articular or in the central nervous system) may influence drug treatment and specifically if there is drug resistance e.g. to INH. Other first line antituberculosis drugs penetrate obstacles such as the blood-brain barrier poorly and the use of a fourth drug such as ethionamide is often recommended.[47-49]

2.6.3 Children often have a different response to antituberculosis drugs than their adult counterparts. On the one hand, adverse reactions such as hepatotoxicity with INH,

RMP and PZA, and gastro-intestinal side effects with ETH may be less common in children. On the other hand, some drugs are not generally recommended for use in children because of the difficulty of evaluating adverse reactions e.g. optic neuritis with the use of EMB and arthropathy with the use of the fluoroquinolones.[45]

2.6.4 Antituberculosis drugs are difficult to administer to children because of the lack of paediatric formulations. Combinations of the first line drugs specifically designed for paediatric use have recently become available for use in South Africa. Often it is necessary to use second line drugs in the treatment of resistant TB. Except for ciprofloxacin, however, no paediatric formulations are available. Furthermore, the dosages recommended for children have often not been prospectively evaluated in children, but have been extrapolated from dosages used in adult studies.

2.7   Management of drug-resistant TB in children.

Although no meaningful clinical trials have assessed the effectiveness of treatment for drug-resistant TB infection or disease,[45] the following basic principles for drug management of drug-resistant TB are important:

a) Never add a single drug to a failing regimen.

b) Drug-resistant strains of *M. tuberculosis* should be treated with at least 2 or preferably 3 drugs to which the strain is susceptible.[45, 50]

c) When possible, use drugs that are active in vitro and have not been employed previously. Continuation of previously used drugs even if the relevant isolate is reported to be susceptible to them, is of uncertain value.[50] Use bactericidal drugs as far as possible.[46]

d) If *M. tuberculosis* strains are resistant to INH and/or RMP, treatment duration should be prolonged in order to attain the necessary sterilisation of the lesions. Although guidelines based on experience in adult drug-resistant TB exist, optimal duration of treatment in children is unknown.[11, 45]

e) If the child's culture for *M. tuberculosis* is not available or the susceptibility pattern is not known, the adult source case's strain can be used as an initial guide for constructing an effective treatment regimen.[25]

f) Only daily (5 times a week or more) treatment and not intermittent therapy is advised.

g) All treatment must be directly observed.

h) Children treated for drug-resistant TB should be carefully monitored specifically with regard to adverse reactions.[45]

i) Cases should be managed by specialised units only, because treatment involves second line reserve drugs which are more expensive, less effective and have more side effects than standard drugs.[46, 51]

j) Trace and effectively treat all adult source cases.

k) Combinations of drugs that produce similar toxic effects, such as deafness or liver or renal damage should be avoided unless no other combination is possible.[52]

2.8   Antituberculosis drugs in the management of drug resistant TB.

The basic principles of managing drug-resistant TB have been discussed. (See section 2.7) A summary of available first line, second line and newer antituberculosis drugs has been compiled in tables 1 to 3 (Addendum).

The use of INH in INH-resistant patients is controversial and needs further clarification. Uncertainty remains whether old (previously used) drugs should be continued once the diagnosis of drug resistance is confirmed in adults, since their effectiveness is doubtful and their use may increase the risk of adverse reactions.[53] However, children develop mainly primary resistant TB and a case can be made for using INH in primary INH-resistant or MDR patients.[54]

Many studies have shown that more than half of the *M. tuberculosis* isolates from primary resistant patients have low-level (MIC <5µg/ml) resistance to INH.[55-58] In early studies some benefit was derived from adding INH, some in double dosage, to treatment regimens in adults with primary INH-resistant TB.[55, 59, 60] An International Union against Tuberculosis (IUAT) study, however, indicated no advantage in adding a normal dose of INH to a regimen for INH-resistant cases.[61] Moulding,[54] reviewing the literature at the time, also argued that an increased INH dosage of 16-20 mg/kg/day might be advantageous even in patients with acquired INH resistance since the bacilli vary in their degree of resistance to INH. More recently, a mouse model study suggested that INH might be useful in combination therapy of *M. tuberculosis* infection caused by low-level INH-resistant organisms (INH MIC 0.2-5.0 µg/ml).[62] Donald et al.[63] showed some early bactericidal effect in adults with INH-resistant TB. Short course chemotherapy regimens have now been shown to be effective in some MDR TB cases and the likely explanation is a low-level resistance to INH in those individuals who responded to treatment.[64, 65]

The question remains: what is the optimal dosage of INH to reach effective INH serum-levels in children, those with faster as well as with a slow acetylator status, who have low-level INH resistance. The International Union Against Tuberculosis and Lung Disease's (IUATLD) guidelines recommend an INH dosage of 4-6 mg/kg/day for

childhood TB cases while the American Academy of Pediatrics (AAP) recommends 10-15 mg/kg/day.[66, 67] Donald et al.[68] showed that even cerebro-spinal fluid concentrations of 5 µg/ml could easily be surpassed in most children with TBM by administering a dose of INH of 20 mg/kg.

In addition to the reasons given above, in high TB incidence areas, adding INH to drug regimens for contacts of MDR or INH-resistant TB cases may be of value, because some contacts may have more than one possible source case and may therefore have drug-susceptible TB.[43, 69] INH alone is, however, insufficient for chemoprophylaxis in such cases, because failure of INH prophylaxis after exposure to INH-resistant strains has repeatedly been reported.[22, 69, 70]

2.9   Management of children in contact with adults with drug-resistant tuberculosis. (Preventive treatment)

INH alone is the only drug proven to be efficacious for the treatment of TB infection in children.[71-74] In the case of INH resistance, however, INH prophylaxis might fail and several such failures have been reported.[22, 69, 70] A need for alternative prophylactic regimens with other antituberculosis drugs and of shorter duration was appreciated.

Both experimental evidence [75] and studies in mainly HIV-infected adults showed a 2-month regimen of RMP plus PZA or a 3-month regimen of RMP alone to be comparable in efficacy to INH alone for 6 to 12 months. [76, 77] The only alternative paediatric regimen that was published, is a 3-month regimen of INH plus RMP evaluated retrospectively, which seemed to be as efficacious as treatment with the same drugs for up to 12 months.[78]

In 1992 the AAP recommended treating children infected with an INH-resistant strain with RMP for 9 months.[79] Recent randomised controlled studies in adults showed markedly shorter courses of RMP plus PZA or RMP alone to be efficacious in preventing TB, but these regimens are not yet generally recommended in children. These regimens can, however, be used in children who are contacts of INH-resistant source cases.[74] Although the optimal duration of chemoprophylaxis with RMP in children has not been established, the AAP now recommends a treatment course of at least 6 months.[67]

The chemoprophylaxis of children infected with a MDR *M. tuberculosis* strain is a challenging problem. No regimen has proven efficacy and there are no data on how long such prophylaxis should be taken.[45, 80] Many experts agree on the need for chemoprophylaxis in persons who have contracted infection from a MDR TB patient.[11, 45] Others, however, consider regular clinic follow-up of individual cases without chemoprophylaxis an alternative strategy until more data are available.[74, 80, 81] Infants have a very high risk (up to 40%) of developing disease after infection and are at special risk for developing disseminated disease and should therefore receive chemoprophylaxis when infected.[74]

The drugs that could be used for the prophylaxis of MDR infected persons have not been prospectively evaluated. Use of a regimen containing other agents to which the source case's strain is susceptible and that are active against *M. tuberculosis* should be considered. No single antituberculosis drug is recommended for preventive therapy.[11] In adults who are at high risk of developing TB, PZA plus EMB or PZA plus a fluoroquinolone for 6 to 12 months has been recommended if the index case's strain is susceptible to these agents. All persons with suspected MDR infection should be followed for at least 2 years irrespective of treatment.[74] In children the combination of

PZA plus EMB for 9 to 12 months is recommended if the isolate is susceptible to both drugs. Resistance to PZA is, however, difficult to determine. Several other combinations of drugs to which the source case's strain is susceptible have been recommended such as PZA plus cycloserine, ETH and cycloserine and, if the potential benefits justify the potential side effects, PZA plus a fluoroquinolone.[11, 45, 81, 82]

The optimal duration of chemoprophylaxis in children has not been determined, but most experts recommend a 6 to 12 month course.[11, 45, 80]

2.10 Conclusion

2.10.1 Drug-resistant TB in children reflects the prevalence of drug resistance in the adult population.

2.10.2 Infection and subsequent disease occur with similar frequency following exposure to sputum smear positive adults who are drug-resistant as following exposure to drug susceptible index cases.

2.10.3 The principles of prophylaxis and treatment of drug-resistant TB are similar in adults and children, although children may be more tolerant of higher drug dosages and less likely to develop adverse effects. Some antituberculosis drugs are, on the other hand, not yet recommended for use in children.

2.11 Setting of planned study

The different components of the study will be done in the Western Cape Province of South Africa, an area with a reported TB notification rate of 589 new cases per 100 000 population per year in 1998 (data from Department of Health, Directorate Health Systems

Research and Epidemiology). In this hyperendemic area, transmission of *M. tuberculosis* is high, and the annual rate of TB infection in the suburbs surrounding the tertiary hospital from where the study will be done, is 3.1% (Beyers et al. Unpublished data). Many children have more than one adult TB source case living in the same house either at the same time or over a period of time.[42] These factors may have a role in the outcome of the study.

2.12 AIMS OF THE STUDY

In the light of the problems discussed above and a number of uncertainties regarding management and treatment, the following areas will be addressed in this thesis:

a) Drug-resistant TB in children is of great epidemiologic importance. No information on the occurrence of drug-resistant TB in children is available from developing countries. One of the aims of this thesis will be to determine the prevalence of drug-resistant TB in children in a geographical area surrounding Tygerberg Hospital, a secondary and tertiary referral centre in the Western Cape Province of South Africa. Although the ideal is a population-based study, a hospital-based study is of value when all problem cases in a geographical area are referred to that hospital.[83]

b) In animal studies INH-resistant *M. tuberculosis* strains had attenuated pathogenicity and it was therefore postulated that INH-resistant bacilli might be less able to establish infection or cause disease in humans. Transmission of drug-resistant TB from adult index cases to their child contacts has been documented. These studies involved mainly INH-resistant or multiple drug-resistant TB cases. In this thesis the aim will be to document transmission of MDR TB from adult index

cases to their child contacts in the short (first two months) and long term (30 months) and the frequency of development of disease in these children. A further objective will be to compare *M. tuberculosis* isolates obtained from both the index case and child contact not only by drug-susceptibility pattern, but also by DNA fingerprinting and gene mutations.

c) The role of INH in the treatment of INH-resistant TB is still controversial, but there is some evidence that it has a place in the management of primary INH-resistant cases. The final aim of this thesis will be to determine the pharmacokinetics of INH in children and to determine whether sufficient concentrations of INH could be achieved to overcome low-level INH-resistant tubercle bacilli in children.

Each chapter will have its own introduction, material and methods, results and discussion. A final chapter will be included to summarise the findings of the thesis.

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Table 1: First line antituberculosis drugs: recommended dosage, effects and possible adverse reactions.

Antituberculosis Drug	Recommended daily dosage (paediatric)	Bactericidal or Bacteriostatic	Adverse reactions	Protective effect against resistance	Continuation phase drug	Maximum daily dose
Isoniazid♦	10-20 mg/kg WHO: 4-6 mg/kg	Bacteriocidal	Hepatitis, hypersensitivity, peripheral neuropathy, drowsiness, skin rash, mood changes	Good	Yes	300 mg
Rifampicin	10-20 mg/kg	Bacteriocidal	Hepatitis, rash, orange discolouration of urine and secretions, gastro-intestinal upset, thrombocytopenia	Good	Yes	600 mg
Pyrazinamide♦	20-40 mg/kg	Bacteriocidal	Hepatitis, arthralgia, hyperuricaemia	Poor	Specific indications	2 g
Ethambutol	15-25 mg/kg	15 mg/kg – bacteriostatic; 25 mg/kg – moderately bacteriocidal	Optic neuritis (dose related and reversible), rash	Good – moderate	Yes	2.5g
Streptomycin	20-40 mg/kg IM	Bacteriocidal	Ototoxic, nephrotoxic	Good - moderate	No	1g

♦ Good CSF penetration

Table 2: Second line antituberculosis drugs: recommended dosage, effects and possible adverse reactions.

Antituberculosis Drug	Recommended daily dosage (paediatric)	Bactericidal or Bacteriostatic	Adverse reactions	Protective effect against resistance	Continuation phase drug	Maximum daily dose
Ethionamide[•] or prothionamide	10-20 mg/kg 2 or 3 divided doses/day	Bacteriostatic; bacteriocidal at higher dose?	Gastric intolerance, hepatitis, convulsions, hypersensitivity	Good	Yes	1g
Kanamycin	15 mg/kg IM	Bacteriocidal	Ototoxic, nephrotoxic	Good - moderate	No	1g
Amikacin	15 mg/kg IM or IV	Bacteriocidal	Ototoxic, nephrotoxic	Good - moderate	No	1g
Capreomycin	15 mg/kg IM	Bacteriocidal	Ototoxic, nephrotoxic	Moderate	No	1g
Viomycin	15 mg/kg IM	Bacteriostatic	Ototoxic, nephrotoxic	Poor	No	1g
Cycloserine[•]	10-20 mg/kg	Bacteriostatic	Psychosis, personality changes, depression, seizures, rash	Good	Yes	1g
Thiacetazone	4 mg/kg	Bacteriostatic	Gastrointestinal intolerance, vertigo, rash, Stevens Johnson's syndrome (HIV-infected patients)	Moderate - poor	Yes	150mg
Para-aminosalicylic acid	150-300 mg/kg 4 divided doses/day	Bacteriostatic	Gastritis, hypersensitivity reactions, hepatitis,	Moderate - poor	Yes	12g

[•] Good CSF penetration

Table 3: New antituberculosis drugs: recommended dosage, effects and possible adverse reactions.

Antituberculosis Drug	Recommended daily dosage (paediatric)	Bactericidal or Bacteriostatic	Adverse reactions	Protective effect against resistance	Continuation phase drug	Maximum daily dose
Ofloxacin (not yet recommended)	10-15 mg/kg	Moderately bacteriocidal	Arthropathy, arthralgia, headaches, insomnia, gastrointestinal upset, photosensitivity	Moderate?	Yes	800mg
Levofloxacin (not yet recommended)		Moderately bacteriocidal	Arthropathy, arthralgia, headaches, insomnia, gastrointestinal upset, photosensitivity	Moderate?	Yes	1000mg
Ciprofloxacin (not yet recommended)	20-30 mg/kg	Moderately bacteriocidal	Arthropathy, arthralgia, headaches, insomnia, gastrointestinal upset, photosensitivity	Moderate?	Yes	1500mg
Azithromycin	10-20 mg/kg		Gastrointestinal disturbance, ototoxic			500mg
Clarithromycin	15-30 mg/kg		Gastrointestinal disturbance, rash			1g

Table 3 (continued): New antituberculosis drugs: recommended dosage, effects and possible adverse reactions.

Antituberculosis Drug	Recommended daily dosage (paediatric)	Bactericidal or Bacteriostatic	Adverse reactions	Protective effect against resistance	Continuation phase drug	Maximum daily dose
Rifabutin (up to 90% cross resistance with RMP) Amoxicillin-clavulanate	10-20 mg/kg	Bacteriocidal	Polyarthralgias, arthritis, neutropenia, myositis, skin hyperpigmentation, uveitis	Good	Yes	600mg
Clofazamine	1-2 mg/kg	Effect not known or no effect on *M. tuberculosis*	Headache, diarrhoea, peripheral neuropathy, red skin discoloration			100mg

CHAPTER 3

Primary drug-resistant tuberculosis in children

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Primary drug-resistant tuberculosis in children

Setting: The Western Cape Province of South Africa, an area with a high tuberculosis (TB) incidence where initial isoniazid (INH) resistance and multidrug resistance (MDR) among adults was 3.9% and 1.1%, respectively, during 1992-1993.

Objective: To determine the drug resistance incidence among children as compared to adults, to compare the clinical features of drug-resistant and drug-susceptible TB, and the degree of INH resistance in isoniazid-resistant isolates.

Methods: All *Mycobacterium tuberculosis* cultures obtained from children (0-13 years) at a regional hospital were prospectively collected from August 1994 to April 1998 and susceptibility testing done on each child's specimens. Degree of INH resistance was determined in available resistant isolates. The children's clinical records were reviewed.

Results: Susceptibility results were available in 306/338 children with cultures of *M. tuberculosis*; 21 isolates (6.9%) were INH-resistant, and seven were MDR. Taking into account study limitations, the incidence of INH resistance was 5.6% and MDR 1% in children aged <5 years. Clinical features were similar in children with drug-susceptible and drug-resistant TB.

Conclusion: The incidence of drug resistance in childhood tuberculosis in Western Cape is low, and probably reflects the level of primary drug resistance amongst organisms currently circulating in the community.

INTRODUCTION

In the last decade there has been an increased awareness that drug-resistant tuberculosis (TB) poses a major threat to patients as well as to tuberculosis control programmes. Surveillance of drug resistance is therefore essential, because trends in primary drug resistance (i.e., resistance in cultures from patients with no previous tuberculosis treatment) or initial drug resistance (i.e., primary resistance plus undisclosed acquired resistance) provide an indication of the effectiveness of treatment regimens, while drug resistance rates in patients with a history of previous treatment (acquired resistance) can indicate failures in the management of the disease.[1] Accuracy in determining whether patients have initial/primary or acquired drug resistance is often unsatisfactory, as obtaining a history of previous treatment is difficult.[2] Van Rie et al.[3] demonstrated that some patients who previously had drug-susceptible TB and thereafter presented with drug-resistant tuberculosis were reinfected with drug-resistant strains and thus had primary drug-resistant tuberculosis, despite having had previous tuberculosis treatment.

Children have mainly primary drug resistant TB, and previous treatment is easier to exclude. They should therefore be a good source for surveillance of true primary drug-resistant TB, and may accurately reflect the transmission of these organisms in the community.[4] There are few studies of drug-resistant TB in children or surveillance data that include children in significant numbers,[5,6] and no data from developing countries could be traced.

The use of isoniazid (INH) in isoniazid-resistant TB patients is controversial.[7] There is reason to believe that adding INH to the treatment of newly diagnosed children

with INH-resistant TB would be beneficial, as the degree of INH resistance differs in patients with primary and acquired resistance, and in early studies some benefit was derived from adding INH to treatment regimens in adults with primary INH-resistant TB.[8,9]

The aim of this study was to determine the prevalence of drug resistance among children with tuberculosis and to compare the clinical and radiological features of drug-resistant and drug-susceptible TB in these children. A secondary aim was to determine the minimal inhibitory concentration of INH-resistant isolates.

PATIENTS AND METHODS

Setting

This prospective study was conducted between August 1994 and April 1998 at Tygerberg Hospital, a secondary and tertiary referral centre in the Western Cape Province of South Africa. This area had a reported TB notification rate of 589 new cases per 100 000 population per year in 1998 (Department of Health: Directorate Health Systems Research and Epidemiology). The rate of initial resistance to INH determined in adult TB cases in the Western Cape Province during 1992-1993 was 3.9% (95% confidence intervals [CI]; 3.3-4.6%) and initial multidrug resistance (MDR-defined as cultures resistant to INH and rifampicin [RMP], with or without resistance to other antituberculosis drugs) was found in 1.1% of isolates (95% CI; 0.7-1.4%).[1] Prevalence of human immunodeficiency virus (HIV) infection in women attending antenatal clinics in the Western Cape Province rose from 1.16% (95% CI; 0.76-1.56) in 1994 to 5.21% (95% CI; 4.2-7.2) in 1998 (Department of Health: Directorate Health Systems Research and Epidemiology).

Drug susceptibility testing

All cultures of *Mycobacterium tuberculosis* obtained at Tygerberg Hospital from children 0 to 13 years of age were collected prospectively. A single specimen from each patient was sent to the South African Institute for Medical Research in Cape Town for susceptibility testing. Initial screening was done for INH resistance only, because RMP resistance was seldom found without INH resistance in primary (initial) drug resistance in the Western Cape.[1] When resistance to INH was found, susceptibility testing for RMP, streptomycin and ethambutol was performed.

Laboratory procedures for determining drug resistance were as follows: Middlebrook 7H12 (Bactec) culture medium was used for selective primary isolation of mycobacterial strains. The niacin production test was used to identify *M. tuberculosis*. Drug susceptibility testing was performed by the economic variant of the indirect proportion method currently in use by the majority of laboratories in South Africa. This method entails the incorporation of the required drug concentration into Löwenstein-Jensen (LJ) egg-based medium before coagulation, while the slants were subsequently inoculated with a standardized inoculum. The following drugs were tested at the indicated concentrations: INH 0.2 μg/ml LJ; RMP 30.0 μg/ml LJ; streptomycin 5 μg/ml LJ and ethambutol 2 μg/ml LJ. The susceptibility of a strain was judged by determining the proportion of bacilli resistant to a specific drug in comparison with growth on a specific control, using international criteria. Resistance was defined as 1% or more bacterial growth. Quality assurance for drug susceptibility results is done locally with every batch and quarterly by the national tuberculosis reference laboratory.[10]

In addition, nine of the clinical isolates that were resistant to the critical concentrations of INH (0,2μg/ml) and which were still available at the time, were

processed to determine the degree of their resistance in MIC values (µg/ml). Briefly, precultures were prepared in 7H9 Middlebrook medium and incubated at 37°C for approximately 14 days before they were adjusted to an optical density of a No. 1 McFarland standard, which is equivalent to 2×10^7 organisms/ml. The latter suspensions were further diluted (1/20) to a concentration of about 1×10^6 colony forming units (cfu)/ml and 20µl aliquots were inoculated on to 7H10 Middlebrook medium, with or without appropriate dilutions of INH, to yield final concentrations of approximately 2×10^4 cfu. INH was incorporated into 7H10 plates at final concentrations of 2, 4, 5, 10 and 20µg/ml and *M. tuberculosis* H37Rv (ATCC 27294) was used as a drug-sensitive control. An organism was considered to be resistant to a specific concentration of the drug if it failed to inhibit more than 99% of the bacterial population, indicating whether the isolate has a high or low level of resistance to INH.

Clinical data

The clinical records of all children with positive cultures for *M. tuberculosis* were reviewed, and all children with drug-resistant TB were recalled. A history was obtained from all children regarding previous TB prophylaxis or treatment, and whether they had close contact with adults with pulmonary TB. Weight at diagnosis and site of tuberculosis were recorded. Results of tuberculin skin test (Mantoux test by intradermal injection, 5 tuberculin units, Japanese purified protein derivative), HIV-serology, and sites of positive *M. tuberculosis* specimens obtained were noted where available. An area of induration of ≥15 mm after Mantoux skin testing was regarded as significantly positive in accordance with World Health Organization criteria, as more than 90% of children in this area receive BCG.[11] Chest radiographs were read according to a standardized method.[12] All children with drug-susceptible TB were treated according to

the National Tuberculosis Programme (NTP) policy with INH, RMP and pyrazinamide. Children with drug-resistant TB were treated according to the drug susceptibility pattern of their *M.tuberculosis* strain and followed up for 2 years.

Categorical data was analyzed using the χ^2 test to compare groups, and Fisher's exact test was applied where appropriate. Statistical analysis was done using Epi-Info version 6.04.

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Stellenbosch.

RESULTS

During the 3 year 9 month period, 538 cultures of *M. tuberculosis* isolates were obtained from 338 children. The children, 193 (57%) boys and 145 (43%) girls, had a median age of 2.6 years (range 0.06 – 13 years). They were divided into two groups; < 5 years of age (n = 241) and 5 – 13 years of age (n = 97). In respectively 23 (9.5%) and 9 (9.3%) children, drug susceptibility testing was not done due to lost specimens (n = 13), contamination (n = 15) or loss of viability (n = 4).

Susceptibility results

Susceptibility results were available in 90.5% of cases (Table 1). Of the 21 isolates (6.9%) that were resistant to INH, seven (2.3%) were multidrug-resistant. Of 56 (19.6%) *M. tuberculosis* isolates that were susceptible to INH and also tested for RMP susceptibility, none was resistant to RMP.

Table 1: Susceptibility test results

	<5 years old n = 218	5-13 years old n = 88	All n = 306
Susceptible to H*†	202 (92.7%)	83 (94.3%)	285 (93.1%)
Resistant to H only	5	4	9
Resistant to H + S	3	1	4
Resistant to H + E	1	-	1
Resistant to H + R	2	-	2
Resistant to H + R + S	3	-	3
Resistant to H + R + S + E + ETH	2	-	2
Total no. resistant to H	16 (7.3%)	5 (5.7%)	21 (6.9%)
Total no. MDR	7 (3.2%)	-	7 (2.3%)

* 56 (20%) also tested for R susceptibility; all were susceptible

† 24 (8%) also tested for S and E susceptibility; all were susceptible

H = isoniazid; R = rifampin; S = streptomycin; E = ethambutol; ETH = ethionamide.

Study limitations

Thirty children whose *M. tuberculosis* strains had been tested for drug susceptibility received previous antituberculosis treatment. Of these, 16 defaulted from treatment. Drug resistance was found in two: one child had received 5 months of INH, RMP and pyrazinamide and did not respond to treatment. The initial culture was done before the study began, but susceptibility testing was not done. Follow-up culture was multidrug-resistant. No close contact could be identified. The second child had previous TB and was treated for more than a year due to extensive pulmonary TB. Her father had INH-resistant TB but died after her initial TB diagnosis. She again presented 4 years later with culture-confirmed TB and bronchiectasis with no known TB source case. The *M.*

tuberculosis strain was resistant to INH only. Both of these cases could therefore still have had primary drug-resistant TB.

The second limitation of the survey is that it was conducted concurrently with a study of childhood contacts <5 years of age of adults with MDR pulmonary TB at the same hospital. Four of seven children identified with MDR TB were part of the latter study. If these children were excluded from the study, only 17 (5,6%) children had INH-resistant and three (1,0%) MDR-TB.

Clinical data

Clinical data of children with drug-susceptible TB, drug-resistant TB and those in whom susceptibility testing was not done are compared and summarized in Table 2.

In 138 (46%) of 297 children an adult pulmonary TB source case could be identified. Specific attention was paid to source cases of the 21 children with drug-resistant TB. Six (28%) children (four from MDR contact study) had a known drug-resistant source case, five of whom had the same drug susceptibility pattern. A further five (24%) children had known adult source cases, but the drug susceptibility patterns of the adults' *M. tuberculosis* strains were not known. Three of the 11 children had additional contact with adults who had drug-susceptible TB. No source cases were found in nine (43%) children, and one (5%) child could not be traced.

Table 2: Clinical data and special investigations

Drug susceptibility results	Susceptible to H*† n = 285	Resistant to H ± other drugs* n = 21	Not done n = 32
Age (median [range]) in years	2.64 (0.15-13)	2.67 (0.22-10.3)	2.43 (0.06-12.3)
Sex: male	157 (55%)	14 (67%)	22 (69%)
Previous antituberculosis drugs (including defaulters)	28 (10%)	2 (10%)	2 (6%)
Household TB contact	116/248 (47%)	11/20 (55%)	11/29 (38%)
Weight for age <3rd percentile (NCHS)	116 (41%)	8 (38%)	11 (34%)
Pulmonary TB	248 (87%)	21 (100%)	32 (100%)
Extra pulmonary TB (including pleural effusion and miliary TB)	171 (60%)	8 (38%)	15 (47%)
Mantoux test ≥10mm	185/222 (83%)	13/18 (72%)	17/22 (77%)
Mantoux test ≥15mm	168/222 (76%)	10/18 (56%)	17/22 (77%)
HIV-seropositive	12/137 (8,8%)	1/20 (5%)	0/9
CXR			
done and read	275 (96%)	20 (95%)	32 (100%)
hilar/mediastinal lymphadenopathy	152 (55%)	9 (45%)	9 (28%)
opacification lobar/segmental	112 (41%)	8 (40%)	12 (38%)
bronchopneumonic opacification	43 (16%)	3 (15%)	4 (13%)
perihilar opacification	27 (10%)	4 (20%)	6 (19%)
cavities	32 (12%)	3 (15%)	0
miliary	24 (9%)	1 (5%)	5 (16%)
pleural effusion	30 (11%)	2 (10%)	6 (19%)
calcification	12 (4%)	0	0
normal	40 (15%)	4 (20%)	2 (6%)
Died in hospital	11 (4%)	0	3 (9%)

* The differences between drug-susceptible and drug-resistant groups were not statistically significant. ($P<0.05$)

† Of 56 (20%) children tested for susceptibility to RMP, all were susceptible. As primary RMP monoresistance in Western Cape 0.0-0.2%, RMP resistance is unlikely.

H = isoniazid; NCHS = US National Center for Health Statistics; HIV = human immunodeficiency virus; CXR = chest X-ray; RMP = rifampicin.

HIV test results were available in 166 (49%) children of whom 13 (7.8%) were positive. Pulmonary TB was present in 89% of children. A diagnosis of pulmonary TB was based on chest radiograph changes and/or culture of *M. tuberculosis* from gastric aspirate, sputum or bronchial aspirate. Extra pulmonary TB (including miliary TB and pleural effusion) was found in 57% of all of the children, and in seven of 13 (54%) HIV-infected children.

Degree of isoniazid resistance

The MICs of INH against five of the nine *M. tuberculosis* isolates were between 0.2 and 2.0 µg/ml; these results imply that the specific strains were moderately susceptible to the drug. However, as MIC values >20µg/ml were observed in the remaining four isolates, this group was considered to be very resistant to INH.

DISCUSSION

Surveillance of antituberculosis drug resistance in a community provides a measure of the success of the NTP and gives an indication of suitable drug regimens for future use.[13] Primary or initial resistance patterns reflect transmission of drug-resistant strains, but as tuberculosis in children is a mark of recent TB transmission in a community, the frequency of drug resistance in children, particularly those <5 years of age, reflects a precise evaluation of the current situation.[4, 13] Few surveillance studies are available, however, on drug resistance in childhood TB.

This study was a hospital-based study, and therefore represents mainly children referred for secondary or tertiary care problems. Specimens for culture of *M. tuberculosis* are difficult to obtain in children, however, and will often be done only when children are hospitalised. The most comprehensive study to date, by Steiner et al., was also a hospital-

based study over a 24 year period (1961-1984) conducted in King County Hospital Center, New York.[5,14] Of 374 children screened for primary drug resistance during this period, 16,3% had resistance to one or more antituberculosis drugs. INH resistance was relatively stable at 10%, but RMP resistance, which was introduced during this period, was on the increase.[5] As far as we could determine, no studies on the prevalence of drug resistance in children from developing countries are available.

Isolated RMP resistance is reported to be rare.[1,15,16] Weyer et al. showed initial RMP resistance and multidrug resistance both to be 1.1% in adult patients in their survey,[1] and Bohmer et al. found two of 808 (0.2%) cases with monoresistance to RMP in the greater Cape Town area in 1990-1991.[17] Due to cost constraints we therefore tested initially for INH susceptibility only; none of the 20% *M. tuberculosis* strains which were susceptible to INH, and were tested for RMP susceptibility, was resistant to RMP.

Initial INH resistance is a sensitive indicator of the overall success of a treatment programme in a country.[1] Failure to control its availability adequately will soon lead to the appearance of INH resistance. The prevalence of primary INH resistance in this study is below the median reported for the 35 countries in the global surveillance for antituberculosis drug resistance from 1994 to 1997 (7,3%).[18] This prevalence nevertheless still requires the use of a four-drug regimen in newly diagnosed cases of adult type pulmonary TB, as the level of primary INH resistance is more than the cut-off of 4% recommended by the US Centers for Disease Control and Prevention.[19] The Western Cape Province had an initial INH resistance rate of 3.9% among adult pulmonary tuberculosis patients during 1992-1993.[1] The increase found with the current survey may be due to several factors. Firstly, age is said to be inversely related to drug resistance. A survey of drug resistance in the USA during the first quarter of 1991

showed a decreasing incidence of drug resistance with increasing age, with 21,6% of children ≤14 years of age resistant to one or more drugs compared to 9,4% adults ≥65 years of age.[6] Age was shown not to be significantly associated with drug resistance in developing countries, however.[13, 20] Secondly, hospital-based studies are biased towards patients referred with problems such as possible drug resistance and will therefore probably predict the worst case scenario.[21] When the known study limitations were removed, the prevalence of INH and MDR is not different from the results obtained in the adult study. Thirdly, primary resistance may be higher than determined by Weyer et al.,[1] as some of the patients in the acquired resistance group may well have had primary drug-resistant TB.[3] Lastly the possibility exists that the increase is a true increase in prevalence of drug resistance in our community. Our results suggest that the observed incidence of drug resistance in childhood tuberculosis, even though determined in a hospital-based population, is a good reflection of the incidence of primary drug resistance in a community.

The clinical presentation and chest radiograph results among those infected with a resistant strain were not significantly different from those infected with a drug-susceptible strain (Table 2). This is in agreement with findings from previous studies.[14,22] An unexpected finding was the high rate of extra-pulmonary tuberculosis (57% overall), which is higher than the expected 25% - 35% previously described in children.[23, 24] This can be explained by the fact that more complicated cases of suspected tuberculosis are referred to our hospital, and that there is a higher yield from tissue cultures than from pulmonary sources.[15] Eighty-nine per cent of all our patients did, however, have pulmonary tuberculosis on chest radiography and/or culture of *M. tuberculosis* from gastric or bronchial aspirate or sputum.

The use of INH for the treatment of INH-resistant *M. tuberculosis* infection is controversial.[7] A distinction could probably be made between the use of INH in primary or acquired (retreatment) cases of INH resistance. Canetti[25] and Tripathy et al.[8] have both shown larger numbers of low level INH-resistant strains (MIC <5µg/ml) in primary resistance compared to acquired resistance. Several studies have shown some benefit by adding INH to the treatment regimen. Devadatta et al.[9] and Tripathy et al.[8] showed an initial improvement in patients with primary INH resistance treated with INH alone or with PAS, and Petty and Mitchell[26] demonstrated significant benefit by the addition of high dose INH to a regimen of ethionamide and pyrazinamide in the retreatment of INH-resistant cases. An International Union Against Tuberculosis (IUAT) study, however, indicated no advantage by adding INH in conventional doses to a retreatment regimen for INH-resistant cases.[27] More recently, a mouse model study suggests that INH may be useful in combination therapy of *M. tuberculosis* infection caused by low-level INH-resistant organisms (INH MIC 0.2-5µg/ml).[7] Our data showed that five of nine (56%) strains from children with primary drug-resistant tuberculosis had low level INH resistance. This is similar to findings in previous studies in primary INH-resistant strains.[8, 27] These concentrations are attainable in children at normal to high dosages (10-20 mg/kg/day) of INH. Further studies to evaluate a possible role of INH in primary INH-resistant patients are needed.

In conclusion, our data suggest that the incidence of drug resistance in childhood tuberculosis in the Western Cape is low, and probably reflects the level of primary drug resistance amongst organisms currently circulating in a community.

ACKNOWLEDGEMENTS

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CHAPTER 4

Evaluation of young children in household contact with adult multidrug-resistant pulmonary tuberculosis cases

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Evaluation of young children in household contact with adult multidrug-resistant pulmonary tuberculosis cases

Background: The prevention and management of multidrug-resistant (MDR) tuberculosis has received much attention, but little attention has been given to children with MDR tuberculosis or children in contact with adults with MDR tuberculosis. The aim of this study was to determine the prevalence of tuberculous infection and disease in childhood contacts of adults with MDR pulmonary tuberculosis.

Method: All children <5 years of age in household contact with 75 recently diagnosed adults with MDR pulmonary tuberculosis were evaluated. Evaluation included clinical examination, tuberculin skin test, chest radiography and culture for *Mycobacterium tuberculosis* from gastric aspirates.

Results: One hundred twenty-eight children, median age 27 months, were evaluated. Fifty children had recent contact with other adult tuberculosis cases. Sixty-six children previously had chemoprophylaxis or treatment of which 36 defaulted treatment or received insufficient chemoprophylaxis. One child had HIV-infection. Forty-seven children were classified as non-infected, 66 were considered infected only (Mantoux test, ≥15mm) and 15 had disease. Three children, who had not previously received antituberculosis drugs, had positive cultures for *M. tuberculosis*; all were multidrug-resistant.

Conclusion: This study documents the transmission of multidrug-resistant *M. tuberculosis* to childhood contacts, the development of disease in these contacts and the importance of knowing the index case's *M. tuberculosis* susceptibility pattern in choosing a proper treatment regimen for the childhood contact.

INTRODUCTION

In recent years there has been an increased awareness of drug-resistant tuberculosis. Outbreaks and transmission of multidrug-resistant (MDR) tuberculosis have been documented in both HIV-infected and noninfected adults.[1-3] The risk factors for acquired drug resistance such as previous tuberculosis (TB) treatment, noncompliance with antituberculosis treatment (unsupervised treatment) and the incorrect prescription of antituberculosis treatment by medical staff are well known.[4-6]

The prevention and management of drug-resistant TB in adults has received much attention, but relatively little attention has been given to drug-resistant tuberculosis in childhood and the management of the childhood contacts of adults with drug-resistant TB and specifically multidrug-resistant TB.[4, 7-10] With the advent of the HIV era it is likely that more children than in the past who are infected with drug-resistant *Mycobacterium tuberculosis* will later develop disease if they are not properly managed as contacts. Furthermore any program designed to control or eliminate TB must focus a greater effort on children because they are a future reservoir for disease.[11]

The debate about whether drug-resistant TB is as infectious as drug-susceptible *M. tuberculosis* in humans seems to be settled.[12] Although there was some doubt in the past as to whether drug-resistant *M. tuberculosis* bacilli were less likely to give rise to infection, there is now little doubt that these organisms are transmitted, and in a controlled study the rate of infection was even higher in the childhood contacts of drug-resistant index cases than in the childhood contacts of a drug-susceptible group of index cases.[13] Many children in communities with a high incidence of tuberculosis are in contact with more than one adult with pulmonary TB during their first 5 years of life, and it is still uncertain how these children

should be managed regarding chemoprophylaxis.[14, 15] Moreover if some of the index cases have drug-susceptible and some have drug-resistant TB, the proper choice of chemoprophylaxis becomes even more complex.[10] There is also little doubt that drug-resistant organisms can give rise to disease.[13, 16]

The purpose of this study was to determine the prevalence of tuberculous infection and disease in young children in contact with newly identified adults with MDR pulmonary TB in an area with a high incidence of TB.

PATIENTS AND METHODS

Primary resistance was defined as resistance in *M. tuberculosis* cultures from patients with no previous TB treatment. Initial resistance is a term used for drug resistance in new TB patients and allowing for undisclosed previous TB treatment (i.e. primary resistance plus undisclosed acquired resistance). Acquired resistance is resistance found in cultures from patients with one or more previous TB treatment episodes. MDR TB is defined as cultures resistant to isoniazid and rifampin with or without resistance to other antituberculosis drugs.

This prospective study was conducted between April, 1994, and April, 1997, in the Western Cape Province of South Africa, an area with a TB incidence of ~700 new cases per 100 000 population per year.[17] The rate of initial resistance to isoniazid determined in adult tuberculosis cases in the Western Cape Province during 1992-1993 was 3.9% [95% confidence intervals (CI), 3.3 to 4.6%] and initial resistance to both isoniazid and rifampin (multidrug resistance) was found in 1.1% of isolates (95% CI 0.7 to 1.4).[18] HIV seroprevalence in women attending antenatal clinics in the Western Cape Province rose from 1.16% (95% CI 0.76 to 1.56) to 3.09% (95% CI 2.34 to 3.84) from 1994-1996.[19]

For the purpose of this study an index case was defined as an adult >15 years of age with sputum culture positive for *M. tuberculosis* and resistant to at least isoniazid and rifampin. Adult index cases were from suburbs surrounding the Tygerberg Hospital a tertiary referral center for the area. They were identified as MDR cases by the South African Institute for Medical Research (SAIMR) in Cape Town, which is responsible for the mycobacteriology services in the Western Cape. Information collected regarding the adult index case included gender and age, their relation to the childhood contact, whether they were previously treated for TB (initial or acquired drug resistance), whether their sputa were smear- and/or culture-positive for *M. tuberculosis* and the susceptibility pattern of the *M. tuberculosis* culture.

Laboratory procedures for determining drug resistance were as follows: Middlebrook 7H12 (Bactec) culture medium was used for selective primary isolation of mycobacterial strains. The niacin production test was used to identify *M. tuberculosis*. Drug susceptibility testing was performed by the economic variant of the indirect proportion method currently in use by the majority of laboratories in South Africa.[20] This method entails the incorporation of the required drug concentration into Löwenstein-Jensen (LJ) egg-based medium before coagulation, and the slants were subsequently inoculated with a standardized inoculum. The following drugs were tested at the indicated concentrations: isoniazid, 0.2 µg/ml LJ; rifampin, 30.0 µg/ml LJ; streptomycin, 5 µg/ml LJ; ethambutol, 2 µg/ml LJ; and ethionamide, 20 µg/ml LJ. The susceptibility of a strain was judged by determining the proportion of bacilli resistant to a specific drug in comparison with growth on a specific control using international criteria. Resistance was defined as 1% or more bacterial growth.[18] Quality assurance for drug susceptibility results is done locally with every batch and quarterly by the national tuberculosis reference laboratory.

Childhood contacts were defined as children 5 years of age or less living and sleeping in the same house or group of clustered houses/shacks on the same plot as the index case for at least one month. Parents or guardians were asked to bring these children to the hospital for evaluation, even if the parents considered the child to be asymptomatic. Transport was arranged when necessary.

As part of the research protocol children were admitted for 3 days for assessment. A history was obtained regarding previous TB prophylaxis or treatment and whether there were any other adults in the same house who had or had recently had TB. This information was verified with personnel at the local authority health clinics. Evidence regarding recent weight loss or failure to gain in weight and documentation of previous bacillus Calmette-Guérin (BCG) immunization was obtained from the patient's "Road to Health" clinic card when available or from clinic records.

Each child had a clinical examination, tuberculin skin test [Mantoux test, 5 tuberculin units (0.1ml) of Japanese purified protein derivative by intradermal injection read after 48 to 72 h], anteroposterior and lateral chest radiographs and two gastric aspirates for *M. tuberculosis* culture. Gastric aspiration was performed in the early morning before feeding and without adding any lavage fluid. Sodium bicarbonate was added to the specimens to neutralize gastric acid. Culture for *M. tuberculosis* was by a radiometric method (Bactec). Erythrocyte sedimentation rate was determined, and HIV serology with enzyme-linked immunosorbent assay (ELISA) was done on all children with informed consent and pre- and posttest counselling of the parent. An area of induration ≥15 mm after Mantoux skin testing was regarded as significantly positive in accordance with World Health Organization criteria given that >90% of children in this area receive BCG.[21]

Bronchoscopy was performed only when the chest radiograph was abnormal and showed persistent non-resolving opacification, but was not suggestive of TB.

The children were reevaluated after 2 months when all the relevant results including gastric aspirate culture results and, where indicated, bronchoscopy and follow-up chest radiograph were available. Children were classified as noninfected, infected or diseased. Non-infected children were asymptomatic, had a non-significant (<15 mm induration) Mantoux test, normal chest radiograph and negative cultures for *M. tuberculosis*. Children with a Mantoux test of ≥15 mm who were asymptomatic had a normal chest radiograph or only calcifications in the lung parenchyma or regional lymph nodes on the chest radiograph, and negative cultures were regarded as having infection and not disease.[22] Diseased children were those who on chest radiograph had clearly visible, well defined hilar/mediastinal adenopathy, miliary TB or endobronchial TB (as evidenced by hilar or mediastinal adenopathy with bronchial compression or segmental/lobar consolidation or both), those with adenopathy identified by bronchoscopy or those with a positive culture for *M. tuberculosis*. The chest radiograph and bronchoscopy findings could be present with or without a positive Mantoux test with or without positive cultures for *M. tuberculosis*.

All infected children and all children <2 years of age who had received no previous treatment or chemoprophylaxis of any kind for TB were given chemoprophylaxis with high dose isoniazid 15 to 20 mg/kg/day, pyrazinamide 25 to 35 mg/kg/day, ethionamide 10 to 15 mg/kg/day and/or ethambutol 15 to 20 mg/kg/day for 6 months, the latter two drugs depending on the susceptibility of the MDR *M. tuberculosis* strain of the adult index case. Infected children who had received previous TB treatment or chemoprophylaxis with isoniazid with or without rifampin/pyrazinamide were not prescribed another course of chemoprophylaxis. Children who had TB were treated with at least two drugs to which the

adult index case's organism was susceptible. They were treated with a 4 or 5 drug regimen (isoniazid, pyrazinamide, ethionamide, ethambutol, and ofloxacin) for 9 to 12 months. All children are included in a follow-up study to establish whether this treatment and duration of therapy are sufficient.

Children older than 5 years of age and adults were not assessed as part of this study, but were referred to the local authority health clinics in their area.

Categorical data were analysed by the chi square test to compare groups, and Fisher's exact test was applied where appropriate. Chi square for trend was performed on data in Table 4. Statistical analysis was done using Epi-Info version 6.03.

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Stellenbosch, and informed written consent was obtained from the parent or guardian for participation in the study.

RESULTS

During the three-year period of the study, 75 index cases with 128 childhood contacts < 5 years of age were identified and studied. The median age of the index cases was 30.5 years (range, 16 to 63 years; mean age, 33.2 years) and the male:female ratio was 1:1.2. Index cases were not routinely tested for HIV, but 3 of the mothers (with 4 childhood contacts) were HIV infected. Only 13 (17%) index cases, all women, had primary drug resistance.

All 128 childhood contacts were evaluated. Of these, 116 (91%) lived in the same dwelling and 12 (9%) stayed in the cluster of houses on the same plot. Ninety-nine children (77%) were in contact with sputum smear positive index case and 29 (23%) with a smear negative but culture positive MDR TB index case. Thirty nine percent of adult source cases were resistant to isoniazid and rifampin only, whereas 44 (59%) were resistant to at least

isoniazid, rifampin and streptomycin. Resistance patterns of the source cases and the number of children in contact with them are summarized in Table 1.

In 47 (37%) children the index case was a parent, in 22 (17%) it was a grandparent and other family members, mainly uncles, were the index case in 32 (25%) of children. Boarders, caretakers and people living on the same plot were the index case for the remaining 27 (21%) children.

Fifty (39%) children (34 of 75 households) had contact with other known adult pulmonary TB cases in the same household at the same time. Of these, 17 (34%) were in contact with known drug susceptible cases, 26 (52%) were in contact with another MDR TB case and in 8 (16%) the susceptibility pattern was unknown.

Table 1: Drug resistance patterns of index cases and number of children contacts

Resistance Pattern to First Line Drugs	No. of Index Cases (n = 75)		No. of Children in Contact (n = 128)	
Isoniazid, rifampin	29	(39)*	46	(36)
Isoniazid, rifampin, streptomycin[†]	31	(41)	54	(42)
Isoniazid, rifampin, ethambutol[‡]	2	(3)	2	(2)
Isoniazid, rifampin, streptomycin, ethambutol[§]	13	(17)	26	(20)

*   Numbers in parentheses, percent.
†   Additional resistance to second line antituberculosis drugs in 3 (10%) index cases (kanamycin 2; thiacetazone 1; cycloserine 1).
‡   Additional resistance to second line antituberculosis drugs in 1 index case (kanamycin).
§   Additional resistance to second line antituberculosis drugs in10 (77%) index cases: 1 to 3 drugs (ethionamide 3, thiacetazone 9, cycloserine 3, kanamycin 2, ofloxacin 2).

The children, 59 boys and 69 girls, had a median age of 27 months (range, 1 to 60 months, mean age 29.25 months). Of the 128 children 62 (48%) had received no previous chemoprophylaxis or treatment and 46 (36%) had previously been given chemoprophylaxis,

but 30 (65%) of these either defaulted within the first month or received isoniazid for only 3 months. In 20 children (16%) treatment for TB with isoniazid, rifampin and pyrazinamide were previously prescribed for 6 months, but 6 (30%) defaulted after 3 to 4 months of treatment.

One hundred nineteen children (93%) had received BCG [BCG scar on arm seen in 84 (71%), no BCG scar but administration of BCG noted on child's clinic card in 35 (29%)] and 1 child did not receive BCG. In 8 children no BCG scar was present and the Road to Health clinic card could not be found.

All children were primarily evaluated because there was an adult household member with MDR pulmonary TB. Some children, however, did have symptoms and these together with the clinical signs and special investigations are summarized in Table 2. The Mantoux skin test was done and read in all children and was positive (≥ 15 mm) in 76 (59%). Chest radiograph features were not statistically analysed because some features like lymphadenopathy and opacification were used as criteria for diagnosis of disease. The lobar and bronchopneumonic opacification in the children classified as noninfected and infection only responded to antibiotics and was therefore considered to be pneumonia.

Bronchoscopy was done in six children and confirmed the presence of adenopathy in three. *M. tuberculosis* was cultured from gastric aspirates in three children, none of whom had received previous TB prophylaxis. Susceptibility testing of the *M. tuberculosis* strains cultured from the children's gastric aspirates was identical to that of the adult source case in two instances. The third child's isolate was resistant to isoniazid, rifampin, streptomycin, ethambutol and ethionamide whereas the isolate from the adult contact was resistant to isoniazid, rifampin and streptomycin only.

After investigation we classified 47 (37%) children as noninfected, 66 (51%) as infected only and 15 (12%) as having disease. Ten children in the disease group had a Mantoux result of ≥15 mm, in one the Mantoux test was ≥10 to 14 mm and in 4 children, one of which was HIV-infected who previously had a positive tuberculin skin test (Tine), the Mantoux result was <5 mm. The diagnostic features in the diseased children were hilar or mediastinal adenopathy in 10 children (identified on bronchoscopy only in one child) and endobronchial TB in 2 children. Three children who had not previously received antituberculosis drugs had a positive culture for *M. tuberculosis*. Two of them had adenopathy on chest radiograph and in the remaining child the chest radiograph appeared normal. Because none of the children had received previous prophylaxis or treatment appropriate for drug-resistant TB, the children were divided into 3 groups according to whether they had received previous TB prophylaxis or treatment or not to detect any difference in the prevalence of infection or disease. The final classification taking into account previous TB chemoprophylaxis or treatment is summarized in Table 3.

Table 2: Clinical features and special investigations in childhood contacts

Clinical Feature or Special Investigation	Noninfected (n = 47)		Infection Only (n = 66)		Diseased (n = 15)	
Fever/sweating at night	2	(4)*	5	(8)	3	(20)
Cough >14 days	8	(17)	7	(11)	6	(40)†
Documented failure to thrive‡	10/41	(24)	12/57	(21)	7/12	(58)†
Weight <3rd centile§	2	(4)	6	(9)	3	(20)
Lymphadenopathy	11	(23)	10	(15)	4	(27)
Respiratory signs (rhonchi, crepitations, wheezes)	4	(9)	7	(11)	4	(27)
Hepatomegaly ≥2 cm	4	(9)	5	(8)	3	(20)
Splenomegaly ≥2 cm	3	(6)	1	(2)	1	(7)
Mantoux ≥15 mm	0		66	(100)	10	(67)
ESR >50 mm/h	4	(10)	7	(11)	5	(33)†
CXR features						
Lymphadenopathy	0		0		12	(80)
Lobar/segmental opacification	0		1	(2)	3	(20)
Bronchopneumonic opacification	1	(2)	2	(3)	3	(20)
Perihilar opacification	1	(2)	9	(14)	3	(20)
Calcified parenchymal focus and/or nodes	0		10	(15)	0	

*   Numbers in parentheses, percent.

† $P < 0.05$; chi-square test with Fisher's exact. "Diseased" group compared with "noninfected" and "infection only" groups combined. CXR features not compared (see text).

‡   Weight loss or failure to gain weight in >3 months.

§   NCHS centile chart.

CXR, chest radiograph; ESR, erythrocyte sedimentation rate.

Table 3: Age and diagnosis according to previous TB prophylaxis or treatment

Characteristic and Final Diagnosis	No Previous Prophylaxis or Treatment (n = 62)		Previous Prophylaxis (n = 46)		Previous Treatment (n = 20)	
Defaulted or insufficient prophylaxis/reatment	0		30	(65%)	6	(30%)
Median age in mo. (range)	22,5	(1-60)	22,5	(1,5-60)	48	(14-60)
Noninfected	27	(43%)	18	(39%)	2	(10%)
Infected only	24	(39%)	25	(54%)	17	(85%)
TB disease	11	(18%)	3	(7%)	1	(5%) *
Positive cultures	3		0		0	

* HIV-infected patient.

There was no statistically significant difference between the number of patients with disease in each group, but there was a trend towards more disease in those children who had not received any previous antituberculosis drugs. Of further significance is that all 3 culture-confirmed TB cases were from the group who received no previous chemoprophylaxis or treatment. The one child with disease in the previously treated group was the only child in the study who was HIV-infected.

Although the percentage of diseased children stayed constant with increasing age, the number of infected children increased markedly (Table 4).

HIV infection was confirmed in only one child, with three different HIV enzyme-linked immunosorbent assay serology tests positive at 36 months of age. Three other children

initially had positive HIV serologic tests, but they were clinically well and HIV serology became negative before 12 months of age.

Table 4: Mantoux result and diagnosis by age

Age (mo.)	No. of children	No. with Mantoux Result		Diagnosis		
		10-14 mm	≥ 15 mm	No. non-infected	No. infected	
					Infected only	Diseased
<12	29	5 (17)†	10 (34)	18 (62)	8 (28)	3 (10)
12-<24	29	2 (7)	13 (45)	14 (48)	11 (38)	4 (14)
24-<36	18	1 (6)	11 (61)	7 (39)	9 (50)	2 (11)
36-<48	25	1 (4)	19 (76)	4 (16)	18 (72)	3 (12)
48-60	27	0 (0)	23 (85)	4 (15)	20 (74)	3 (11)

* Chi-square for trend test for tuberculin positivity by age categories: Mantoux ≥10 mm, $P < 0,001$; Mantoux ≥15 mm, $P < 0,001$; combining infected + diseased, $P < 0,001$.

† Numbers in parentheses, percent.

DISCUSSION

Several studies have shown that isoniazid-resistant and multidrug-resistant organisms can be as infectious as drug-susceptible *M. tuberculosis*.[3, 13, 16, 23] The presence of infection in contacts is usually evaluated by tuberculin skin testing. In our study 81 (63%) childhood contacts of adults with MDR pulmonary TB were infected or had disease. This is significantly higher than ($P < 0.02$) the 48% rate of infection and disease found in a similar study in childhood contacts of mainly drug-susceptible adult TB cases in the same area.[14] The disease rate was, however, higher in the latter study (34% *vs.* 12%). No data on previous prophylaxis or treatment in this drug-susceptible TB group were collected, and the number of households with >1 adult TB case was not systematically determined.

The cutoff for a positive tuberculin test in South Africa and other developing countries where BCG is routinely given is ≥15 mm induration. This differs from the recommended cutoff of >5 mm induration for children in contact with an adult with contagious TB and ≥10 mm for all children <5 years of age used in the US. It is therefore possible that in our study some children might have been misclassified as noninfected.

In a controlled trial Snider et al.[13] also found the infection rate to be higher in the drug-resistant TB contacts than in the drug-susceptible TB contacts. This higher rate of infection could be explained in several ways, even in children younger than 5 years of age where a positive tuberculin test usually indicates recent infection. In children living with adults with MDR pulmonary TB, it is possible that the child was already infected before the adult developed acquired drug resistance or that because of the delay in making a diagnosis of drug resistance, the children had prolonged contact with adults chronically "expectorating" *M. tuberculosis* organisms. In our study and earlier ones[14, 15] it is also clear that in an area with high TB incidence, often more than one adult in a household has or recently had TB, and it need not necessarily be drug-resistant TB.

The disease rate was relatively low in this study, possibly because there were children who had previously received prophylaxis and treatment. Because of small numbers there was no significant difference in the number of disease cases in those who previously received antituberculosis drugs and those who did not. It is interesting, however, that all three culture-proven cases arose among those children who had not received any previous treatment with antituberculosis drugs. A disturbing finding is that even in those children in whom the index case's drug susceptibility pattern was known, it was not considered in the choice of antituberculosis drugs given to the children either as chemoprophylaxis or treatment. A further problem was that the drug regimen used for prophylaxis (isoniazid for 3 months only)

was inadequate for protection against TB disease and was further compromised by poor compliance amongst the 36% of children receiving chemoprophylaxis.

Three children had a positive *M. tuberculosis* culture, representing 20% of those who had disease. All were drug-resistant and in two the drug resistance pattern matched that of the adult index case, suggesting transmission of MDR *M.tuberculosis* from the adult index cases to their child contacts. In the third patient the child's *M. tuberculosis* strain was not the same as that of the index. The reason for this difference in drug susceptibility is unclear, and we were unable to detect any other adults with MDR TB among the child's household contacts.

There was no difference in disease rate or infection rate in the children who were in contact with smear-positive or culture-positive adult pulmonary TB cases. This could be the result of low numbers in the study, other adults in the same household with smear-positive TB (many adults were never screened) or the fact that sputum samples were not obtained from the index case at a time that they were smear-positive (taking into account the potential long duration of disease).

Although all children were screened for TB primarily because they were contacts of adult MDR PTB, some did have clinical symptoms and signs compatible with TB disease. The most significant of these was a documented failure to gain weight for a period of ≥3 months and a history of cough for >14 days. These are indicators for the diagnosis of TB used by the World Health Organization.[24]

Taking into account that a significant proportion of adults in this community probably develop tuberculosis as a result of reactivation rather than reinfection,[25] the very high infection rate in our childhood MDR TB contacts is disturbing. With poor chemoprophylaxis and increasing HIV infection in the community, this may lead to an increasing pool of MDR

TB-infected patients in the next generation who will develop MDR TB disease. It may therefore be rewarding to concentrate on the childhood contacts of adult MDR TB cases with regard to chemoprophylaxis and compliance. We would suggest that more attention be paid to the drug susceptibility pattern of the adult case's *M. tuberculosis* culture in choosing the chemoprophylactic drugs in children.

In a study of MDR *M. tuberculosis* isolated from adult patients in our area half of the isolates were susceptible to isoniazid concentrations easily achieved in most children.[26] Earlier studies in adults have also provided evidence of a clinical response to isoniazid in patients with primary drug resistance,[27, 28] whereas a slight but significant response was reported recently in the early bactericidal activity of isoniazid despite primary isoniazid resistance.[29] These findings suggest that isoniazid may still be of value in chemoprophylaxis and treatment despite documented isoniazid resistance. We do, however, suggest that a larger daily dosage of 15 to 20 mg/kg be used and this is well-tolerated by young children.[30] This should be combined with pyrazinamide and one or two other drugs to which the adult strain is susceptible, e.g. ethambutol (only 20% resistance in this study), ethionamide (tolerated better in children than in adults) and/or a fluoroquinolone such as ofloxacin or ciprofloxacin. Chemoprophylaxis should continue for at least 6 months and should be administered under direct observation only. We preferred to keep the currently available fluoroquinolones in reserve for the treatment of tuberculosis in that they are thought not to be ideal for the sterilization of lesions.[31] Although the fluoroquinolones are contraindicated for use in small children, they are often used for treatment of resistant organisms. Serious adverse effects are rare.[32] A 30 month follow-up study of these patients is in progress.

This study documents the transmission of MDR *M. tuberculosis* to childhood contacts, the development of disease in these contacts and the importance of knowing the index case's

M. tuberculosis susceptibility pattern in choosing a regimen for chemoprophylaxis or the treatment for the childhood contact.

A further disturbing factor is the prevalence of 1.1% MDR TB among new adult cases of TB pre-HIV, a situation that is likely to deteriorate as HIV-infected patients are increasingly exposed to MDR TB. Preventive and curative therapy options for children exposed to adult MDR TB cases are quite limited and unresearched. Therefore the development of MDR TB in adults and the subsequent transmission to children should be prevented. The first step to achieve this is to identify new adult TB cases in the community and to give them combination therapy under direct observation.

ACKNOWLEDGEMENTS

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CHAPTER 5

Transmission of multidrug-resistant tuberculosis

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Transmission of multidrug-resistant tuberculosis

Aim: To compare the *Mycobacterium tuberculosis* isolates of adult index cases with multidrug-resistant (MDR) tuberculosis to the isolates obtained from their child contacts.

Patients and methods: A 4-year prospective study in the Western Cape Province of South Africa. We evaluated 149 child contacts of 80 adult MDR pulmonary tuberculosis cases. This report includes those cases where a culture for *M. tuberculosis* was obtained from both the adult source case and child contact. Isolates were compared by drug susceptibility pattern and restriction fragment length polymorphism analysis.

Results: Six adult-child pairs with cultures for *M. tuberculosis* were identified. Two children had contact with more than one adult tuberculosis case. One child received previous isoniazid prophylaxis. Drug susceptibility pattern and restriction fragment length polymorphism analysis were identical for five adult-child pairs. One child, with no other known source case, had a strain different from that of the identified source case, but the MDR *M. tuberculosis* strain with which he was infected was prevalent in the community in which he resided. All children responded well to treatment.

Conclusion: This study confirms that most of the childhood contacts of adults with MDR tuberculosis are likely to be infected by these MDR source cases despite their exposure to other drug susceptible adults with tuberculosis in some instances. Child contacts of adults with MDR tuberculosis should be treated according to the drug susceptibility patterns of the likely source cases' *M. tuberculosis* strains unless their own strain's susceptibility testing indicates otherwise. Contact tracing remains of fundamental importance in identifying children at risk.

INTRODUCTION

Drug-resistant *Mycobacterium tuberculosis* strains had been postulated to be less infectious and to be less likely to cause disease than their drug-susceptible counterparts[1] but recent studies have shown them to cause infection and disease as often as drug-susceptible organisms.[2-4] It is further presumed that a person in contact with both drug-susceptible and drug-resistant source cases will be less likely to develop drug-resistant tuberculosis (TB).[5] When young children develop drug-resistant TB, it is most likely primary resistance caused by transmission.[6,7] Studies have compared the characteristics of *M. tuberculosis* strains from children in contact with adults with single and multiple (excluding rifampin) drug-resistant TB by drug susceptibility patterns only.[2,3] These studies found a high degree of correlation between the susceptibility patterns of the source cases and the child contacts.

Restriction fragment length polymorphism (RFLP) analysis is a useful epidemiologic tool to trace the transmission of particular strains of *M. tuberculosis* during investigations of outbreaks mainly in large epidemiologic studies in adults.[8,9] It has been used in a nosocomial outbreak of TB in pediatric wards[10] and to prove adult to child transmission of drug-susceptible TB.[11] The aim of this study was to compare the isolates of adult index cases with multidrug-resistant (MDR) TB and their child contacts by drug susceptibility pattern as well as by RFLP analysis.

PATIENTS AND METHODS

This prospective study was conducted between April, 1994, and March, 1998, in the Western Cape Province of South Africa, an area with a reported tuberculosis notification rate of 589 new cases per 100 000 population per year in 1998 (data from Department of

Health: Directorate Health Systems Research and Epidemiology). For the purpose of this study an index case was defined as an adult >15 years of age with a sputum culture positive for MDR *M. tuberculosis*, i.e. resistance to at least isoniazid and rifampin. Child contacts were defined as children who, at the time of diagnosis of the index case, had lived in the same house or on the same residential plot as the index case for at least 1 month.

Adult-child pairs described in this report are those in whom a culture of *M. tuberculosis* was obtained from both the index case and the child contact. Information collected regarding the adult index cases included age, their relation to the childhood contact, history of prior TB treatment, HIV status, results of acid-fast bacilli smear (AFB) and culture for *M. tuberculosis* and the drug susceptibility pattern of the *M. tuberculosis* culture. Childhood contacts were evaluated regarding age at diagnosis, duration of contact with the smear-positive index case, HIV status, treatment and outcome. Clinic records were reviewed to ascertain whether other household members also had active pulmonary tuberculosis.

Cultures from adult index cases and child contacts were obtained at different times and in different laboratories. Drug susceptibility testing was performed by the economic variant of the indirect proportion method by the South African Institute for Medical Research and drug resistance was defined as 1% or more bacterial growth.[4] Quality assurance for drug susceptibility results is done locally with every batch and quarterly by the national tuberculosis reference laboratory.

At a tertiary hospital in the province, 149 child contacts of 80 index cases were evaluated during the study period and 2 gastric aspirates or sputum specimens were obtained from each child for culture of *M. tuberculosis*. Eight children had confirmed TB.

Seven children had a positive culture for *M. tuberculosis* of which one isolate was contaminated and could not be processed further. The diagnosis was confirmed by Ziehl-Neelsen stain of a lymph node biopsy in the remaining child, and no tissue was sent for culture of *M. tuberculosis*.

Isolates from index cases and child contacts were genotyped by RFLP analysis performed by the internationally standardized method based on the IS6110-3' probe.[12] To enhance the accuracy of strain typing, especially in cases of low IS6110 copy numbers, three additional probes were used (ECL labeled IS*6110*-5', DRr probe for *Pvu*II digests and ^{32}P-labeled MTB484(1) probe for H*inf*I digests).[13]

Child contacts were treated for 9 to 18 months according to the adult index case's susceptibility pattern with at least two drugs to which the index case was susceptible (Table 1).

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Stellenbosch. Written informed consent was obtained from the parents of the children for inclusion in the study.

RESULTS

During the 4-year period six adult-child pairs were identified (Table 1).

Except for Case 1 all adult index cases lived in the same house and were related to the child contact. The duration of contact of the child with the AFB smear-positive index case varied from 2 months to >1 year. Two children had additional adult household members with active TB. In Case 6 the child's uncle had MDR TB and the child's father had AFB smear-positive drug-susceptible TB. In Case 5 where the child's maternal

grandmother had MDR TB, the child's father had smear- and culture-positive TB, but no drug susceptibility test was done on the isolate. The paternal grandmother in case 5 who also lived in the same house had MDR TB with the same resistance pattern as the index case and child.

The median age at diagnosis of TB in the child contacts was 32 months (range, 7.5 months to 15 years). All children received *Bacillus Calmette-Guerin* (BCG) at birth. Only one child received prophylactic antituberculosis treatment 7 years before the diagnosis of MDR TB. All children and the 4 adults in whom HIV serology was done, were HIV negative.

The drug susceptibility and RFLP pattern of the *M. tuberculosis* isolates infecting adult and child was the same in five of the six cases. The RFLP pattern was identical with respect to both the number and molecular size of all bands using all four probes in five of the six cases implying transmission of a multidrug-resistant *M. tuberculosis* strain from adult to child in these five cases (Fig 1). In one case (Case 1) the adult was infected with a different *M. tuberculosis* strain resistant to isoniazid, rifampin and streptomycin, whereas the organism infecting the child had in addition ethambutol and ethionamide resistance. This implies that the child was infected by a different strain from an unknown source as proved by RFLP (Fig 1, Lane 1). The use of three additional probes showed that the isolates from Adult-Child Pair 1 are quite different from each other and that this is not simply a gain or loss of one IS6110 locus which may sometimes occur.

The one child with a different strain (Case 1) was inappropriately treated because the child, owing to technical difficulties experienced with the drug resistance testing of the child's strain, was treated according to the adult index case's results. The drug

susceptibility pattern of the infecting strain was eventually found to be different, with the child's isolate being resistant to more drugs than the strain of the suspected source case. However, clinically and on chest radiography the child improved, and for this reason further treatment was considered unnecessary. No relapse occurred during the 30 months of follow-up.

TABLE 1: Summary of adult-child pair data

Adult/Child Pair	Adult MDR Index Cases				Child Contacts									
	Relationship to child	Same house/ plot	Minimum duration smear-positive contact (mo)	Resistance pattern	Other household contacts with TB	Age at diagnosis (mo)	Prior anti-TB drugs	Mantoux result (mm)	Resistance pattern	Chest radiograph at diagnosis	Treatment	Duration follow-up	Chest radiograph at last follow-up	RFLP pattern (pairs)
1. Cm-CW	Boarder	Plot	2	H, R, S	No	32	No	17	H, R, S, E, Eth	Hilar nodes + perihilar opacification	H, Z, E, Eth for 9 mo	30	Normal	Different
2. AH-MH	Mother	House	2	H, R, S	No	7.5	No	15	H, R, S	Perihilar opacification	H, R, Z, E, Eth for 9 mo	36	Calcified hilar node	Same
3. RdV-EdV	Father	House	>12	H, R, S, E, T	No	180	Isoniazid prophy-laxis 7 yr ago	Not done	H, R, S, E, T	Cavities left upper lobe	H, Z, K, T O for 8 mo + HZTO for 8 mo	30	Fibrosis left upper lobe, residual pneumatoceles	Same
4. NP-BP	Aunt	House	10	H, R, S	No	9.5	No	18	H, R, S	Paratracheal + hilar nodes and segmental opacification	H, Z, E, Eth, O for 9 mo	18	Normal	Same
5. SdP-JdP	Grandmother	House	>12	H, R, S	Yes	54	No	14	H, R, S	Perihilar opacification	H, Z, E, Eth, O for 6 mo (then defaulted treatment)	30	Normal	Same
6. CA-RA	Uncle	House	3	H, R	Yes	32	No	40	H, R	Hilar nodes + segmental lesion	H, Z, E, Eth, O for 12 mo	30	Normal	Same

H, Isoniazid; R, Rifampicin; S, streptomycin; E, ethmabutol; Eth, ethionamide; O, olfloxacin; T, thiacetazone; K, kanamycin
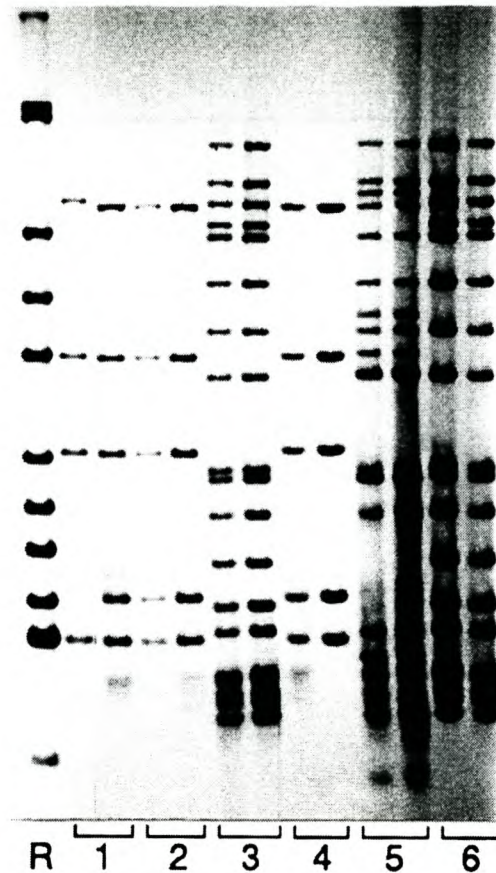
FIG 1. RFLP (IS6110-3' probe) pattern of the infecting *M. tuberculosis* strain of six children (first lane of each of six pairs) and the corresponding adult MDR TB source case (second lane of each of six pairs). *Lane R* corresponds to the control strain MTB 14323.

None of the children had serious side effects from the antituberculosis drugs used. Ethionamide caused some gastrointestinal side-effects, but in all five children in whom it was used it could be continued for the full duration of treatment. Four children, of whom three were <5 years of age, received ofloxacin (15 mg/kg/day) for 6 to 16 months with no clinical side effects. All children were clinically well after their treatment and remained so at median follow-up of 30 months.

Three of the adult index cases died during the study (two of TB and one from a gunshot), two were still culture-positive at the end of the study (both defaulting treatment) and one was cured.

DISCUSSION

As far as we could determine this is the first prospective study making use of drug susceptibility patterns and RFLP analyses to confirm transmission of MDR TB from adult source cases to their childhood contacts.

In a study of 29 cases of isoniazid- and/or streptomycin-resistant TB in children and/or their source cases, Steiner et al.[2] found that the drug susceptibility patterns of source cases and children were similar in 69% (n = 20). In our study 5 of 6 pairs (83%) had the same multidrug-resistant *M. tuberculosis* strain as shown by susceptibility and RFLP analysis, and this despite the fact that in 2 households there were other adults with presumably drug-susceptible TB. In the one case (Case 1) in which the strains were different, no other source case could be identified even after a detailed social history. The strain with which the child was infected is, however, part of a cluster of MDR TB strains circulating in the community in which the child resides. This implies that transmission of MDR tuberculosis to children can occur through unknown contact.[14]

Four of the six adult-child pairs (Pairs 1 to 4) come from a well-studied community while two pairs are from geographically distant communities. In the well-studied community, only eleven five-bander isolates (during a 5-year period) have been detected with the genotype presented, of which five are shown in this report. (unpublished data). For this reason it is more likely that transmission occurred within the household than within the community.

The long duration of smear-positive contact in this study was probably a result of insufficient contact tracing. It emphasizes the importance of contact tracing especially in young children where the incubation period can be as short as 6 to 8 weeks and who are in danger of developing disseminated and more severe forms of disease.[15] Treatment for infection and disease should be initiated as soon as possible.[15]

Transmission of MDR TB from adults to their child contacts has important implications for the management of these children. Young children ≤5 years of age should be considered for prophylactic therapy after active TB has been excluded.[5] Isoniazid is the only drug proven to be effective as prophylactic therapy, and rifampin is recommended as an alternative in cases of isoniazid resistance.[5,16] However, both these drugs are probably of limited value in prophylactic therapy in MDR TB contacts. No controlled trial has evaluated any prophylactic regimen for contacts of MDR TB cases. A potential prophylactic regimen could be a combination of pyrazinamide and ethambutol or pyrazinamide and a fluoroquinolone for a minimum period of 6 months.[5,16]

The treatment of MDR TB in children is a controversial issue. It has been postulated that persons exposed to several sources of *M. tuberculosis*, including infectious TB patients with drug susceptible *M. tuberculosis*, are less likely to have been infected with a MDR strain than are those whose only known exposure to TB was to an infectious MDR TB case.[5] The presumed intermediate to low risk has not been confirmed by our study and we suggest that child contacts of infectious MDR index cases should be treated according to the drug susceptibility pattern of the MDR TB index cases and not the drug-susceptible source case. These children should be treated with at least two or three drugs to which the *M. tuberculosis* strain of the child or adult source case is susceptible. However, much uncertainty exists as to which drugs to use and for what

duration. Ethionamide is much better tolerated in children than in adults and therefore plays an important role in the treatment of MDR TB in children.[7] No serious side effects were experienced by our patients. Ethambutol is a valuable drug in the treatment of drug-resistant TB and no case of optic neuritis has been reported in a child.[17] Eyesight should still be tested when possible. On the grounds of the effect of the quinolones on cartilage growth in animal studies, it is suggested that the quinolones should not be used in children.[17] We found no side effects in the four children treated with quinolones for up to 16 months.

The suggested duration of treatment in adults and children with MDR TB is 12 to 24 months.[16,17] The number of *M. tuberculosis* organisms found in caseating lung lesions in children is, however, much less than in the cavitatory lung disease of adults and probably not many more than the number of organisms found in children who are infected but do not have disease.[17] In this study, we treated children without cavitatory disease for 9 to 12 months, and they have had satisfactory weight gain and no sign of relapse on chest radiograph after 18 to 36 months.

This study confirms that most of the childhood contacts of adults with MDR TB are likely to be infected by these adult MDR source cases despite their exposure to other drug-susceptible adults with TB in some instances. Contact tracing remains of fundamental importance in identifying children at risk. We recommend that appropriate samples for *M. tuberculosis* cultures and susceptibility testing be taken from all children in close contact with adults MDR pulmonary TB cases. However, if the child contact's *M. tuberculosis* culture is not available, we suggest that children in contact with adults who have MDR TB should be treated according to the drug susceptibility patterns of the source cases' *M. tuberculosis* strains.

ACKNOWLEDGEMENTS

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CHAPTER 6

Evaluation of young children in contact with adult multidrug resistant pulmonary tuberculosis: A 30-month follow-up

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Evaluation of young children in contact with adult multidrug resistant pulmonary tuberculosis: A 30-month follow-up

Setting: The Western Cape Province of South Africa, an area with a high tuberculosis (TB) incidence, where initial multidrug resistance (MDR) among adult TB cases was 1.1% during 1992-1993.

Objective: To determine the long-term prevalence of TB infection and disease in children in household contact with adults with MDR pulmonary TB, and to establish the efficacy of chemoprophylaxis in preventing disease in these children.

Method: Children < 5 years old in contact with 73 MDR TB adults were evaluated. Disease was treated by prescribing at least 2 drugs to which the adult's strain was susceptible. The remaining children were classified as infected or noninfected and received chemoprophylaxis according to the index's strain susceptibility or were followed up and treated when indicated. All were followed up for 30 months.

Results: At the initial evaluation 125 children were seen, median age 27.5 months. Of these, 119 were followed up. Fourteen (12%) had disease, 61 (51%) were infected only and 44 (37%) were noninfected. By 30-month follow-up, 29 (24%) had developed disease and 64 (54%) were infected only. Four adult-child pair *M. tuberculosis* isolates were compared by DNA fingerprinting; 3 were identical. All children who developed TB disease were clinically cured. Two (5%) of 41 children who received appropriate chemoprophylaxis and 13 (20%) of 64 who did not, developed TB during follow-up. (odds ratio: 4.97)

Conclusion: The study confirms MDR TB transmission to childhood contacts. Seventy-eight percent of children were infected or developed disease. Appropriate chemoprophylaxis may prevent disease in these children.

INTRODUCTION

Tuberculosis (TB) contact tracing has produced a significant yield of new TB cases and newly infected patients in the past.[1] Children in close contact with drug-susceptible adult pulmonary TB have a high risk of becoming infected and developing disease.[2,3] It is generally accepted that 30 to 50% of household contacts of adults with infectious forms of pulmonary TB will become infected.[3] The risk for young children with untreated infection to develop TB is up to 43% in children <1 year of age and about 24% for children 1 to 5 years of age.[3] Little is known, however, about the long-term outcome of children in contact with multidrug-resistant (MDR) adult pulmonary TB cases. Although studies in guinea pigs suggested that isoniazid-resistant strains are less infectious and cause less disease than the drug-susceptible strains, this diminished infectiousness and pathogenicity was not confirmed in human studies.[4,5] The management of adults or children in contact with infectious MDR pulmonary TB cases is still very uncertain and, although many suggestions for different regimens for MDR chemoprophylaxis have been made, there are no prospective studies to verify their effectiveness.[6] Furthermore, the optimal duration of chemoprophylaxis with these drugs is uncertain.[6,7] On the other hand, the implications of not being able to give adequate chemoprophylaxis to children infected with MDR strains of *Mycobacterium tuberculosis* are serious, because about 10% or more of infected children will develop TB disease in their lifetime, and they will have the potential to continue the transmission of MDR TB in future.[8]

Drugs used in the treatment of MDR TB cases are generally much more toxic than first line drugs. In children, the use of fluoroquinolones is not generally recommended.[9]

Although their safety in short and medium term treatment have been documented, uncertainty still exists about the long-term treatment with the fluoroquinolones.[9,10]

The main purpose of this study was to determine the long-term prevalence of tuberculous infection and disease in young children in household contact with adults with MDR pulmonary TB in a geographical area with a high incidence of TB. An additional aim was to establish whether chemoprophylaxis is effective in preventing active disease in these children.

PATIENTS AND METHODS

Primary resistance was defined as resistance in *M. tuberculosis* cultures from patients with no previous TB treatment. Initial resistance is drug resistance in new TB patients but allowing for undisclosed previous TB treatment (i.e., primary resistance plus undisclosed acquired resistance). Acquired resistance is resistance found in cultures from patients who have had 1 or more previous TB treatment episodes. MDR is resistance to isoniazid and rifampin with or without resistance to other anti-TB drugs.[11]

A prospective study was conducted between April 1994 and January 2000 in the Western Cape Province of South Africa, an area with a TB incidence of 589 new cases per 100 000 population per year in 1998 (Department of Health: Directorate Health Systems Research and Epidemiology). The rate of initial resistance to isoniazid determined in adult tuberculosis cases in the Western Cape Province during 1992-1993, was 3.9% (95% confidence intervals [CI]: 3.3-4.6%) and initial resistance to both isoniazid and rifampin was found in 1.1% of isolates (95% CI: 0.7-1.4).[12] Human immunodeficiency virus (HIV) seroprevalence in women attending antenatal clinics in the Western Cape Province rose from 1.16% (95% CI: 0.76-1.56) to 5.21% (95% CI: 3.2-

7.2) from 1994 to 1998 (Department of Health: Directorate Health Systems Research and Epidemiology).

An index case was defined as an individual >15 years of age with sputum culture positive for *M. tuberculosis*, which was resistant to at least isoniazid and rifampin. The sputum specimens were processed by the South African Institute for Medical Research in Cape Town, which is responsible for the mycobacteriology services in the Western Cape. Laboratory procedures for determining drug resistance were described previously.[11] Briefly, Middlebrook 7H12 (Bactec; Becton Dickenson and Company, Sparks, MD) culture medium was used for isolation of mycobacterial strains. The niacin production test was used to identify *M. tuberculosis*. Drug susceptibility testing was performed by the indirect proportion method. The following drugs were tested at the indicated concentrations: isoniazid 0.2 μg/ml LJ; rifampin 30.0 μg/ml LJ; streptomycin 5 μg/ml LJ; ethambutol 2 μg/ml LJ; and ethionamide 20 μg/ml LJ. Resistance was defined as 1% or more bacterial growth. Quality assurance for drug susceptibility results is done locally with every batch and quarterly by the national TB reference laboratory.

Index cases were resident in suburbs surrounding the Tygerberg Hospital, a tertiary referral center for the area. Information collected regarding the adult index case included gender and age, their relation to the childhood contact, whether their sputa were smear-positive or smear-negative, the susceptibility pattern of the *M. tuberculosis* culture, and the index case's outcome after 30 months.

Childhood contacts were defined as children 5 years of age or less living and sleeping in the same house or group of clustered houses/shacks on the same residential site as the index case for at least one month.[11] Parents or guardians were asked to bring

these children to hospital for initial and follow-up evaluations, even if the parents considered the child to be asymptomatic. All the children in these households were seen.

Initial evaluation included obtaining a history regarding previous TB chemoprophylaxis or treatment and whether there were other adults in the same house who had or had recently had TB. This information was verified by contacting the local authority health clinics where patients reside. Evidence regarding recent weight loss or failure to gain in weight and documentation of BCG immunization was obtained from the patient's "Road to Health" clinic card or from clinic records.

Each child was subjected to an initial clinical examination, tuberculin skin test (Mantoux test - 5TU [0.1ml] Japanese purified protein derivative by intradermal injection read after 48-72 hours), antero-posterior and lateral chest radiographs, and two early morning gastric aspirates for *M.tuberculosis* cultures.[11] The erythrocyte sedimentation rate (ESR) was determined and HIV serology with enzyme-linked immunosorbent assay was done in all children with written informed consent and after counselling of the parent. An area of induration ≥15mm in transverse diameter after Mantoux skin testing was regarded as significantly positive in accordance with World Health Organization criteria and National TB program guidelines as > 95% of children in this area receive BCG at birth.[13] Bronchoscopy was performed only when the chest radiograph was persistently abnormal but was not diagnostic of TB. As part of the initial diagnostic evaluation, children were seen again after 2 months when the relevant results of investigations including cultures and, where indicated, follow-up chest radiograph were available.

Children were classified as noninfected, infected, or diseased. Noninfected children were asymptomatic, had a nonsignificant (<15mm induration) Mantoux test, normal chest radiograph, and negative cultures for *M.tuberculosis*. Children with a Mantoux test of ≥15mm who were asymptomatic, had a normal chest radiograph or only calcifications in the lung parenchyma or regional lymph nodes on the chest radiograph, and negative cultures were regarded as having infection and not disease.[14] Diseased children were those who on chest radiograph had well-defined hilar or mediastinal adenopathy, miliary TB or endobronchial TB (as evidenced by hilar or mediastinal adenopathy with bronchial compression or segmental/lobar consolidation or both), those with adenopathy compressing airways identified by bronchoscopy, acid-fast bacilli on biopsy histology specimen or those with a positive culture for *M. tuberculosis* from any source. The chest radiograph and bronchoscopy findings could be present with or without a positive (≥15mm) Mantoux test, and with or without positive cultures for *M. tuberculosis*. A cough lasting >2 weeks, weight loss or failure to gain adequately in weight for ≥3 preceding months, and an ESR of >50mm/hour Westergren substantiated the diagnosis of disease but was not regarded in itself as confirming the presence of tuberculous disease.

All infected children and all children <2 years of age who had received no previous treatment or chemoprophylaxis of any kind for TB were offered chemoprophylaxis with high-dose isoniazid 15 to 20 mg/kg/d, pyrazinamide 25 to 35 mg/kg/d, ethionamide 10 to 15 mg/kg/d and/or ethambutol 15 to 20 mg/kg/d and/or ofloxacin 15 mg/kg/d for 6 months, the latter 2 drugs being included depending on the susceptibility of the MDR *M.tuberculosis* strain of the adult index case. Children who had received previous TB treatment or chemoprophylaxis with isoniazid with or without rifampin/pyrazinamide

were not routinely prescribed another course of chemoprophylaxis except when it was preferred by the parent rather than follow-up only.

Children who had TB disease received individualized treatment. This consisted of a 4- or 5-drug regimen (isoniazid, pyrazinamide, ethionamide, ethambutol, and ofloxacin) that included at least 2 to 3 drugs to which the adult index case's isolate was susceptible. Prescribed duration of treatment was for 6 to 12 months, determined by extent of disease, eg, hilar adenopathy only was initially treated for 6 months (later 9 months), whereas extensive pulmonary infiltrates were treated for 12 months.

All treatment and chemoprophylaxis was given as directly observed therapy. Caregivers were expected to take the children to the local health clinic daily (5 days a week) where health care workers had to observe the children taking their treatment. When patients did not return for treatment, several home visits were made to motivate caregivers to bring the children for treatment. If despite all efforts (including motivation for hospital admission for treatment) children did not receive any treatment for >1 month, treatment was discontinued. Follow-up visits were, however, continued.

Follow-up visits were arranged for all children at 4 months, 6 months and 6-monthly thereafter to 30 months. Other evaluations occurred as were clinically indicated. Evaluations at follow-up comprised a clinical examination, Mantoux skin testing if a previous test was not significantly positive, and chest radiographs every 6 months or more often, when clinically indicated. Culture for *M. tuberculosis*, lymph node biopsy and bronchoscopy were done only when clinically or radiologically indicated.

In those cases where cultures of *M. tuberculosis* were obtained from both the adult index case and the child contact, isolates were genotyped by restriction fragment length

polymorphism (RFLP) analysis using the internationally standardized method (IS6110-3') and three additional probes as previously described.[15,16]

Children older than 5 years of age and adults were not assessed as part of this study, but were referred to the local authority health clinics in their area.

Categorical data was analysed using the χ^2 test to compare groups and Fisher exact test was applied where appropriate. Statistical analysis was done using Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA).

The study was approved by the Ethics Committee of the Faculty of Health Sciences of the University of Stellenbosch and informed written consent was obtained from the parent or guardian for participation in the study.

RESULTS

Seventy-three index cases and 125 childhood contacts <5 years of age were identified and studied. Two index cases with 3 childhood contacts identified in the original study were excluded because the 1 index case was not culture positive after the birth of the 2 children, and the second index case was subsequently shown to have a drug susceptible *M. tuberculosis* strain.[11]

The median age of the index cases was 30 years (range: 16-63 years; mean: 33.1 years) and the male:female ratio was 1:1.2. Thirteen (18%) index cases had primary drug-resistant TB. At 30-month follow-up, 24 (33%) of the index cases had died, in 2 of whom the cause was not TB-related; 8 (11%) were still smear- and/or culture-positive for MDR *M. tuberculosis*, and 5 (7%) were lost to follow-up. Thirty-six (49%) index cases were alive and culture-negative.

There was no significant age difference between the index cases of children who developed disease (median: 32 years old; range: 19-62), had infection only (median: 32 years old; range: 16-63) and those that were not infected (median: 30 years old; range: 17-60).

Forty-one households (56%) affecting 57 (46%) children had >1 adult source case, consisting of additional MDR TB source cases in 38 children (68%), drug-susceptible source cases in 19 children (28%), and cases with unknown susceptibility in 11 children (16%).

At initial evaluation of all 125 childhood contacts there were 58 boys and 67 girls with a median age of 28 months (range: 1-60 months). Of these, 14 (11%) children had tuberculous disease, 66 (53%) were infected only and 45 (36%) were not infected. Six (5%) children, 5 infected only and 1 noninfected, did not return for any follow-up and were excluded from the analysis.

One hundred nineteen children attended follow-up visits, 116 (97%) of whom were seen at the 30-month appointment. Except for the 4-month follow-up attendance of 71%, all 6-monthly evaluations had an attendance of >90%. The median number of follow-up evaluations per child was 6 (range: 1-16) with only 6 children having <4 follow-up evaluations. Twenty-three (19%) children were followed up beyond 30 months.

On follow-up, an additional 15 children (12%) developed disease by 30 months. Diagnosis at 6-month follow-up evaluations is summarized in Table 1. There was no significant difference in the rate of infection or disease by age. Twelve (80%) of the 15 children that developed disease during the 30-month follow-up, were diagnosed by 12 months, and only 1 child developed disease between 18 and 30 months.

Ninety-five (80%) children were in contact with an acid-fast bacilli smear-positive MDR TB index case and 24 with a culture only confirmed index case. Of the smear-positive contacts, 80 (84%) were infected of whom 27 (34%) developed disease and of culture only positive contacts 14 (58%) were infected of whom 2 (14%) developed disease. Infection occurred significantly more in the smear-positive group [$P = .012$; odds ratio 3.81 (95% CI: 1.28-11.36)], but disease among those infected was not significantly more frequent. ($P = .21$) Ten (40%) of 25 noninfected children had a smear-negative adult index case.

Table 1. Diagnosis at follow-up evaluations*

TB diagnosis	TB disease	Infected only	Noninfected	Lost to follow-up
Initial evaluation	14 (11%)	66 (53%)	45 (36%)	
6-month evaluation	24 (19%)	58 (46%)	37 (30%)	6 (5%)
12-month evaluation	26 (21%)	62 (49%)	31 (25%)	6 (5%)
18-month evaluation	28 (22.5%)	63 (50%)	26 (21%)	8 (6.5%)
24-month evaluation	28 (22.5%)	65 (52%)	24 (19%)	8 (6.5%)
30-month evaluation	29 (23%)	64 (51%)	23 (19%)	9 (7%)

*n = 125

Continued contact with the adult index case after the initial evaluation was shorter for noninfected children (median: 2 months; range: 1-7 months) than for infected only ($P = .002$; Kruskal-Wallis test) and diseased children ($P = .04$; both groups, median: 4 months; range: 1-30 months).

Tuberculosis was confirmed in 6 (21%) of 29 children with disease, 5 by culture of *M. tuberculosis* and 1 by finding acid-fast bacilli on microscopy of a peripheral lymph node biopsy. Drug susceptibility testing was done in 4 cases and these isolates were all MDR. The 1 isolate not tested was contaminated and the histology specimen was regrettably not submitted for culture. RFLP analysis was performed on 4 adult-child pair isolates, 3 of which were identical. In 1 adult-child pair the drug susceptibility pattern and RFLP were dissimilar, but both were MDR and a community infection was likely in the child.[11]

Of the 29 children diagnosed with tuberculosis, 27(93%) had hilar lymphadenopathy on chest radiography, 16 of whom had other parenchymal or pleural changes. A Mantoux of ≥15mm induration was present in 22 (76%), a cough for >2 weeks in 16 (55%) and documented weight loss or failure to gain weight in 12 (41%). In 3 children hilar lymphadenopathy compressing the airways was confirmed by bronchoscopy. In 1 of these the chest radiograph did not clearly show hilar adenopathy. The ESR was >50 mm/hour in both children who did not have hilar lymphadenopathy on chest radiography. No child had disseminated disease.

At the initial evaluation, calcification was present on chest radiography in 10 (8%) of 119 children, all of whom were tuberculin skin test-positive. Subsequently, a further 29 (24%) children developed calcification on chest radiograph. In 10 cases, in 2 of whom the Mantoux test remained 0 mm, calcifications developed 6 to 18 months after diagnosis of TB disease. In 4 children with infection only, the diagnosis was based solely on the presence of calcifications on chest radiograph. In 3 of these cases, the Mantoux test showed no reaction and in 1 an induration of 10mm was found.

Tuberculin skin test results are summarized in Table 2. Only 1 child, whose Mantoux test was 0 mm and was diagnosed as having TB disease, was HIV-infected.

Table 2. Mantoux test results during 30-month follow-up of children in contact with adult MDR pulmonary TB cases.*

Induration	0-9 mm	10-14 mm	≥15 mm
Initial evaluation	42 (35)	8 (7)	69 (58)
6-month evaluation	40 (34)	7 (6)	72 (60)
12-month evaluation	38 (32)	5 (4)	76 (64)
18-month evaluation	36 (30)	5 (4)	78 (66)
24-month evaluation	35 (29)	5 (4)	79 (67)
30-month evaluation	33 (28)	5 (4)	81 (68)

*n=119; % in parenthesis.

Individualized treatment according to the drug susceptibility pattern of the index case was prescribed in 25 (86%) of 29 children that had disease. (Table 3) Fourteen (56%) children completed a 4- to 5-drug regimen of 9 to 12 months, 6 (24%) children defaulted from a 9-month treatment course after 4 to 8 months, and 5 (20%) children, all with only hilar adenopathy on chest radiograph, completed a 6-month treatment regimen. Of the 4 children who did not receive treatment, in 3, one of whom had received previous TB treatment, the diagnosis was made retrospectively. These children were followed up but no treatment was given. The remaining child missed his 6-month follow-up and was diagnosed at the local authority health clinic and received isoniazid, rifampin and pyrazinamide for 6 months. All 29 children were clinically and radiologically well after 30 months follow-up.

Table 3. List of regimens used as chemoprophylaxis and treatment

Chemoprophylaxis regimens	(n = 41)	(%)
5 days a week for 6 months		
H Z Eth	20	(49)
H Z E	9	(22)
H E Eth	4	(14)
E Eth	2	(5)
H Z E Eth	2	(5)
Z E Eth	2	(5)
H Z Eth O	2	(5)
Treatment regimens	(n = 25)	(%)
6 H Z E Eth	4	(16)
6 H Z Eth O	1	(4)
9 H Z E Eth	8	(32)
9 H Z Eth O	2	(8)
9 H Z E Eth O	4	(16)
6 H Z E O / 6 H Z E Eth*	1	(4)
12 H Z E Eth	1	(4)
12 H Z Eth O	1	(4)
12 H Z E Eth O	3	(12)

H indicates isoniazid; Z, pyrazinamide; E, Ethambutol; Eth, Ethionamide; O, ofloxacin.
*Ofloxacin was stopped because of arthralgia.

Forty-one children received prophylaxis for MDR TB. Three- or 4-drug combinations for 6 months were prescribed. (Table 3) Isoniazid and pyrazinamide were included in almost all regimens together with ethionamide and/or ethambutol or ofloxacin. A comparison between the groups of children that did and those that did not receive appropriate prophylaxis once they were identified as MDR TB contacts is summarized in Table 4. Fifty-seven of these contacts had no other adult source case

identified. None of the 29 children who received appropriate chemoprophylaxis and 6 of 28 children who did not, developed disease during follow-up ($P = .01$; Fisher exact). Of the remaining 9 children who developed disease and did have other source cases, 6 source cases had MDR (including both cases in the chemoprophylaxis group), in 2 the source cases' susceptibility patterns were unknown and one child had an additional drug-susceptible contact.

Ethionamide was used in the treatment or prophylaxis of 61 (51%) children. Thirty (49%) children experienced gastrointestinal side effects, and the drug had to be stopped in 4 cases. There was no difference in the occurrence of side effects attributable to ethionamide in children less or more than 2 years of age.

Ofloxacin, used mainly for treatment, was administered in 15 children for durations of 6 to 12 months. Median age of onset of treatment with ofloxacin was 37 months with a range of 7 to 63 months. In combination with ethionamide, it was not possible to establish whether it caused gastrointestinal side effects. Only 1 child, a girl in whom treatment was started at 19 months old, complained of pain in the knees after 6 months, and ofloxacin was immediately stopped. The arthralgia cleared but it could not be determined whether ofloxacin was the actual cause. No radiologic studies were done to evaluate potential toxicity in any of these children.

Table 4. Comparison of children who received and those who did not receive appropriate chemoprophylaxis during this follow-up.

Characteristic	Appropriate Prophylaxis n = 41 (%)	No Prophylaxis n = 64 (%)
Age (mo)	Median: 19 (range: 1-60)[*] average: 25	Median: 31 (range: 1-60) average: 31.5
Male:Female	20:21	27:37
Previous prophylaxis	10 (24)[*]	32 (50)
Previous TB treatment	2 (5)[*]	16 (25)
Mantoux first evaluation		
≥15 mm	28 (68)[*]	33 (52)
5-14 mm	5 (12)	4 (6)
0-4 mm	8 (20)	27 (42)
Adult index case		
Smear positive	39 (95)[*]	43 (67)
Duration of contact after first evaluation (mo)	Median: 6 (range: 1-30)[*] average: 9.15	Median: 2 (range: 1-30) average: 5.0
Initial diagnosis		
Infected only	28 (68)[*]	33 (52)
Noninfected	13 (32)	31 (48)
Outcome:		
TB disease[#]	2 (5)	13 (20)
Confirmed	0	3
Probable	2	10
Infected only:	34 (83)	31 (48)
Noninfected:	5 (12)	20 (31)

[*] Group in which more disease is expected.
[#] $P = 0.05$; odds ratio: 4.97 (95% CI: 1.06-23.33).

DISCUSSION

Although TB contact tracing has produced a significant yield of new cases in the past, the duration and value of long-term follow-up has been debated.[1,17] Data from previous studies suggest that contacts of smear-positive pulmonary TB and adult pulmonary TB cases in socioeconomically deprived areas should be followed up after the initial examination.[1,17,18] In several retrospective contact tracing studies, 90% or more of TB cases were identified within the first 12 months after identification of the index case.[1,17,19] Furthermore, studies have shown that drug-resistant organisms can be as infectious as drug-susceptible *M. tuberculosis*, but no long-term follow-up of childhood contacts of MDR TB index cases could be found.[4,5] In this study 29 (23%) children developed disease, 90% of whom were diagnosed as diseased by 12-month follow-up. Tuberculous infection with or without disease was present in 78% of children by 30 months, 95% of whom were already infected by 12 months. The low yield after completion of the first 12 months of follow-up is similar to findings in other studies.

It is generally accepted that between 30 and 50% of all household contacts of infectious adults will have a positive tuberculin skin test.[3] In children 0 to 5 years of age this infection rate was reported to be as high as 72% in earlier studies.[20,21] The very high infection rate in this study is therefore not unexpected. Possible explanations are that 80% of the children had smear-positive adult index cases, MDR TB usually remains infective for longer periods [4] and 46% of children had more than one adult household source case with active pulmonary TB. Other studies of households with a drug-resistant index case have shown similar high infection rates.[22-24]

Twenty percent of household contacts <5 years of age of mainly drug-susceptible index cases developed TB disease during a 5-year follow-up in India.[2] This is similar to

the 15 (14%) cases that developed disease during follow-up in our study. The rate of developing disease was the highest for children <5 years of age, but chemoprophylaxis significantly reduced disease in this age group to 5% in the Indian study.[2] This is similar to the group without appropriate chemoprophylaxis in our study in whom 20% developed disease compared with the 5% who developed disease in the group given appropriate chemoprophylaxis.

The age of the adult index cases of those children who developed disease and those who did not was not significantly different. This is in contrast to the findings of Snider et al.[4] where adults with drug-resistant TB causing infection and disease amongst childhood contacts were significantly younger. Nearly half of the children, however, had >1 adult source case. This may influence results but it is a common problem in communities with a high incidence of TB and complicates the management of these patients.[25,26] Childhood contacts of smear-positive adults were more likely to be infected than contacts of smear-negative adults. This again emphasizes the importance of smear positivity as a determinant of the transmission of infection.

DNA fingerprinting of *M. tuberculosis* isolates from 3 of 4 adult-child pairs that were analysed by RFLP were identical which confirms transmission from adult index case to the child contact.[16]

All 29 children with TB disease had primary disease. It is known that a large proportion of these children will improve even without treatment, [27] but effective treatment remains important to prevent progression of disease especially in the very young child. Furthermore, the eradication of live bacilli may prevent relapse with MDR disease in future, which is an important consideration in the face of the rapidly spreading

HIV/AIDS epidemic. The optimal duration of anti-TB treatment in children even in cases of drug-susceptible TB is still uncertain and many children are probably overtreated.[9]

Although directly observed therapy is practiced mainly in the urban areas, it has limitations, as the patients are expected to pay a daily visit to the local clinic. Children are therefore dependent on their caregivers to take them for treatment. Problems arise when this does not happen despite home visits by nursing staff. Resources at clinic level are inadequate to treat children at home and if hospital admission is not possible, treatment is stopped and patient declared a defaulter.

Isoniazid was given to almost all children either as treatment or chemoprophylaxis. There is evidence that about half the patients with primary isoniazid resistance have low-level resistance (minimal inhibitory concentration ≤ 2.0 µg/mL) and these serum levels are easily achievable in children with a dose of 15-20 mg/kg/d.[28,29] Furthermore, child contacts in this study often had contact with >1 adult source case, of whom a number had either drug susceptible TB or their susceptibility pattern was unknown.

Ethambutol has been accepted as first-line agent in TB and can even be recommended in children aged 5 years or more for routine treatment. Reviews of clinical trials in children <5 years of age did not reveal any serious ocular complications during treatment with ethambutol at a dose of 15 mg/kg/d.[30,31] It is an essential drug in the treatment of MDR TB cases, even for younger children.[9, 30,31]

Of the second-line anti-TB drugs, ethionamide has significant activity against tubercle bacilli but causes considerable gastrointestinal discomfort. It seems to be better tolerated in children than in adults and therefore plays an important role in the treatment of MDR TB in children. About half of the 61 children who received ethionamide in this

study experienced gastrointestinal side effects but by dividing the daily dose, drug-induced nausea and vomiting subsided so that it could be continued once again as a single dose in all but 4 patients.

The fluoroquinolones are generally not recommended for use in children because of their possible effect on cartilage growth in immature animals following long-term administration.[9] A number of reports have shown low rates of side effects with arthralgia episodes in only 1,3 to 3,5% of children even when used for periods of 150-300 days.[10,32,33] Three women who were receiving ofloxacin treatment became pregnant but no adverse effects were noted in their infants.[34] Ofloxacin has a higher early bactericidal activity of 0.32-0.39 against *M. tuberculosis* compared with the early bactericidal activity of 0.205 of ciprofloxacin, and the pharmacokinetic profile of ofloxacin is better than that of ciprofloxacin.[35-37] Ofloxacin is therefore the fluoroquinolone preferred by many experts in the treatment of MDR TB.[34,38] Arthralgia was experienced in only 1 of 15 children receiving ofloxacin for 6 to 12 months in our study. Because of the gastrointestinal discomfort caused by ethionamide, it was difficult to evaluate this side effect of ofloxacin in these children.

Chemoprophylaxis was successfully administered to 41 children, and 64 children received no appropriate chemoprophylaxis. This was, however, not a randomized, controlled study and results should therefore be interpreted with caution. The children who received chemoprophylaxis were clearly the group with the higher risk for developing disease since they were significantly younger, had more sputum smear-positive index cases, had a higher rate of infection and had had previously received treatment or chemoprophylaxis less often (Table 4). Despite the higher risk for disease, they developed significantly less disease than those that did not receive

chemoprophylaxis, This is particularly true of the children that had no other adult TB source case. Several combinations have been advocated such as pyrazinamide and ethambutol, pyrazinamide and a fluoroquinolone, a fluoroquinolone alone, and ethionamide and cycloserine.[8,9] We have used several combinations according to the drug resistance pattern of the index case, but most children received a combination of isoniazid, pyrazinamide and ethionamide with good effect.

The optimal duration of chemoprophylaxis for MDR TB contacts is uncertain. Our experience suggests that 6 months may be adequate if not optimal. Twelve months chemoprophylaxis has been advised in at least 2 official recommendations.[6,7] An alternative strategy to chemoprophylaxis, because of the lack of data, is regular follow-up without chemoprophylaxis, an option which was also offered to our patients.[7] However, with the high incidence of infection and disease in our study we believe that giving chemoprophylaxis should be the preferred management. In areas with a high burden of disease and in poorly resourced countries where treating smear-positive TB cases is the priority, 6 months of directly observed chemoprophylaxis may be more appropriate than giving 12 months of unsupervised chemoprophylaxis to MDR TB contacts.

CONCLUSION

This study confirms the transmission of TB infection from MDR adult index cases to children in close household contact and the subsequent development of disease in these children. The incidence of infection and disease was comparable to that occurring in children in contact with drug-susceptible adult index cases. Our results suggest that in resource limited situations the follow-up of such children with a view to detecting the development of disease can be limited to 12 months, as only a minority of children

developed disease after this period. Appropriate chemoprophylaxis, taking into account the resistance profile of the index case, seemed to be effective in preventing the development of disease. There is, however, an urgent need for a multicenter, randomized, controlled trial to identify the most effective drug combinations and the optimal duration of chemoprophylaxis in contacts of MDR pulmonary TB adults.

ACKNOWLEDGEMENTS

This article was written in partial fulfillment of a MD-thesis registered at the University of Stellenbosch.

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CHAPTER 7

Clinical pharmacokinetics of isoniazid in children with tuberculosis

Clinical pharmacokinetics of isoniazid in children with tuberculosis

7.1 LITERATURE REVIEW

Historical perspective

Fifty years after its introduction as an antituberculosis agent,[1] isoniazid (INH) continues to form the basis of all first-line antituberculosis regimens. Furthermore, INH is the agent of choice for the prevention of tuberculosis and is the only agent that has been evaluated in randomised controlled trials for the prevention of tuberculosis in children.[2, 3]

Isoniazid has a potent bactericidal action against *Mycobacterium tuberculosis* (*M. tuberculosis*) organisms, especially against actively multiplying bacilli such as those found in the walls of cavities. There is also evidence that INH suppresses the growth of non-multiplying organisms and that prolonged exposure of these organisms to supra-minimal inhibitory concentrations results in bacteriolysis.[4, 5]

Efficacy of INH

a)   Minimal inhibitory concentration (MIC)

The efficacy of INH is largely dependent on the MIC of INH against the *M. tuberculosis* organism. Susceptible strains are usually classified as organisms that have a MIC of ≤0.2 mg/L (break point).[6] The majority of susceptible strains will, however, have a MIC of approximately 0.05 mg/L or less.[6] Not all INH-resistant strains are equally resistant and strains with an MIC ≤5.0 mg/L are particularly likely to occur in patients with primary drug resistance.[7, 8]

b) Concentration vs. time of exposure (exposure integrals [mg L^{-1} h])

There is at present some uncertainty as to what pharmacokinetic parameters correlate best with INH efficacy against *M. tuberculosis*. One opinion is that the peak INH concentration following an oral INH dose provides the best correlation with efficacy.[9] The views referred to should, however, be interpreted with caution for reasons that will be discussed in context (*vide infra*).

Although peak serum INH concentrations achieved by oral administration are only slightly lower in fast acetylators compared to slow acetylators,[10] the systemic INH levels decrease much more rapidly in the fast acetylators because of a shorter half-life. Both the INH concentration versus time integral (AUC), and by implication the time that optimal concentrations in excess of the MIC are maintained, are significantly reduced in fast acetylators. The time available to penetrate effectively into the different body fluid compartments and TB lesions is reduced leading inevitably to lower peak concentrations where they are most needed.[11]

It is likely that not only is the peak concentration important but also a level near the peak concentration maintained for some time. Although INH diffuses freely into caseous lesions and cells, the peak concentrations in the lesions are likely to be somewhat lower and flatter than in the serum as a result of diffusion.[12] It has also been shown in vitro that it takes several hours for INH to reach an intracellular plateau.[12, 13]

INH has no post antibiotic effect (PAE) like other antituberculosis drugs such as rifampicin and streptomycin, and any escape from the influence of the drug in vitro leads to diminished efficacy.[14] Even among drug susceptible *M. tuberculosis* strains, bacilli differ in their response to INH; some bacilli need higher concentrations and/or longer

duration of exposure for INH to have a bacteriostatic or bacteriocidal effect.[15] The effect of exposing a *M. tuberculosis* culture to INH is proportional to the product of the concentration and the duration of exposure.[14]

On the other hand Awaness and Mitchison [16] and others have also shown that a single daily dose with higher peak levels is better than divided doses with smaller peak levels.[17] Since INH does not exhibit a PAE, the observed improvement of efficacy is due either to an improvement in the exposure of the *M. tuberculosis* organism to INH (balance between concentration and exposure time) or to better penetration of INH to the sites of antimycobacterial action. *M. tuberculosis* organisms exposed to high concentrations of INH for relatively short periods of time are thought to suffer damage that requires an adequate period of time to repair. If the time needed to effect these repairs is greater than the dosage interval, initial bacteriostasis followed by ultimate bacteriolysis ensues. In fast acetylators not receiving an optimal dose of INH, the balance between effective INH concentration in relation to an adequate period of time may be lost, with a consequent loss of efficacy.

Burman [6] cautions that in vitro and in vivo activity of drugs, and specifically antituberculosis agents, are not always comparable and that MIC, MIC/C_{max} and serum half-life are often not relevant predictors of clinical outcome. Given the complexity of mycobacterial disease this observation is not surprising. However, it is rational to assume that both therapeutic efficacy and INH related toxicity are primarily determined by exposure to INH. Both the maximal systemic concentration and the elimination half-life determine the exposure of organism and host to INH and are relevant. The extent to which the two separate factors contribute to efficacy and toxicity remain to be better defined.[18]

The apparent volume of distribution (Vd, vol.mass^{-1}) of a medicinal agent is the apparent volume of aqueous medium into which the agent distributes after absorption has taken place. This apparent volume is determined with reference to the concentration of the agent in the plasma and varies from agent to agent. In computational terms Vd = D/C$_0$, in which D and C$_0$ represent the administered dose (mass) and subsequent elevation in concentration (mass.vol^{-1}), respectively.

The volume of distribution (Vd) of a drug is a basic pharmacokinetic parameter required for the determination of therapeutic concentration versus time exposure integrals at the site of therapeutic action. In the context of the use of INH in the treatment of tuberculosis, the systemic concentration of INH should be high enough to exceed the MIC of the *M. tuberculosis* organism wherever it is located or sequestered.

As INH has no PAE, it is necessary to maintain concentrations at the target site for as long as practically possible, while taking into account possible toxicity. Since the MIC of non-resistant *M. tuberculosis* organisms is low (0,05 to 0.2 mg/L) therapeutic concentrations are easily achieved and adequately maintained in the well-perfused central compartment (brain, heart and lungs) in most instances.

The *M. tuberculosis* organism may, however, invade any tissue, no matter how peripheral, and enhance its survival by forming impenetrable clusters in denatured and necrotic tissue. The therapeutic challenge from a pharmacokinetic perspective should thus not be viewed solely in terms of MIC-values, but rather in terms of the pharmacokinetic imperatives required to ensure adequate exposure integrals of INH at peripheral sites where blood perfusion is poor, or wherever organisms may lurk in sequestered lesions.

The area under the time versus concentration curve (AUC) or "exposure integral" of INH is the most informative basic therapeutic parameter, since it allows assessment of the mean effective concentration of INH within a dosage interval. The mean concentration embodies both elements of therapeutic importance: 1) the magnitude of the therapeutic concentrations and 2) the length of time during which effective therapeutic concentrations are maintained.

c)   Pharmacodynamics

The aim of antituberculosis treatment, as with any other antibiotic treatment, is to achieve adequate drug concentrations at the site of the infection for an appropriate length of time in order to ensure the eradication of the organisms and optimise clinical success.[19] This desired outcome depends not only on the pharmacokinetics of the agent, but also on the characteristic of the pathogen, e.g., microbial susceptibility, and host related factors.

A new approach to this problem seeks to combine the microbiologic activity with antibacterial pharmacokinetic data into a discipline called pharmacodynamics. The best pharmacodynamic parameter is the minimum effective antimicrobial action in an area under the inhibitory titre (AUIC), which is calculated as the 24-hour serum AUC divided by the MIC of the pathogen.[20] According to Schentag the minimum effective action is an AUIC of 125, but this is mainly of value in extracellular organisms (personal communication). This target can be achieved by a single antibiotic or by the sum of AUIC-values of two or more antibiotics. This could possibly explain why, even in the event of low INH doses in adults or children or in fast acetylators, patients still have a good clinical outcome with combination therapy.

d)   Early bactericidal activity (EBA)

The rate at which an antituberculosis drug kills rapidly metabolising and multiplying bacilli during the first two days of therapy is termed the early bactericidal activity (EBA). EBA can be used to determine the lowest effective dose size of these drugs.[21] In an evaluation of the EBA of INH there was a significant drop in the EBA in adult patients receiving 300 mg (6 mg/kg body weight) compared to those receiving a dose of 150 mg (3 mg/kg body weight), while there was no further increase in the EBA at a dose of 600 mg (12 mg/kg). This would imply that 3 mg/kg body weight is a less effective dose for the killing of metabolically active organisms. In these same patients the mean 3-hour INH serum concentration dropped from 6.05 mg/L to 1.22 mg/L at doses of 12 mg/kg and 3 mg/kg, respectively. It would therefore appear that a mean INH concentration of between 3.0 to 3.7 mg/L at 3 hours, as could be expected from a dose of 6 mg/kg body weight, would provide an optimal response.[21]

e)   Potential impact of polymorphic metabolism on efficacy

There are contradictory reports in the literature in respect of the extent to which pre-systemic or first-pass extraction of INH takes place. Some investigators have found the differences between polymorphic subtypes to be marginal,[10, 13, 22] whilst others have found the differences to be considerable and significant.[12, 23] The apparent contradiction may be easily explained in terms of recently generated data in which it was shown that the metabolism of isoniazid is saturable, consistent with Michaelis-Menten concepts.[22, 24] Furthermore, the polymorphic acetylator subtypes all share the same V_{max}-value (about 10 mg L^{-1} h^{-1}), but have distinctly different K_m-values and hence affinities for INH substrate: K_m fast (FF) = 6.57 mg/L; K_m intermediate (FS) = 14.43 mg/L; K_m slow (SS) =

32.82 mg/L.[25] It is clear from these data that fast and intermediate acetylators have a far greater affinity for INH substrate than do slow acetylators. Consequently, the pre-systemic extraction fraction of INH will be maximal in fast (FF) acetylators receiving a low dose of INH (e.g., 3 mg kg^{-1}) in whom absorption is retarded (for whatever reason), and minimal in slow (SS) acetylators receiving a high dose of INH (e.g., 10 mg kg^{-1}), and in whom absorption is rapid; intermediate acetylators would appear to resemble fast acetylators, rather than slow acetylators, in this regard. It is clear from the foregoing that the pre-systemic absorption of INH cannot validly be assessed in the absence of corresponding C_{max} and T_{max} pharmacokinetic parameters.

Simplistic approaches to a complex problem will thus fail to shed light on the most appropriate pharmacokinetic parameter with which to correlate antimycobacterial efficacy. However, it is rational to assume that a parameter that reflects the total exposure of the *M. tuberculosis* organism to the pharmacodynamic effects of isoniazid, with due regard to the MIC applicable to the specific organism, would be most appropriate. The area under the isoniazid concentration versus time curve (a direct measure of exposure) is precisely such a parameter and should be determined as accurately as possible when assessing the antimycobacterial activity of INH in the research setting.

An instance of therapeutic failure in a fast acetylator being treated for tuberculous meningitis with an INH containing regimen, has recently been reported.[11] Because the patient was being treated with a regimen comprising higher than usual dosages of isoniazid and rifampicin, and the infecting *M. tuberculosis* organisms had been shown to be susceptible to both isoniazid and rifampicin, the reason for the therapeutic failure was not immediately apparent. However, subsequent investigation showed that the patient

was a fast acetylator of INH. The INH dosage was increased and thereafter therapeutic progress was uneventful and the child progressed to cure. The authors expressed the view that the most likely cause of therapeutic success was the increase in the dosage of INH, which was large enough to compensate for the rapid elimination. This report suggests that acetylator status assessment may be valuable in the face of unexplained therapeutic difficulties. Furthermore, and equally important, whenever higher than usual dosages of INH are contemplated, for whatever reason, acetylator status should be determined in order to allow assessment of the individual risk of dose-related INH induced toxicity. Several authors have expressed the view that INH dosage should be adjusted to allow for, or accommodate, the acetylator capacity of the individual, in order to ensure optimal treatment and limit toxicity.[13, 26]

Pharmacokinetics of isoniazid in adults

The pharmacokinetic characteristics of INH have been studied extensively in adults.[18] However, data in respect of children, and especially in children younger than 6 years of age, are limited.[26, 27] INH is well absorbed from the gastrointestinal tract but is subject to first-pass metabolism, which may impact on the systemic concentrations of the parent compound. INH does not bind appreciably to plasma proteins and distributes to the total body water compartment; it crosses membranes readily despite its high water solubility and has a volume of distribution in adults of 0.67 L/kg.[28] Very little parent compound is excreted unchanged in the urine and the greater proportion is acetylated (in the liver and the small intestine) to acetylisoniazid prior to excretion in the urine.

Although the initial work on the pharmacokinetics of INH seemed to indicate that INH is bimodally eliminated, and that alleles coding for slow acetylation are recessive to

the fast wild type, it has now been established that INH is eliminated in accordance with a trimodal distribution of subtypes [fast (FF), intermediate (FS) and slow (SS)], and that fast (F) and slow (S) alleles are in fact co-dominant.[22] This knowledge, coupled with developments in modern analytical technology, allows for rapid and accurate assessment of pharmacokinetic parameters, and their potential impact on therapy.

Pharmacokinetics of isoniazid in children

Observations, perceptions and practical experience of research-orientated clinicians seem to indicate that the pharmacokinetic parameters for INH differ in children and adults. Furthermore, the extent of these differences should probably be taken into account when determining the most appropriate dosage regimen of INH in the paediatric patient. Finally, certain practical aspects of paediatric patient management also have a direct bearing on the clinical pharmacokinetics of isoniazid in this age group. These factors are summarised briefly below:

a)   **Elimination rate and metabolism**

It has been shown that children eliminate INH faster than adults despite having similar acetylation phenotypes.[29, 30] This may be due to the relatively larger size of the liver in children.[31] Calculations based on body surface area indicate that younger children need higher doses than adults do to achieve an equivalent exposure to the agent.[9]

b)   **Volume of distribution**

The total body water in children and the extracellular fluid make up a larger percentage of the total body weight than in adults and the volume of distribution in children might therefore be increased.[9, 30, 32]

c) Absorption of INH

Concurrent food intake decreases the bioavailability of INH.[33] It is difficult in practice to keep a hungry child from eating for two or three hours in order to improve drug absorption. Furthermore antituberculosis drugs will often be administered together with something sweet to eat or to drink.

d) Isoniazid toxicity: Children vs. adults

Advancing age is a risk factor for the development of INH-induced toxicity.[34, 35] According to some reports, even children receiving INH at 20 mg/kg daily together with other hepatotoxic drugs such as rifampicin, pyrazinamide and ethionamide during treatment for tuberculous meningitis, show relatively low rates of hepatotoxicity. [36] There are, however, reports from different populations in which children who were treated with INH 15-20 mg/kg together with rifampicin had very high rates of hepatotoxicity.[37-39] INH toxicity seems to be dose-related with very little hepatotoxicity at 8-10 mg/kg, and INH without rifampicin also has a lower rate of hepatotoxicity.[37,38]

The reason for the lower risk of toxicity in children has never been explained satisfactorily but may be due to metabolism related causes. INH itself has little (if any) toxic potential but is biotransformed in the liver and a variety of toxic metabolites are generated, most notably hydrazine and mono-acetyl hydrazine.

Hepatotoxicity is the most serious risk factor limiting the upper extremes of isoniazid dosage regimens. Mono-acetyl hydrazine is thought to be the most important mediator of hepatotoxicity and is converted by the cytochrome mixed function oxidase enzymes into a highly reactive hepatotoxic acylating agent.

The cytochrome mixed function oxidase system is comprised of a large variety of oxidative isoenzymes, all of which are easily inducible. Both the induction profile (the specific isoenzymes induced) and the extent of the induction are very variable, and depend upon inducing substrate(s) involved and the duration and extent of the exposure of the enzymes to the substrate. It is possible that the cytochrome system of adults is more mature (and hence has a greater capacity and wider metabolic variety) than is the case in children. It is possible that conversion of non-toxic mono-acetyl hydrazine to a highly reactive acylating agent is more efficient and extensive in adults and that the higher incidence of hepatotoxicity is due to this factor.

It has been demonstrated that hydrazine levels in the plasma of adults are on average higher than those in children following a comparable dose for mass.[40] The reason for this difference has never (to date) been satisfactorily explained and these elevated levels of toxic hydrazine in adults may be of greater significance in the pathogenesis of hepatotoxicity than has previously been supposed. This aspect should be investigated in depth at the molecular level.

e)   **Pathology related considerations**

Young children are more prone to the lymphohaematogenous dissemination of organisms and the development of extrapulmonary TB than are adults. Furthermore, children in the South African population are being infected by the human immunodeficiency virus at an escalating rate. HIV-infected patients (both adults and children) who contract TB also have a greater predisposition to extrapulmonary TB.[41] Consequently, as indicated elsewhere appropriate therapeutic INH concentrations should not be viewed simplistically in terms only of MIC-values, the well-perfused central compartment, and the concentrations achieved in the blood, but rather in terms of levels

achieved and maintained (as far as this is possible) in peripheral tissue and in tissues protected by physiological barriers (e.g. the blood brain barrier).

f) Population differences in acetylation type

It is well known that acetylation type varies widely in different populations. Caucasians (Europeans) are mainly slow acetylators compared to white North Americans who have more or less equal numbers of slow and fast acetylators. Japanese, Chinese, Eskimos and African blacks are mainly fast acetylators.[18] In the Western Cape Province, fast and intermediate acetylators constituted 65% of the population studied and 35% were slow acetylators.[22] Acetylation phenotype may have a role in determining the optimal INH dose for children. Although children seem to metabolise INH more rapidly than adults, this is not related to the acetylator status, which is genetically determined, and which matures early.[31] Acetylation type is, however, not the only consideration in choosing appropriate dosage regimens, as within each acetylator group the range of recommended doses can vary widely.[26] According to Rey et al. [26] dose can be calculated by use of the inactivation index method. (*vide infra*)

Dosage regimens of INH in general

a) Inactivation index (I$_3$). [13]

When an individual is given different doses of oral INH in mg/kg, the resulting 3-hour INH serum levels (y-axis) plotted against the mg/kg oral dose (x-axis) will result in a straight line if the points are connected. This line will, however, not pass through the point of origin (zero on both axis) but will be displaced to the right because of the first pass effect on INH. Although the slope of the straight line will be different for each individual patient (a way in which acetylation type can be determined), the intersection
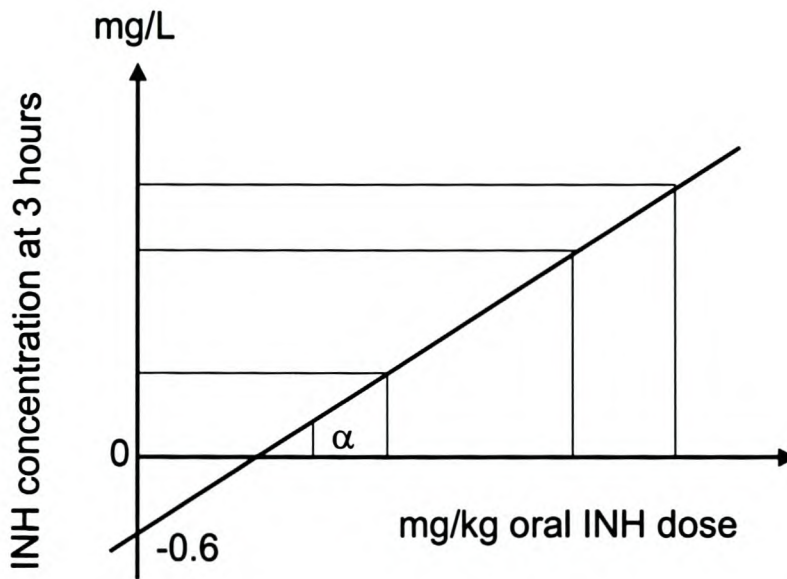
Figure 1: Correlation between 3h INH concentrations and INH oral dose (3 estimations, different doses, same patient). Straight line passes through at -0.6 (constant), $\propto$ is the angle of the line that varies from patient to patient.

of the straight line with the y-axis has been shown to be a well-defined average value that can be fixed at –0.6. From this information the *index of inactivation of INH* (I_3) was developed. The index is calculated by the equation:

$$I_3 = C_{3h} + 0.6 / D \text{ (mg/kg)} \qquad (C = \text{concentration of INH; } D = \text{dose in mg/kg})$$

The inactivation index has since been used to adjust individual children's INH dosages and to determine optimal INH dosages for the different acetylation types.[26, 42] To achieve this it was necessary to accept that the efficacious therapeutic plasma concentration of INH should range from 1 to 2 mg/L, three hours after oral administration as previously suggested.[43] The advantage of this index is that only a single

serum specimen at 3 hours after an INH dose is necessary to calculate the "optimal dose".

b) Reference integral

According to the literature 5 mg/kg of INH in an appropriate antituberculosis regimen yields satisfactory treatment results in patients with INH-susceptible *M. tuberculosis* organisms.[9, 44-46] A satisfactory outcome implies that the patient responds satisfactorily to treatment, that treatment is not associated with undue toxicity, that treatment is completed within the scheduled time frame (six months), and that the incidence of relapse is acceptably low (2-4%) on follow-up.[45, 47] Caution is, however, necessary in accepting the appropriateness of a dose of 5 mg/kg of INH since publications emanate from populations in which slow and intermediate acetylator phenotypes are predominant. The minority fast acetylator phenotypes in these populations probably are at a relative therapeutic disadvantage as to the most appropriate dose of INH. They may nevertheless respond satisfactorily to treatment because of the synergistic potential of the treatment regimen as a whole.

The apparent satisfactory response of fast acetylators should not however be cause for complacency, as the relative disadvantage may be exposed in circumstances in which other negative factors play a role: poor absorption of INH itself as well as other components of the regimen - most notably rifampicin; non-compliance with the therapeutic regimen, and protected organisms sequestered in lesions in poorly perfused tissues.[11, 48, 49]

In populations in which fast acetylators are predominant, the dosage recommendations for INH appear to be somewhat higher, and 7-10 mg/kg is apparently

not perceived by clinicians as excessive (Japanese Tuberculosis Society, personal communication).[50] Their use of a higher dosage is presumably the result of clinical experience that evolved in a natural way, rather than on true experimental data. From the foregoing it seems reasonable to assume that fast acetylator phenotypes may benefit from an upward adjustment of the dosage of INH, although the extent to which the dosage should be adjusted, if at all, is a matter to be investigated.

For purposes of rational assessment of an appropriate dose of INH, a reference standard should be set in terms of a firm pharmacokinetic base. The most appropriate pharmacokinetic parameter is the area under the concentration versus time curve. Since a 5 mg/kg dose is universally accepted as being appropriate and adequate in slow acetylators the AUC applicable to slow acetylators following this dose would seem to be a rational reference parameter.

If 5 mg/kg of INH is administered to a slow acetylator once daily, and rapid and complete absorption of INH and a volume of distribution for INH of 0.67 L/kg [28] is assumed, then the following parameters will apply:

C_{max} = $[INH]_0$ = 7.5 mg/L

C_{24h} = $[INH]_{24h}$ = 0.006 mg/L

$AUC_{(0-\infty h)}$ = 37.3 mg/L/h

$[INH]_{(ave,0-24h)}$ = 1.56 mg/L/d

Dosage regimens of isoniazid appropriate to children.

a)   **Current practice**

A review of currently suggested INH doses for children shows wide variations. (Table 1) The World Health Organization[51] and the International Union against

Tuberculosis and Lung Disease[52,53] recommend an INH dose (daily) of 4 to 6 mg/kg body weight, while the American Academy of Pediatrics (AAP)[50] advises 10 to 15 mg/kg. In TBM the suggested dose ranges from 15 to 20 mg/kg daily with a maximum total dose of between 300 and 500 mg. None of these dosing schedules take into account either the acetylation phenotype of the patient or the incidence of primary INH resistance in the community involved.

b) Childhood INH dosage regimens: The potential role of INH in the management of primary INH-resistant TB

The dosages of INH that are used in regimens in the treatment of tuberculosis in children have largely been derived from and reflect the regimens used in adults. This tendency continues to the present time and is cause for some concern. Optimal INH dosage regimens in adults are still a matter of lively debate and considerable uncertainty, and the constitutional differences between adults and children may impact on the most appropriate dosage regimens applicable to children.[9] These uncertainties, and related relevant factors, require careful appraisal. Although earlier studies in adults and children should be taken into account, newer technological developments allow for more accurate assessment of INH pharmacokinetics and genetic analysis.

INH-resistant and multidrug-resistant TB has become a global problem. This development compromises the usefulness of INH in these patients both in chemoprophylaxis and in treatment.[54-56] However, it has been shown that about half of the primary INH-resistant bacilli have low-level INH resistance (vide supra Chapter 3, Results: Degree of isoniazid resistance),[7, 8] and consequently the potential role of INH in the management of primary INH-resistant *M. tuberculosis* infected patients should be

assessed. The implications would be far-reaching if it could be shown that appropriate INH dosage regimens contribute to the eradication of partially resistant *M. tuberculosis* organisms.[57] Such information would better define the most appropriate use of INH and prolong the usefulness of this invaluable agent in tuberculosis control programs, particularly in resource poor countries.

Table 1: Recommended daily isoniazid dosage for use in children

INH dosage (mg/kg)		Recommended by:	Year	Population	Reference
Dosage	Maximum				
5 (3-6) 20 if TBM		NTCP South Africa	2000	South Africa	Dept of Health 2000 [58]
5 (4-6)		IUATLD	1991 & 2000	International	IUATLD 1991 [52] IUATLD 2000 [53]
5		WHO	1996	International	WHO 1996 [51]
5	300	V. Seth, India	1997	India	Seth V 1997 [59]
7-10	300	Japan Anti-tuberculosis Association	2000	Japan	Japan Anti-tuberculosis Association 2000*
10-15	300	American Academy of Pediatrics (AAP)	2000	North America	AAP 2000 [50]
10-20	300	Nelson and Bradley	2000-2001	International	Nelson & Bradley 2000 [60]
10 15-20 TBM	300 500	Frank Shann, Australia	2001	Australia, International	Shann 2001 [61]
10-20	300	American Thoracic Society Ad Hoc Committee	1993	North America	Ad Hoc Committee ATS 1995 [62]

NTCP = National Tuberculosis Control Programme
IUATLD = International Union Against Tuberculosis and Lung Disease
WHO = World Health Organization
ATS = American Thoracic Society
* Japan Anti-tuberculosis Association Guidelines. 2000 (Personal communication)

7.2 EVALUATION OF CLINICAL PHARMACOKINETICS OF ISONIAZID IN CHILDREN, AND ISONIAZID'S POTENTIAL ROLE IN PRIMARY ISONIAZID-RESISTANT TB IN CHILDREN

STUDY OBJECTIVE

The study was undertaken in order to assess:

1.) the potential role of INH in patients with low-level INH resistance;

2.) INH pharmacokinetics in children, making use of improved analytical technology and advances in the understanding of the polymorphism governing INH metabolism;

3.) the potential impact of the polymorphism governing INH metabolism, on therapeutic outcome.

METHODS

Patients:

Children less than 13 years of age of mixed [22] and black race were enrolled in this study. All children were diagnosed with primary tuberculosis and were included randomly on the basis of being available for study on a Thursday.

Ethical approval was obtained from the Institutional Ethical Authority and parents/guardians had to give written informed consent.

The age, gender and weight of each child were recorded and a detailed clinical history was taken. In addition, the presence or absence of extrapulmonary tuberculosis

was established, the extent of the pulmonary disease was assessed and recorded, and the human immunodeficiency virus (HIV) status was determined.

Inclusion criteria: As above.

Exclusion criteria: Children with stage 2 or 3 tuberculous meningitis or severely sick children.

Dosage and Sampling:

All children participating in the trial were hospitalised for the required procedures. Those children not already hospitalised (for other than trial related reasons) were admitted for the morning on which the procedures were scheduled.

The INH used for this study was standard pharmaceutical grade in powder form from Fluka Chemie AG (Buchs, Switzerland). The dose of INH was accurately weighed at 10 mg/kg according to the child's exact weight the previous day. INH powder was dissolved in 5-10 ml of water and administered orally with a syringe or, in the case of very young children, through a nasogastric tube, and washed down with water. The drug was given by one of two people between 07:00 and 07:30 a.m. after abstaining from solids from the previous evening. A light breakfast was served between 08:00 and 08:30.

Blood samples were drawn at 2, 3, 4, and 5 hours post INH dose. After collection into EDTA-coated tubes, the blood samples, 1 to 1.5 ml in all instances, were chilled and delivered to the laboratory on ice for determination of the INH levels in plasma using the HPLC-method of Seifart et al.[63] A further single sample of 3 to 4 ml was collected into an EDTA-coated tube from each child for DNA analysis and the HIV status was determined in those children where it was not previously known.

Pharmacokinetic Parameters:

a) Determination of first-order elimination rate constant (k, h⁻¹)

The apparent first-order elimination rate constants (discriminant for phenotypification) were calculated over the interval 2 to 5 hours after the dose (k, h^{-1}) for all children from the linear regression of $\ln C_t$ and t in the range 2, 3, 4 and 5 hours, $\ln C_t$ being the natural logarithm of the concentration at time t.

b) Concentration at zero time post dose (C₀, mg/L)

The maximal increase was measured as the concentration $(C_0, mg/L)$ at time designated zero, i.e., the time at which the test dose of medication was administered. C_0 was determined from back-extrapolation to the ordinate of the best fit linear regression line in respect of the natural log of the concentration of INH as a function of time, over the time range 2 to 5 hours after the dose (involving four concentrations vs. time data pairs).

C_0 was also calculated for each individual making use of the population data in respect of Vd as follows: $C_0 = $ Vd (population data) divided by the dose/kilogram that each individual received.

c) Determination of volume of distribution (Vd)

The volume of distribution (Vd) of INH was determined in each patient from the maximal increase in the INH concentration following an accurately weighed oral test dose. The maximal increase was measured as the concentration $(C_0, mg/L)$ at time designated zero, i.e., the time at which the test dose of medication was administered. Vd was calculated by dividing the INH dose (D) by C_0 (Vd = D/Co L/kg)

d)   First-order elimination rate constant (k, h^{-1}) related variables

First-order elimination rate constant was compared to age (in groups: 0 to 2 years, >2 to 5 years and >5 to 13 years) by analysis of variance (Anova). First-order elimination rate constant was compared separately by student t-test for independent samples (with Levene's test for homogeneity of variances) to gender, ethnicity, human immunodeficiency virus (HIV) status and the presence of abdominal TB to determine whether there were any significant differences or whether all results could be compiled.

e)   Vd-related variables

The influence of age-related variation in physical development on the magnitude of Vd (if any), and the potentially distorting effect of polymorphic INH elimination on the determination of Vd (*vide supra*), was assessed prior to determination of truly representative paediatric population Vd data. Regression analysis between these variables and Vd was done. Population data in respect of Vd was calculated after excluding values deviating more than one standard deviation from the mean if Vd was not influenced by the above mentioned variables.

f)   Area under the time concentration curve (AUC)

AUC (zero to infinity) for each individual was determined by dividing the calculated C_0-value of each child by its first-order elimination rate constant (k, h^{-1}).

g)   Phenotypification

Slow acetylator phenotype was defined as a first-order elimination rate constant of ≤0.31 h^{-1} and intermediate (FS) to fast (FF) acetylator status as >0.31 h^{-1}.[22]

h)   Statistical Methods

Statistical methods used are described in each section. Statistica version 5, 1997 edition, was used for all statistical analysis.

RESULTS

Demographics and clinical profile of participants

INH levels were determined in 65 children, of which one was excluded because of unreliable laboratory results due to technical problems. Of the remaining 64 children, 44 were of mixed race and 20 were black children. There were 36 boys and 28 girls, and median age was 3.75 years with a range of 0.6 to 13 years.

All children had primary tuberculosis, although a number had progressive or disseminated disease. The clinical features, including age, HIV-status and extrapulmonary TB status, are summarised in table 2.

Dosage commentary and INH concentrations versus time data

The mean dose of INH administered was 10.14 mg/kg with a standard deviation of ± 0.70 mg/kg.

The raw data of age, gender, dose in mg/kg, actual post dose time of each specimen and INH concentration at that time is summarised in table 3. (Addendum)

INH concentrations corrected for exact post dose times of 2, 3, 4 and 5 hours were calculated and are presented in table 4. (Addendum)

Derived pharmacokinetic parameters of individuals

a)   First-order elimination rate constant (k, h^{-1})

The results for each individual are presented in table 5. (Addendum) There was no statistical difference when first order elimination rate constant was compared to age groups (p = 0.81) (Figure 2), ethnicity (p = 0.69), gender (p = 0.50), HIV status (p = 0.56) and presence or absence of abdominal TB (p = 0.53).

Age vs. elimination rate constant
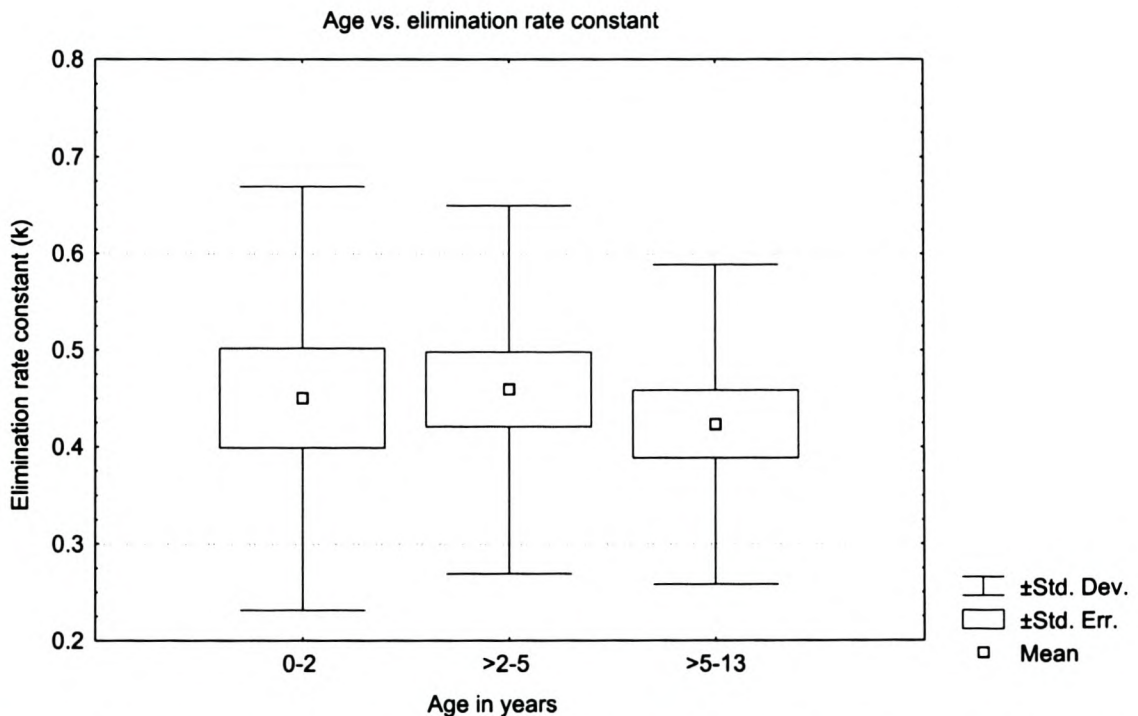
Figure 2: First order elimination rate constant compared by age group (Anova: p = 0.82)

b)   Concentration at zero time post dose (C$_0$, mg/L)

The results of both the extrapolated and calculated concentrations at zero time post-dose are presented in table 5. (Addendum)

c)   Calculated volume of distribution (Vd, L/kg)

The calculated Vd values for each individual patient are summarised in table 5.
(Addendum)

d)   Area under the concentration versus time curve (AUC, mg l^{-1} h)

The AUC (time zero to infinity) values of each individual is presented in table 5.
(Addendum)

Table 2. Clinical features and special investigation results of the study children

Clinical feature or special investigation	Number n = 64	Percentage
Age groups		
0-2 years	18	28%
>2-5 years	24	38%
>5-13 years	22	34%
Weight loss	50	78%
Weight <3rd percentile	31	48%
Cough >2 weeks	39	61%
Household TB contact	35	55%
Mantoux tuberculin test:		
0-4 mm	13 (7 = HIV-infected)	20%
5-14 mm	6	9%
≥15 mm	41 (6 = HIV-infected)	64%
Not done	4	6%
HIV-infected	13	20%
Chest radiograph		
Lymphadenopathy	44	69%
Collapse/opacification	33	52%
Pleural effusion	14	22%
Miliary	8	13%
Extra pulmonary TB		
Peripheral lymph node	14	22%
Pleural effusion	14	22%
Miliary	8	13%
TB meningitis stage 1	4	6%
Pericardial effusion	1	2%
Culture or histology confirmed tuberculosis	41	64%

Variables with potential to impact directly or indirectly on Vd

Age: The calculated Vd values were plotted as a function of age (years) as shown in figure 3. Inspection of the distribution of the data reveals that age *per se* did not influence the magnitude of Vd over the paediatric age range (birth to adolescence) (p = 0.082). It is also evident that there were a number of outliers but that these were randomly distributed over the entire age-range and hence due to experimental variation only.

First order elimination rate constant: The calculated Vd values were plotted as a function of the first order elimination rate constant (k, l/h) as shown in figure 4. Inspection of the distribution of the data reveals that the rate constant *per se* did not influence the magnitude of Vd as calculated over the paediatric age range (p = 0.84). It is also evident that there were a number of outliers but that these were randomly distributed over the entire range of first order rate constants and hence due to experimental variation only.

Derived pharmacokinetic population parameters

a)   Population first-order elimination rate constant (k, h^{-1})

Twenty-four (37.5%) children were slow and 44 (62.5%) were intermediate to fast acetylators of INH. The populations' mean first-order elimination rate constant for slow acetylators was 0.2433 ± standard deviation (SD) of 0.0341h^{-1}, with a range of 0.1731 - 0.3085 h^{-1}. The populations' mean for the faster acetylators was 0.5654 ± SD 0.1295 h^{-1} with a range o 0.3317-0.9715 h^{-1}. There was no clearly distinctive antimode between the intermediate and fast acetylator groups.

VD vs. AGE_IN_Y (Casewise MD deletion)

AGE_IN_Y = 6.6563 - 2.323 * VD
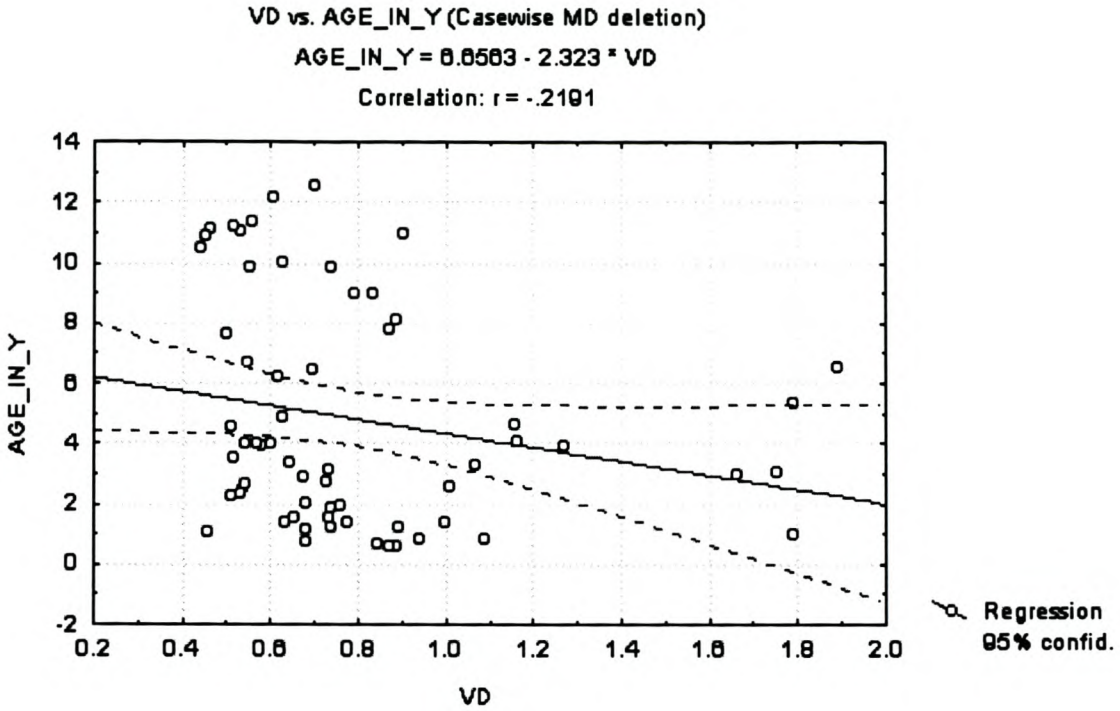
Correlation: r = -.2191

Figure 3: Volume of distribution vs. age in years. Correlation not significant (p = 0.082)

VD vs. K_VALUE (Casewise MD deletion)

K_VALUE = .46251 - .0194 * VD
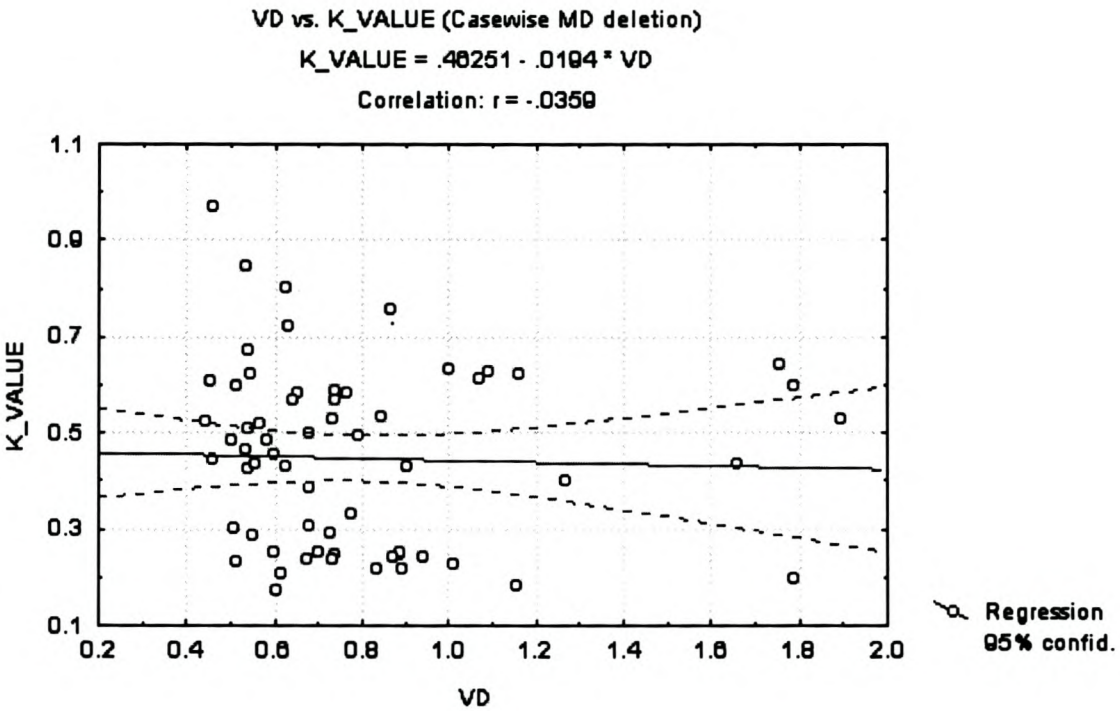
Correlation: r = -.0359

Figure 4: Volume of distribution vs. first-order elimination rate constant. Correlation not significant (p = 0.84)

b) Volume of distribution (Vd, L/kg)

The mean (0.7962 L/kg) and standard deviation (0.3439 L/kg) of the Vd values of the entire population was calculated as a point of departure, since the magnitude of this factor was not influenced either by age or by acetylator capacity. Values deviating from the mean by more than one standard deviation (n = 8) were then identified and excluded, followed by calculation of a corrected mean (0.6876) and its standard deviation (0.1646 L/kg), in order to approach more accurately truly representative population values. Population data in respect of Vd used was therefore 0.6876 +/- 0.1646 L/kg. The data in respect of the entire population and of the modified population are shown in table 5.

Dosage comparisons and assessment relative to experimental data

a) Isoniazid Inactivation Index

The inactivation index using the 3-hour INH concentration was calculated for each individual according to Vivien's formula (see introduction and table 6).[13] If a 3-hour INH concentration of 1.5 mg/l is accepted as optimal,[13,26] 17 (27%) children, all of whom are classified as fast acetylators, would need an INH dosage regimen containing >7mg/kg/d. The mean and SD INH dose for the slow acetylators according to this method of calculation would be 3.2 mg/kg ± 0.8 mg/kg/d, while the mean dose for the faster acetylators would be 7.2 mg/kg ± 2.9 mg/kg/d.

b) Isoniazid Exposure Integrals

If it is assumed that 5 mg/kg/d of INH is the optimal dose for an average adult slow acetylator (k-value 0.2 h^{-1}), the AUC would be 37.3 mg/l/h.(see introduction) At 10

mg/kg 5 (8%) children had an AUC of <20 mg/L/h and a further 23 (36%) had an AUC of between 20-30 mg/L/h (table 5). The mean AUC for the faster acetylators was only 27.578 mg/L/h, while the slow acetylators had a mean AUC of 61.182 at 10 mg/kg INH.

Results of HIV-infected children compared with HIV-uninfected children

Thirteen (20%) children were HIV-infected, of whom 6 had clinical category B and 7 had clinical category C infection according to the Centers for Disease Control and Prevention 1994 revised classification system for HIV-infected children <13 years of age.[64] There was no statistically significant difference between the first order elimination rate constant (k-values) of the HIV-infected children compared with the HIV-uninfected children (p = 0.56) The mean and standard deviation (SD) of the 2- and 3-hour corrected INH-concentrations, k-value and AUC are presented in table 7. No significant difference in INH-levels was found and the marginally higher mean-AUC in the HIV-uninfected group most likely reflects the slightly lower mean k-value (slower acetylation) in this group.

Table 7: Comparison of mean (±SD) of corrected INH-concentrations, k-values and AUCs in HIV-uninfected and HIV-infected children

	HIV-uninfected children n = 51 Mean (±SD)	HIV-infected children n = 13 Mean (±SD)
2-h INH-concentration (mg/L)	6.21 (± 2.73)	6.26 (± 2.34)
3-h INH-concentration (mg/L)	4.30 (± 2.43)	4.12 (±2.02)
k-value (h^{-1})	0.4376 (± 0.1933)	0.4721 (± 0.1723)
AUC (mg.L^{-1}.h)	41.18 (± 18.93)	36.24 (± 14.86)

DISCUSSION

The primary aim of the experimental work was to assess the pharmacokinetics of INH in children in the context of practical antituberculosis chemotherapy. This objective was approached in two ways: (1) a prospective accurate determination of INH concentrations versus time data and the standard pharmacokinetic parameters deriving therefrom; (2) a comparison of the experimental data with existent data. Existent data are limited, fragmentary and in many respects outdated.

Although it was demonstrated that the pharmacokinetics of INH in children reflect the pharmacokinetic profiles applicable to adults to a large extent, there are differences in children that should be taken into account in practical pharmacotherapy. The experimentally determined pharmacokinetic parameters, which reflect the profiles applicable to children, are discussed for completeness prior to the discussion of the practical therapeutic implications.

Volume of distribution: Although the volume of distribution of isoniazid was marginally higher in children than in adults (0.69 vs. 0.67 L/kg, respectively), the difference was smaller than anticipated.[9, 32] With a Vd of 0.67 L/kg (adults), isoniazid appears to distribute into the total body water compartment. The similarity in distribution between children and adults would seem to indicate that the total body water is a stable compartment and does not vary markedly with age. However, it should be borne in mind that the Vd data generated in our trial is in respect of lean body mass, since all our patients were suffering from tuberculosis with its inevitable impact in this regard.

First order elimination rate constant: The elimination rate constant (k; h[-1]) of the children in the trial group ranged from 0.1731 to 0.9715 h^{-1}. There was clear phenotypic

separation between the slow acetylators and the intermediate and fast acetylators. Although the fast and intermediate acetylators formed a single composite group, the fast and intermediate individuals tended to cluster together in separate domains. Since there is a clear separation of phenotypes in adults, using the k-values as discriminant,[22] the non-separation of phenotypes in children is probably a reflection either of a degree of developmental immaturity or of the relatively high test dose (INH 10 mg/kg) that was used in most instances.

Since the first order elimination rate constant (k; h^{-1}) is the complete primitive of clearance (Cl = kVd, Vol t^{-1}), it follows that the individual k-value may be used as a means of individualising INH dosaging if, and when necessary. In this regard the considerable variation between individual children, as in the case of adults, should be taken into consideration when planning an optimal INH containing dosage regimen. How best this should be done remains a matter of conjecture rather than controversy.

Area under the isoniazid-concentration versus time curve (AUC mgL^{-1}h): The AUC of INH following a standard oral dose (INH 10 mg/kg) varied in accordance with the k-value as was to be expected.[22] As in adults, the magnitude of the differences in children was extensive and varied from 14.97 to 85.78 mgL^{-1}h. This, in the presence of polymorphic metabolism, implies irrevocably that this variation will carry over to differences in overall antimycobacterial efficacy and therapeutic response, and risk of INH-induced toxicity following a standard INH dose.

The finer nuances of these differences have never successfully been demonstrated, presumably because of the complexity of antituberculosis treatment (application of multiple component dosage regimens over extended [months] periods of treatment).

However, basic pharmacological principles and good clinical practice would seem to serve as motivation that appropriate (to best knowledge) dosage adjustment be effected in order to confer comparable, if not equal, therapeutic efficacy/toxicity risk profiles on each individual patient.

In adult TB patients, it seems as if a dose of 4 to 6 mg/kg daily in a population of mainly slow acetylators will be sufficient for the majority of patients, specifically if used in combination with other antituberculosis drugs. This will give a concentration at time zero (C_0), calculated as dose per kg divided by Vd (0.67 for adults), of 5.97 to 8.96 mg/l. The AUC for an average slow acetylator ($k = 0.2$ h^{-1}) will therefore be between 29.9 and 44.8 mg/l/h. From the calculations of AUC in our study, it is clear that the mean AUC for fast acetylators in children will fall below the optimal AUC for adults even at a dose of 10 mg/kg and an elevation of dosage may be required, while the majority of slow acetylators will have exceeded the desired AUC at 10 mg/kg body weight.

It seems therefore that in children the dose will vary for slow and fast acetylators and that the optimal dose will vary between 5 to 10 mg/kg daily in most cases, taking into account that toxicity may increase considerably with doses of more than 10 mg/kg in slow acetylators.[9, 50]

In an area where the majority of tuberculosis occurs in the ethnic populations that are intermediate to fast acetylators, and taking into consideration the factors discussed that could negatively affect INH-concentrations in children, it seems appropriate to advise that children should receive a higher dose of INH of between 6 to 10 mg/kg body weight compared to the WHO and IUATLD recommended dose of 4 to 6 mg/kg (table 1).[51-54]

Inactivation Index (I₃): Although the dosage adjustment according to the 3-hour INH concentration and the inactivation index formula [13] has the advantage of making use of a single INH concentration point, there are now more accurate methods available to determine the dose requirements for individual patients. However, when the inactivation index and the 3-hour INH concentration was used to calculate the dose requirement for our patients, using the accepted therapeutic 3-hour level of 1.5 mg/L,[13,26] the required dosages (approximately 4 mg/kg for slow acetylators and approximately 7 mg/kg for intermediate to fast acetylators) were similar to the AUC-derived dosages (table 6).

What constitutes an optimal INH dosage regimen?: In attempting to determine optimal INH dosage regimens, suitable for general application to large populations of children, the characteristics of the specific population should properly be taken into account. Our target population is comprised of a preponderance of fast acetylators in the ratio slow:fast of 33-35%:65-68% (own data and Parkin et al.).[22] Furthermore, a considerable percentage of this population is HIV-infected (8.7%; 95% CI: 6.0-11.4% in the Western Cape province, antenatal survey 2000, Department of Health), and the primary INH-resistant rate as determined by our study (chapter 3) was about 7%, half of which will probably have low-level INH resistance (MIC = ≤2 mg/L).

The optimal dosage regimen should consequently contain the highest dose of INH that can safely be given to slow acetylators who are at the highest risk of developing toxicity. In our children a dose of 10 mg/kg is well tolerated provided that there is no underlying hepatic pathology. Directly observed therapy (DOT) should be applied in the treatment of all patients. With DOT hepatic toxicity (nausea, jaundice, hepatic tenderness) can be adequately assessed. If such an approach is adhered to, maximal therapeutic benefit may be achieved in large populations in field conditions. If however

INH at this dosage is ineffective or toxicity ensues, the specific needs of the patient need to be assessed, e.g. toxicity in slow acetylator needing a reduced dose or a fast acetylator needing an increased dose.

The use of INH in primary INH-resistant or MDR children: Resistance of *M. tuberculosis* to INH is usually defined as a MIC of >0.2 mg/l. Studies have shown varying degrees of resistance to INH, but when the MIC is >5.0 mg/L, resistance is complete.

In approximately half the patients who present with primary INH resistance, the bacilli have low-level resistance of between 0.2 and 2.0 mg/L.[7, 8] This was confirmed in a small study sample in our study (Chapter 3). Victor et al.[65] showed that up to 50% of strains of a large number of random specimens had a MIC of ≤5 mg/L.

The use of INH in the treatment of INH-resistant patients remains a matter of conjecture. This issue becomes more relevant as the incidence of primary INH and multidrug resistance rises in communities, and specifically developing communities with restricted resources. Devadatta et al.[66] showed a significantly beneficial response to high-dose INH (14 mg/kg) in patients with low-level INH resistance and Tripathy et al.[8] agreed that primary INH resistance should not be regarded as an indication for stopping further treatment with the drug.

Mitchison [57] discovered that the efficacy of an INH-only regimen increased as the dose was increased. His explanation was that it seemed likely that an increased dose of INH is more efficacious because it suppresses the growth of mutants with lower degrees of resistance.

Even in retreatment cases with low-level INH resistance (MIC between 0.2 to 1.0 mg/L), the addition of high dose (16 to 20 mg/kg) INH may be advantageous if given together with other drugs to which the organisms are susceptible.[67] No advantage was, however, shown when the normal dose of 300 mg INH was used in addition to a similar regimen in another study.[68]

In a recent experimental study in mice a marked reduction in low-level (MIC ≤5 mg/L) INH-resistant *M. tuberculosis* bacilli was achieved (although significantly less than in an INH-susceptible strain) with INH at 25 mg/kg. However, INH at 100 mg/kg was not more active than INH at 25 mg/kg against the low-level INH-resistant strain of *M. tuberculosis*.[69]

Considering the relative low toxicity rate of INH in children, it should be possible to use INH at increased doses (15 to 20 mg/kg) in selected cases, such as children with primary INH resistance or TBM. In our study of childhood contacts of adult MDR TB cases, none of 62 children who had received either chemoprophylaxis or treatment that included INH at 15 to 20 mg/kg daily, developed jaundice because of therapy. (Chapter 6, table 3) Furthermore, only one of 38 children treated with INH 20 mg/kg together with three other antituberculosis drugs for TBM developed significantly raised liver enzymes and bilirubin levels.[70]

Mean INH peak serum concentrations of 19.1 mg/l +/- SD 4.2 mg/l have been achieved by giving INH doses of 20 mg/kg, which is approximately 10 times the MIC of 2 mg/ml.[70] A C_{max} of ten times the MIC should have an effect on these low-level resistant organisms even though the AUC will be significantly decreased and an AUIC of 125 may not be achieved. In the case of INH, if even smaller pulses that in themselves may

not have a detectable effect, are followed on successive days by further small pulses, the overall effect, measurable by bactericidal activity or lag in growth, is cumulative.[10] It is therefore advisable that INH treatment should be given daily if added to a drug-resistant treatment regimen.

In this study we have shown that even at a dose of only 10 mg/kg , 51 (80%) children had an 3-hour INH-concentration of >2 mg/L. All children with 3-hour INH-concentrations <2 mg/L were phenotypically fast (n = 11) and intermediate (n = 2) acetylators. Furthermore 28 (44%) children, including all the slow acetylators, had INH-levels of >2 mg/L at 5-hours post INH administration. Therefore, in an area where primary INH resistance is high, a dosage of 10 mg/kg of INH may contribute to the efficacy of treatment regimens in low-level INH-resistant cases mainly in the slow acetylators. An increased INH-dosage of 15-20 mg/L in the faster acetylators will most likely have a similar effect in this group. INH should, however, not be given as a replacement drug for other antituberculosis drugs to which the bacilli are susceptible, but as an additional drug to such a regimen.

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Table 3: Raw data tabulation of age, gender and INH levels at 2 to 5 hours post dose.

Patient ID	Age (years)	Gender	INH d/kg	2-hour time	2-hour concentration	3-hour time	3-hour concentration	4-hour time	4-hour concentration	5-hour time	5-hour concentration
1	9.00	Male	10	2	4.8354	3.016	2.6118	4	1.8244	5.0333	1.0217
2	11.17	Male	10.26	2.0667	8.4644	3	5.942	4.0833	3.9603	5.1167	2.1134
3	2.25	Male	10	2.0167	10.6173	3.1167	7.7778	4.0333	5.8908	5.0833	4.2015
4	2.92	Male	10	1.9667	9.1346	3	7.5492	4	5.8439	5.0833	4.4007
5	3.92	Male	13.27	1.95	2.1831	2.9833	2.9833	3.95	2.3518	5.1	1.2816
6	4.00	Female	10	1.9166	7.1133	2.883	4.3116	3.9	2.761	4.933	1.7673
7	6.75	Male	10	1.917	5.9251	2.966	2.7138	3.9	1.5153	5	0.862
8	3.92	Male	10	2.067	6.2507	3.1	3.8725	4.1	2.4194	5.1	1.4257
9	7.83	Female	10	2	2.7367	3	1.1041	4.05	0.5406	5.034	0.2724
10	2.75	Female	10			3	5.6697	4	4.3157	5	3.1516
11	4.67	Male	10.87	2.083	6.3389	3	5.5548	4.083	4.3228	5.033	3.7504
12	2.08	Male	10	2.033	7.7387	3.083	5.811	4.083	4.2697	5.117	2.9883
13	4.00	Male	10.2	2	5.1221	3	2.3443	4	1.3305	5	0.6558
14	2.50	Male	10	1.983	7.8519	2.9667	5.4469	4	3.9141	4.9667	3.3263
15	0.83	Male	10	1.95	6.7381	2.967	4.9591	4	3.9855	4.9667	3.1764
16	4.08	Male	10	2.0833	2.6211	3.0667	1.1771	4	0.6139	5.233	0.3681
17	3.58	Female	10	2.134	5.5043	3	3.1869	4	1.7639	5	0.9862
18	1.25	Male	10.41			3.1	6.1663	3.9667	5.1647	5.2667	3.6099
19	11.08	Female	10	2	7.7494	3	4.944	4.0167	2.8028	5.0167	1.9554
20	0.75	Male	9.75	2.133	5.3125	3	3.108	4.05	1.9052	5.08	1.1928
21	4.00	Female	14.49	2	9.8829	3	7.7941	4	5.5386	5	4.7665
22	3.08	Male	10	2	2.3697	3.016	1.0929	4.05	0.6156	5.07	0.3153

Table 3: Raw data tabulation of age, gender and INH levels at 2 to 5 hours post dose. (continued)

Patient ID	Age (years)	Gender	INH d/kg	2-hour time	2-hour concentration	3-hour time	3-hour concentration	4-hour time	4-hour concentration	5-hour time	5-hour concentration
23	3.00	Female	10.1	2	2.5745	2.96	1.6594	3.95	1.0031	5.03	0.6994
24	10.92	Male	10	1.933	6.5805	3.05	3.6765	4	1.9654	4.98	1.0322
25	0.67	Female	10	2.016	4.2066	3.016	2.3474	4.016	1.2339	5	0.8863
26	9.00	Male	10	1.95	7.9327	2.967	6.0954	3.967	4.9336	4.95	4.0782
27	4.92	Female	10	2.4	2.4829	3.05	1.3441	4	0.6138	5.1	0.2833
28	9.92	Female	10	1.9167	4.4031	2.9167	2.374	3.9333	1.3348		
29	1.42	Female	10	1.9	3.2558	2.983	1.3365	3.967	0.7797	4.95	0.4579
30	10.50	Male	10	2.05	7.9379	3.083	4.4433	4.067	2.5537	5	1.7132
31	8.17	Male	10	1.867	7.1961	3.033	5.1931	4.05	4.2569	4.9667	3.2884
32	3.17	Female	10	2.25	4.3195	2.967	2.6136	4.133	1.7953		
33	5.33	Male	10	1.933	1.7752	2.95	0.9256	4	0.5128	4.95	0.4001
34	2.58	Female	10	2	6.3807	2.883	5.1362	3.917	3.9759	4.917	3.3156
35	3.33	Female	10	1.967	3.3596	2.867	1.7548	3.95	0.9478	4.967	0.5197
36	7.67	Male	10	2.033	7.3902	3	4.743	4.0333	2.9218	5.0167	1.734
37	2.67	Female	10	2	6.5268	2.9666	4.1383	4	2.5305	5	1.394
38	9.92	Female	10	1.85	10.8406	2.9833	7.47	3.9833	5.751	5	4.3066
39	1.25	Female	10	1.95	7.2519	3.0166	6.0108	4	4.3918	5	3.8142
40	0.58	Male	10	1.933	7.0375	3.05	5.0144	4.083	3.9572	5.1166	3.0972
41	1.17	Female	10	2.033	6.6676	3.0833	4.4643	4.1	3.0454	5.1333	1.9997
42	12.58	Female	10	2.0667	8.3661	2.95	6.8566	3.95	5.5876	4.9667	3.9842
43	2.33	Male	10	1.9833	3.4724	2.95	1.5588	4.0667	0.6029	4.95	0.2799
44	11.00	Male	10	2.0333	4.8809	3.0333	2.7867	3.9833	1.9601	4.9666	1.3518

Table 3: Raw data tabulation of age, gender and INH levels at 2 to 5 hours post dose. (continued)

Patient ID	Age (years)	Gender	INH d/kg	2-hour time	2-hour concentration	3-hour time	3-hour concentration	4-hour time	4-hour concentration	5-hour time	5-hour concentration
45	4.58	Female	10	1.95	12.6101	3.0833	9.4362	4.0833	7.4746	5.0333	6.1358
46	4.00	Female	10	2.1667	5.6127	2.95	3.9965	4.0833	2.1316	5	1.3159
47	6.58	Male	10	2	1.9065	3	1.0087	4.1166	0.5962	5	0.379
49	10.08	Male	10.08	2.0833	6.5786	3	4.4561	4.1667	2.7025	4.9	1.9557
50	0.58	Male	10	1.8833	7.2402	3.0167	5.5783	4.0833	4.216	5.05	3.374
51	1.58	Male	10	2	4.7023	2.95	2.7473	4.1	1.3843	4.95	0.8361
52	12.17	Male	10	2	11.4861	3	10.1876	4.033	8.3527	4.95	6.9322
53	1.00	Male	10	2.3166	3.4932	3.2	3.048	4.1667	2.4248		
54	2.00	Male	10	2.1	3.9257	2.95	2.2925	4.0333	1.2554	4.95	0.7348
55	3.42	Female	10	2.1333	4.6051	3.0167	2.7607	4.05	1.574	5.0667	0.8525
56	6.25	Female	10	2	10.7708	3.0167	8.6152	3.95	7.0946	4.9667	5.7846
57	0.83	Male	10	2.1167	2.4584	2.9833	1.3779	4.0167	0.7065	4.9833	0.4031
58	11.42	Female	10	1.8833	7.9778	3	4.7749	3.9667	3.214	4.9333	2.0815
59	1.58	Male	10	2	8.5183	3.0833	6.5491	4.0333	5.1388	5.0333	4.16
60	1.92	Female	10	1.9167	4.663	3.0167	2.382	3.9833	1.3725	5	0.8047
61	6.47	Female	10	2.0167	8.3002	2.9833	6.8854	4	5.4259	4.9667	3.878
62	11.25	Male	10	1.95	12.3481	3.0167	9.712	4.0333	7.3508	4.9837	6.1418
63	1.08	Female	10	2.016	3.1033	3.1333	1.0303	4.0333	0.4378		
64	1.39	Female	10	2.1167	6.5204	3.0667	4.6814	4.0833	3.2884	5	2.5097
65	1.40	Male	9.73	2.05	3.823	3	1.7435	4.067	0.8474	4.9667	0.4627

Table 4: INH concentrations corrected for times 2 to 5 hours post dose.

Patient ID	Age (years)	Gender	INH d/kg	Corrected 2h concentration	Corrected 3h concentration	Corrected 4h concentration	Corrected 5h concentration
1	9.00	Male	10	4.6800	2.8431	1.7272	1.0493
2	11.17	Male	10.26	9.1252	5.8360	3.7324	2.3870
3	2.25	Male	10	10.7757	7.9621	5.8831	4.3470
4	2.92	Male	10	9.2816	7.3268	5.7836	4.5655
5	3.92	Male	13.27	4.6785	3.1261	2.0888	1.3957
6	4.00	Female	10	6.6763	4.2193	2.6665	1.6852
7	6.75	Male	10	5.2814	2.8283	1.5146	0.8111
8	3.92	Male	10	6.5467	4.0284	2.4788	1.5252
9	7.83	Female	10	2.5326	1.1867	0.5560	0.2605
10	2.75	Female	10	7.6575	5.7093	4.2567	3.1737
11	4.67	Male	10.87	6.5037	5.4090	4.4986	3.7414
12	2.08	Male	10	7.9646	5.8504	4.2974	3.1566
13	4.00	Male	10.2	4.9391	2.5191	1.2848	0.6553
14	2.50	Male	10	6.5075	5.0909	3.9827	3.1157
15	0.83	Male	10	6.5075	5.0909	3.9827	3.1157
16	4.08	Male	10	2.4736	1.3252	0.7100	0.3804
17	3.58	Female	10	5.8864	3.2347	1.7776	0.9768
18	1.25	Male	10.41	8.2301	6.4134	4.9978	3.8946
19	11.08	Female	10	7.6968	4.8255	3.0253	1.8967
20	0.75	Male	9.75	5.4238	3.2864	1.9913	1.2066
21	4.00	Female	14.49	9.8139	7.6209	5.9180	4.5956
22	3.08	Male	10	2.3704	1.1893	0.6230	0.3263

Table 4: INH concentrations corrected for times 2 to 5 hours post dose. (continued)

Patient ID	Age (years)	Gender	INH d/kg	Corrected 2h concentration	Corrected 3h concentration	Corrected 4h concentration	Corrected 5h concentration
23	3.00	Female	10.1	2.5172	1.6262	1.0506	0.6787
24	10.92	Male	10	6.5863	3.5733	1.9387	1.0518
25	0.67	Female	10	4.0660	2.3828	1.3963	0.8183
26	9.00	Male	10	7.7068	6.1793	4.9545	3.9725
27	4.92	Female	10	3.2386	1.4540	0.6528	0.2931
28	9.92	Female	10	4.1548	2.2990	1.2721	0.7039
29	1.42	Female	10	2.8061	1.4863	0.7873	0.4170
30	10.50	Male	10	7.9502	4.7044	2.7837	1.6472
31	8.17	Male	10	6.8937	5.3839	4.2047	3.2838
32	3.17	Female	10	4.7384	2.7921	1.6453	0.9695
33	5.33	Male	10	1.6821	0.9226	0.5060	0.2776
34	2.58	Female	10	6.3036	5.0255	4.0065	3.1942
35	3.33	Female	10	2.7389	1.4803	0.8001	0.4324
36	7.67	Male	10	7.6226	4.6975	2.8948	1.7839
37	2.67	Female	10	6.6920	4.0157	2.4097	1.4460
38	9.92	Female	10	10.2098	7.6366	5.7119	4.2723
39	1.25	Female	10	7.2278	5.7946	4.6457	3.7245
40	0.58	Male	10	6.7666	5.2415	4.0601	3.1450
41	1.17	Female	10	6.7853	4.6069	3.1279	2.1237
42	12.58	Female	10	8.6765	6.7499	5.2510	4.0850
43	2.33	Male	10	3.4558	1.4787	0.6327	0.2707
44	11.00	Male	10	4.6882	3.0452	1.9779	1.2847

Table 4: INH concentrations corrected for times 2 to 5 hours post dose. (continued)

Patient ID	Age (years)	Gender	INH d/kg	Corrected 2h concentration	Corrected 3h concentration	Corrected 4h concentration	Corrected 5h concentration
45	4.58	Female	10	12.3168	9.7471	7.7135	6.1041
46	4.00	Female	10	6.2890	3.7449	2.2300	1.3279
47	6.58	Male	10	1.8285	1.0752	0.6322	0.3718
49	10.08	Male	10.08	6.8357	4.4454	2.8909	1.8800
50	0.58	Male	10	7.0659	5.5400	4.3435	3.4055
51	1.58	Male	10	4.7412	2.6368	1.4665	0.8156
52	12.17	Male	10	11.7552	9.8867	8.3153	6.9936
53	1.00	Male	10	3.7686	3.0919	2.5368	2.0813
54	2.00	Male	10	4.0949	2.2842	1.2742	0.7108
55	3.42	Female	10	4.9769	2.8095	1.5860	0.8953
56	6.25	Female	10	10.7173	8.6925	7.0502	5.7182
57	0.83	Male	10	2.5987	1.3811	0.7340	0.3901
58	11.42	Female	10	7.5239	4.8564	3.1346	2.0232
59	1.58	Male	10	8.4745	6.6790	5.2639	4.1486
60	1.92	Female	10	4.3503	2.4597	1.3908	0.7864
61	6.47	Female	10	8.6191	6.6758	5.1706	4.0048
62	11.25	Male	10	12.1887	9.6380	7.6210	6.0262
63	1.08	Female	10	3.1352	1.1867	0.4492	0.1700
64	1.39	Female	10	6.5204	4.6814	3.2884	2.5097
65	1.40	Male	9.73	3.8230	1.7435	0.8474	0.4627

Table 5: Calculated pharmacokinetic parameters

Patient ID	Age (years)	Gender	Calculated 3h concentration	k , h^{-1}	Phenotypification	Co (mg/L) extrapolated	Co (mg/L) calculated	Vd (L/kg) individual	$AUC_{0-\infty}$ (mg/L/h)
1	9	Male	2.8431	0.4984	Intermed	12.6808	14.5433	0.7886	29.180
2	11.17	Male	5.836	0.447	Intermed	22.3101	14.9215	0.4599	33.381
3	2.25	Male	7.9621	0.3026	Slow	19.7369	14.5433	0.5067	48.061
4	2.92	Male	7.3268	0.2365	Slow	14.8951	14.5433	0.6714	61.494
5	3.92	Male	3.1261	0.4032	Intermed	10.479	19.2990	1.2663	47.865
6	4	Female	4.2193	0.4589	Intermed	16.7159	14.5433	0.5982	31.692
7	6.75	Male	2.8283	0.6245	Fast	18.4153	14.5433	0.543	23.288
8	3.92	Male	4.0284	0.4856	Intermed	17.2905	14.5433	0.5784	29.949
9	7.83	Female	1.1867	0.7581	Fast	11.5357	14.5433	0.8669	19.184
10	2.75	Female	5.7093	0.2936	Slow	13.7755	14.5433	0.7259	49.534
11	4.67	Male	5.409	0.1843	Slow	9.4024	15.8086	1.1561	85.776
12	2.08	Male	5.8504	0.3085	Slow	14.7612	14.5433	0.6775	47.142
13	4	Male	2.5191	0.6733	Fast	18.9877	14.8342	0.5372	22.032
14	2.5	Male	5.1825	0.2678	Slow	13.3912	14.5433	0.7468	54.307
15	0.83	Male	5.0909	0.2455	Slow	10.633	14.5433	0.9405	59.240
16	4.08	Male	1.3252	0.6241	Fast	8.6182	14.5433	1.1603	23.302
17	3.58	Female	3.2347	0.5987	Fast	19.4929	14.5433	0.513	24.291
18	1.25	Male	6.4134	0.2494	Slow	13.5528	14.5433	0.7379	58.313
19	11.08	Female	4.8255	0.4669	Intermed	19.582	15.1396	0.5316	32.425
20	0.75	Male	3.2864	0.501	Intermed	14.773	14.5433	0.6769	29.029
21	4	Female	7.6209	0.2529	Slow	16.2745	14.1796	0.599	56.068
22	3.08	Male	1.1893	0.6466	Fast	8.2742	21.0732	1.7512	32.591

Table 5: Calculated pharmacokinetic parameters (continued)

Patient ID	Age (years)	Gender	Calculated 3h concentration	k , h^{-1}	Phenotypification	Co (mg/L) extrapolated	Co (mg/L) calculated	Vd (L/kg) individual	$AUC_{0-\infty}$ (mg/L/h)
23	3	Female	1.6262	0.4369	Intermed	6.0312	14.5433	1.658	33.287
24	10.92	Male	3.5733	0.6115	Fast	22.3762	14.6888	0.4513	24.021
25	0.67	Female	2.3828	0.5344	Intermed	11.8397	14.5433	0.8446	27.214
26	9	Male	6.1793	0.2209	Slow	11.9879	14.5433	0.8342	65.837
27	4.92	Female	1.454	0.8008	Fast	16.0664	14.5433	0.6224	18.160
28	9.92	Female	2.299	0.5918	Fast	13.57	14.5433	0.7369	24.575
29	1.42	Female	1.4863	0.6355	Fast	10.0021	14.5433	0.9979	22.885
30	10.5	Male	4.7044	0.5247	Fast	22.7053	14.5433	0.4404	27.717
31	8.17	Male	5.3839	0.2472	Slow	11.3023	14.5433	0.8848	58.832
32	3.17	Female	2.7921	0.5289	Fast	13.6467	14.5433	0.7328	27.497
33	5.33	Male	0.9226	0.6006	Fast	5.5916	14.5433	1.7884	24.215
34	2.58	Female	5.0255	0.2266	Slow	9.9177	14.5433	1.0083	64.180
35	3.33	Female	1.4803	0.6153	Fast	9.3761	14.5433	1.0665	23.636
36	7.67	Male	4.6975	0.4841	Intermed	20.0719	14.5433	0.4982	30.042
37	2.67	Female	4.0157	0.5107	Fast	18.5842	14.5433	0.5381	28.477
38	9.92	Female	7.6366	0.2904	Slow	18.2497	14.5433	0.548	50.080
39	1.25	Female	5.7946	0.221	Slow	11.2451	14.5433	0.8892	65.807
40	0.58	Male	5.2415	0.2554	Slow	11.2774	14.5433	0.8867	56.943
41	1.17	Female	4.6069	0.3872	Intermed	14.7192	14.5433	0.6794	37.560
42	12.58	Female	6.7499	0.2511	Slow	14.3367	14.5433	0.6975	57.918
43	2.33	Male	1.4787	0.8489	Fast	18.8752	14.5433	0.5298	17.132
44	11	Male	3.0452	0.4315	Intermed	11.1123	14.5433	0.8999	33.704

Table 5: Calculated pharmacokinetic parameters (continued)

Patient ID	Age (years)	Gender	Calculated 3h concentration	k , h^{-1}	Phenotypification	Co (mg/L) extrapolated	Co (mg/L) calculated	Vd (L/kg) individual	$AUC_{0-\infty}$ (mg/L/h)
45	4.58	Female	9.7471	0.234	Slow	19.6675	14.5433	0.5084	62.151
46	4	Female	3.7449	0.5184	Intermed	17.7362	14.5433	0.5638	28.054
47	6.58	Male	1.0752	0.531	Intermed	5.2882	14.5433	1.891	27.389
49	10.08	Male	4.4454	0.4303	Intermed	16.1636	14.6597	0.6236	34.069
50	0.58	Male	5.54	0.2433	Slow	11.4947	14.5433	0.8699	59.775
51	1.58	Male	2.6368	0.5867	Fast	15.328	14.5433	0.6524	24.788
52	12.17	Male	9.8867	0.1731	Slow	16.6181	14.5433	0.6018	84.017
53	1	Male	3.0919	0.1979	Slow	5.5985	14.5433	1.7862	73.488
54	2	Male	2.2842	0.5837	Fast	13.1594	14.5433	0.7599	24.916
55	3.42	Female	2.8095	0.5718	Fast	15.6176	14.5433	0.6403	25.434
56	6.25	Female	8.6925	0.2094	Slow	16.2918	14.5433	0.6138	69.452
57	0.83	Male	1.3811	0.6321	Fast	9.2001	14.5433	1.0889	23.008
58	11.42	Female	4.8564	0.4378	Intermed	18.0598	14.5433	0.5537	33.219
59	1.58	Male	6.679	0.2381	Slow	13.6435	14.5433	0.7329	61.081
60	1.92	Female	2.4597	0.5702	Fast	13.6079	14.5433	0.7349	25.506
61	6.47	Female	6.6758	0.2555	Slow	14.3677	14.5433	0.696	56.921
62	11.25	Male	9.638	0.2348	Slow	19.4941	14.5433	0.513	61.939
63	1.08	Female	1.1867	0.9715	Fast	21.8828	14.5433	0.457	14.970
64	1.39	Female	4.6814	0.3317	Intermed	12.8978	14.5433	0.7753	43.845
65	1.4	Male	1.7435	0.7219	Fast	15.8388	14.1507	0.6313	19.602

Table 6: Dose adjustments according to the inactivation index (I_3)

Patient ID	K-value	Corrected 3-h conc	Inactivation Index$_{3h}$ (I_3)	I_3-adjusted dose (3-h INH conc. = 1.5 mg/L)	I_3-adjusted dose (3-h INH conc. = 2.0 mg/L)
1	0.4984	2.8431	0.3443	6.10	7.55
2	0.447	5.836	0.6273	3.35	4.14
3	0.3026	7.9621	0.8562	2.45	3.04
4	0.2365	7.3268	0.7927	2.65	3.28
5	0.4032	3.1261	0.2808	7.48	9.26
6	0.4589	4.2193	0.4819	4.36	5.39
7	0.6245	2.8283	0.3428	6.13	7.58
8	0.4856	4.0284	0.4628	4.54	5.62
9	0.7581	1.1867	0.1787	11.75	14.55
10	0.2936	5.7093	0.6309	3.33	4.12
11	0.1843	5.409	0.5528	3.80	4.70
12	0.3085	5.8504	0.6450	3.26	4.03
13	0.6733	2.5191	0.3058	6.87	8.50
14	0.4263	5.1825	0.5783	3.63	4.50
15	0.2455	5.0909	0.5691	3.69	4.57
16	0.6241	1.3252	0.1925	10.91	13.51
17	0.5987	3.2347	0.3835	5.48	6.78
18	0.2494	6.4134	0.6737	3.12	3.86
19	0.4669	4.8255	0.5426	3.87	4.79
20	0.501	3.2864	0.3986	5.27	6.52
21	0.2529	7.6209	0.5673	3.70	4.58
22	0.6466	1.1893	0.1789	11.74	14.53
23	0.4369	1.6262	0.2204	9.53	11.80
24	0.6115	3.5733	0.4173	5.03	6.23
25	0.5344	2.3828	0.2983	7.04	8.72

Table 6: Dose adjustments according to the inactivation index (I_3) (continued)

Patient ID	K-value	Corrected 3-h conc	Inactivation Index$_{3h}$ (I_3)	I_3-adjusted dose (3-h INH conc. = 1.5 mg/L)	I_3-adjusted dose (3-h INH conc. = 2.0 mg/L)
26	0.2209	6.1793	0.6779	3.10	3.84
27	0.8008	1.454	0.2054	10.22	12.66
28	0.5918	2.299	0.2899	7.24	8.97
29	0.6355	1.4863	0.2086	10.07	12.46
30	0.5247	4.7044	0.5304	3.96	4.90
31	0.2472	5.3839	0.5984	3.51	4.34
32	0.5289	2.7921	0.3392	6.19	7.66
33	0.6006	0.9226	0.1523	13.79	17.08
34	0.2266	5.0255	0.5626	3.73	4.62
35	0.6153	1.4803	0.2080	10.09	12.50
36	0.4841	4.6975	0.5298	3.96	4.91
37	0.5107	4.0157	0.4616	4.55	5.63
38	0.2904	7.6366	0.8237	2.55	3.16
39	0.221	5.7946	0.6395	3.28	4.07
40	0.2554	5.2415	0.5842	3.59	4.45
41	0.3872	4.6069	0.5207	4.03	4.99
42	0.2511	6.7499	0.7350	2.86	3.54
43	0.8489	1.4787	0.2079	10.10	12.51
44	0.4315	3.0452	0.3645	5.76	7.13
45	0.234	9.7471	1.0347	2.03	2.51
46	0.5184	3.7449	0.4345	4.83	5.98
47	0.531	1.0752	0.1675	12.54	15.52
49	0.4303	4.4454	0.5005	4.20	5.19
50	0.2433	5.54	0.6140	3.42	4.23
51	0.5867	2.6368	0.3237	6.49	8.03

Table 6: Dose adjustments according to the inactivation index (I_3) (continued)

Patient ID	K-value	Corrected 3-h conc	Inactivation Index$_{3h}$ (I_3)	I_3-adjusted dose (3-h INH conc. = 1.5 mg/L)	I_3-adjusted dose (3-h INH conc. = 2.0 mg/L)
52	0.1731	9.8867	1.0487	2.00	2.48
53	0.1979	3.0919	0.3692	5.69	7.04
54	0.5837	2.2842	0.2884	7.28	9.01
55	0.5718	2.8095	0.3410	6.16	7.63
56	0.2094	8.6925	0.9293	2.26	2.80
57	0.6321	1.3811	0.1981	10.60	13.12
58	0.4378	4.8564	0.5456	3.85	4.77
59	0.2381	6.679	0.7279	2.89	3.57
60	0.5702	2.4597	0.3060	6.86	8.50
61	0.2555	6.6758	0.7276	2.89	3.57
62	0.2348	9.638	1.0238	2.05	2.54
63	0.9715	1.1867	0.1787	11.75	14.55
64	0.3317	4.6814	0.5281	3.98	4.92
65	0.7219	1.7435	0.2409	8.72	10.79

CHAPTER 8

General Conclusions

General Conclusions

A.   Background to drug-resistant tuberculosis

Soon after the discovery of the first antituberculosis agents it became clear that the development of drug resistance was not uncommon, and that it could lead to failure of both individual antituberculosis treatment and tuberculosis control programmes.[1-3] Combination therapy introduced in the mid-1950s appeared to overcome most of the problems with antituberculosis drug resistance.[4-6]

The first twenty to twenty-five years after the introduction of antituberculosis treatment saw the development of most of the antituberculosis agents currently in use, as well as research on the mechanisms and effects of drug resistance. So effective was the introduction of combination treatment regimens that research in the field of tuberculosis in general, but more specifically drug-resistant tuberculosis, halted almost completely in the early 1970s.

Despite warnings by experienced researchers such as Canetti[7] and Horne[8], this lack of interest continued into the late 1980s, at which time the incidence of tuberculosis started to rise in developed countries after many years of steady decline.[9, 10] This was mainly due to the effect of HIV/AIDS, but also as a result of increased immigration from the developing countries where tuberculosis had never really been controlled.[10,11] The severity of the new epidemic only struck home, however, when large outbreaks of MDR TB started to occur in developed countries such as the United States.[12,13]

B.   Background to childhood drug-resistant tuberculosis

Tuberculosis in children has been a very neglected field of research, as it was thought to be of relatively little epidemiologic importance.[14] This is, however, an incorrect assumption, because when a child presents with a *M. tuberculosis* infection or tuberculous disease, it usually implies recent transmission from an adult with active pulmonary tuberculosis in the community.[15-17] These infected children, even if they do not immediately develop disease, may undergo reactivation during adolescence or adulthood and so contribute to the infectious pool. This has become a particularly serious possibility in the era of HIV and AIDS.

For many years the dogma that INH-resistant tubercle bacilli are less infectious and less pathogenic than INH-susceptible tubercle bacilli was accepted. This was based on experimental studies in animals,[18-21] but was not confirmed in human studies.[15,22-26] Many researchers still hold this view, and the infectiousness and pathogenicity of MDR TB for immunocompetent children still had to be proven.

With the rise in the incidence of drug-resistant tuberculosis in adults in cities such as New York, it was noticed that a similar increase in drug-resistant tuberculosis in children soon followed.[27-29] Apart from a few surveys in the USA,[28,30,31] very little is known about the incidence of drug-resistant tuberculosis in children. Furthermore, despite much debate on the management of adults in contact with MDR TB cases, children have received little attention, and no prospective studies have been recorded to date to evaluate the management of these childhood contacts.[32-34] Many reports describe the treatment and outcome of adults with MDR TB, but, once again, the treatment of

children with MDR TB is based on a number of case reports, and experience in the treatment of adults with MDR TB.[28,35-37]

The role of individual antituberculosis agents in the management of MDR TB in children needs to be clarified.[34,38] Several reports speculate on the possible therapeutic role of INH in primary INH-resistant tuberculosis, which would include the majority of children with drug-resistant TB.[39-42] Although failure of INH as a single chemoprophylactic agent against INH-resistant strains has been reported,[22,43,44] it is possible that, with partial or low-level resistance to INH, this agent can still be a valuable adjunct to the treatment regimen. Furthermore, the second line antituberculosis agents, which are known to be less effective and to cause more adverse events than the first line drugs, need to be evaluated in the long-term treatment in children.

C. Drug-resistant TB in children as a measure of transmission of drug-resistant TB in the community.

Although the primary aim of any TB control program or service should be to optimise treatment (short course chemotherapy, directly observed) for drug-susceptible cases, the surveillance, either continuous or periodic, of the magnitude of the drug resistance problem remains essential.[45] Trends in primary or initial drug resistance provide an indication of the effectiveness of treatment regimens, while acquired drug resistance rates can indicate failure in the management of disease.[46]

The surveillance of drug-resistant TB in children may be beneficial for several reasons. Firstly, it is often difficult to establish whether adults had received previous antituberculosis treatment, and thus whether they actually have primary drug resistance.[8,17] Young children, because of their age, usually have primary drug resistance

and previous antituberculosis treatment is much easier to exclude with certainty.[17] Secondly, even when it is established that previous TB treatment had been received by an adult, it has been shown by DNA fingerprinting of *M. tuberculosis* strains that in a high TB incidence area, MDR TB is still more often transmitted (i.e. reinfection from an index case with MDR TB) rather than acquired through non-compliance with antituberculosis drug treatment.[47] Finally, few high TB-burden countries have the resources to do susceptibility testing on all *M. tuberculosis* isolates or even to do cultures for *M. tuberculosis* on each patient with suspected TB. Sampling is, therefore, often done, the results of which could easily be distorted by testing mainly those who are at higher risk of having drug-resistant TB.[45] Drug susceptibility testing of all *M. tuberculosis* isolates obtained from children may thus be a cost effective alternative.

We have shown that prospective susceptibility testing of *M. tuberculosis* isolates from children with TB in a specific geographic drainage area yielded results of drug resistance comparable with results obtained from an adult study that had been done in the same area. This was despite the number of childhood cases being much smaller than the number of cases in the adult study.[46] When study limitations were removed, 5.6% of children had primary INH resistance and 1.0% MDR TB compared to a rate of 3.9% and 1.1%, respectively, in adults.

If the total study population was included in the analysis, however, the increase in drug resistance evident in the childhood surveillance study (6.9% INH resistance and 2.3% MDR) could be explained in several ways (see chapter 3, discussion), of which the most likely reasons are a bias because the childhood study was hospital-based and therefore probably represents the worst case scenario,[48] or because the childhood study

truly reflects transmitted drug resistance in a high incidence area as demonstrated by the DNA fingerprint study of van Rie et al.[47]

In conclusion, our findings show that the incidence of drug-resistant TB in children in the Western Cape Province is low. The study probably reflects the level of primary drug resistance amongst organisms currently circulating in our community.

Recommendation: This surveillance study should be repeated on a larger scale to include all the secondary and tertiary hospitals in the Western Cape area. The data should again be compared to current surveillance studies in adults in the same area. If these results are again similar, it will strengthen the case for children's drug susceptibility results to be used as a cost effective, continuous, surveillance method for primary (currently circulating) drug resistant TB organisms in the total population in an area.

D.   Infection and disease in childhood contacts of adult MDR pulmonary TB cases

One of the main study objectives was to determine prospectively the short-term prevalence and the long-term incidence of tuberculous infection and disease in young children in household contact with adult MDR pulmonary TB, and therefore the importance of contact tracing in this group of patients. A further aim was to confirm transmission of MDR *M. tuberculosis* organisms to children in contact with adult MDR pulmonary TB cases by evaluating drug susceptibility patterns and RFLP analysis.

Although the absence of a prospective control group was a limitation of this study, the initial evaluation (0 to 2 months) was compared with a similar study of children <5 years of age who had been in contact with mainly drug-susceptible cases in the same geographical area.[49] The infection rate (63%) at the first evaluation was significantly

higher (P < .02) than in the child contacts of the drug susceptible cases (48%), but the disease rate was much less (12% versus 34%, respectively). Snider et al.[24] had similar results regarding the infection rate in a controlled trial of INH- and/or streptomycin-resistant contacts compared to drug-susceptible contacts. This could be because adults with drug-resistant TB remain infectious for longer periods. A complicating factor in our study was that many children had more than one adult TB contact, not all of whom were multidrug-resistant.

On follow-up to 30 months, a first of its kind for childhood MDR TB contacts, many more children became infected or developed disease. As was previously found in several contact tracing studies, 90% of those who developed disease did so within the first 12 months of follow-up.[50-52] This implies that follow-up beyond 12 months is probably not cost effective in resource poor areas.

Seventy-eight percent of children had developed infection by 30-month follow-up, which is higher than the generally reported infection rate of childhood contacts 0-5 years of age of 30-72%.[29,53,54] Although a number of explanations can be given (Chapter 6, discussion), this demonstrates that MDR TB is not less infectious than drug-susceptible TB. Despite some children receiving chemoprophylaxis, 24% of the children eventually developed disease, which is again similar to the expected disease incidence in childhood contacts <5 years of age of infectious drug-susceptible adult pulmonary TB cases.[29]

Six adult-child pairs were identified in whom both the adult source case's *M. tuberculosis* strain and the child's strain were obtained. Four of these were included in the initial follow-up study and two further child contacts of adult MDR TB cases for which both isolates were available, were subsequently identified. Susceptibility patterns

as well as RFLP analysis were identical in 5 adult-child pairs. In one child, although also infected with a MDR *M. tuberculosis* strain, the strain was different both in susceptibility pattern as well as RFLP analysis. The results confirmed transmission of MDR *M. tuberculosis* strains from adult source cases to their child contacts in most cases, and confirm previous results found in INH-resistant cases by Steiner et al.[25]

Recommendation: Specimens for *M. tuberculosis* culture and susceptibility testing should be taken from all children in close contact with adult MDR pulmonary TB cases. However, if the child's *M. tuberculosis* strain is not available, the child in contact with the MDR source case should be treated according to the drug susceptibility pattern of the source case's strain.

E. Treatment and chemoprophylaxis in childhood MDR TB disease and contacts

No prospective evaluation of treatment of children with MDR TB or chemoprophylaxis for children in contact with adult MDR TB cases has been reported. An additional aim of this study was therefore to establish the role of treatment in children with MDR TB and whether chemoprophylaxis is effective in preventing active disease in the childhood contacts.

Although children with primary uncomplicated TB will often improve without treatment,[55] it is imperative that they should receive treatment because of the risk of progression or dissemination of disease specifically in young children with death or disability the outcome. The possibility of later HIV infection and its propensity to activate dormant organisms is a further cause for concern. In our series of 29 children diagnosed as having disease, 25 (86%) received appropriate antituberculosis treatment according to the drug-susceptibility pattern of the adult source cases' *M. tuberculosis*

isolate. Prescribed treatment regimens consisted of two to three drugs to which the source case's isolate was susceptible and antituberculosis drugs used were INH (see below), pyrazinamide, ethambutol (EMB), ethionamide and ofloxacin. The duration of treatment was generally shorter than that prescribed for adult type cavitary disease, as most children were identified and treated before progression of disease occurred. It is known that the lesions of uncomplicated primary TB contain a much smaller population of organisms than cavitary or disseminated lesions, and that TB in childhood in the absence of cavitation or dissemination is therefore probably overtreated.[7,34]

All children treated for MDR TB were clinically and radiologically well after completion of treatment, despite some children defaulting treatment after as little as 4 months. All treated children were followed up for 30 months and no clinical or radiological relapse occurred in these patients.

The reason for including INH at a high dose of 15 to 20 mg/kg/d in all treatment regimens was that about half of the primary INH-resistant *M. tuberculosis* isolates probably have low-level INH resistance of ≤2 mg/L. (See below) EMB, which is used as first line drug in the treatment of children with drug-susceptible TB in some developing countries, is a relatively safe drug with optic neuritis the main adverse reaction.[56,57] When used at the recommended dosage, this adverse event is very rare and EMB is therefore an essential drug in the treatment of MDR TB cases, including young children.

Ethionamide was the most commonly used second line antituberculosis drug in our study. Although about half the children receiving the drug experienced gastrointestinal adverse events, most of these could be overcome by temporarily splitting the daily dose, and only in 4 (7%) of 61 children did the drug have to be discontinued.

The fluoroquinolones are not generally recommended for use in children because of their possible harmful effect on cartilage growth.[34] Many reports of the safety of these drugs in children in short- and medium-term treatment have been published.[58-60] In our experience of 17 children (including 2 children from chapter 5 not included in chapter 6), ofloxacin for a duration of 6 to 12 months was well tolerated. Possible arthralgia due to the drug occurred in one (6%) child after 6 months of treatment. This is similar to previous reports of arthralgia occurring in 1.3 to 3.5% of children, following 150 to 300 days of treatment.[58-60] Although we used ofloxacin mainly for the treatment of children with MDR TB, it could probably also be recommended for use in MDR chemoprophylaxis in children. The adverse effects of long-term fluoroquinolone use in children needs further study.

An interesting observation during the follow-up of child contacts of adults with infectious MDR TB was that TB disease occurred significantly less often in children that received appropriate chemoprophylaxis (according to the drug susceptibility pattern of the adult source case's isolate). Although this was not a randomised controlled trial, the group that received chemoprophylaxis during the follow-up period was at higher risk for developing disease. This has definite implications for the prevention of TB in MDR contacts in so far as that these children should receive appropriate chemoprophylaxis with at least two drugs to which the infectious source case's strain is susceptible.

Recommendation: A prospective, randomised controlled study is necessary to evaluate the best drug combinations, their safety in long-term treatment, and the optimal duration of such chemoprophylactic regimens.

F.   Isoniazid and its possible role in primary isoniazid-resistant TB cases

Relatively little is known about the pharmacokinetics of INH in children.

This study was undertaken to determine whether a dose of 10 mg/kg INH would provide adequate therapeutic exposure even in children with low-level INH resistance who are fast or intermediate acetylators of INH. Low-level INH resistance occurs in about half the cases with primary INH resistance (Chapter 3).[7, 40]

In our study, using only a 10 mg/kg dose, phenotypic separation could be effected between slow and 'fast' acetylators, but clear separation between fast and intermediate acetylators, as in adults, could not be demonstrated.[61] A high percentage (>60%) of this population is intermediate or fast acetylators (Chapter 7).[61] On studying the exposure integrals and adjusted dosage regimens for INH according to Vivien's inactivation index,[62] it seems likely that in a population with mainly fast acetylators, and because children in general having a lower propensity to toxicity, an INH dosage regimen of 6-10 mg/kg/d is more appropriate than the 4-6 mg/kg/d suggested by the IUATLD and WHO.[63, 64] Supervision for adverse events, although rarely expected at this dose, can be done together with directly observed therapy.

Our results also suggest that peak levels of 10 times the MIC of low-level (≤2 mg/L) INH-resistant organisms can be achieved in most patients by giving higher dosages of INH. In case of low-level resistance to INH the optimal management will be to determine both the MIC of the INH-resistant *M. tuberculosis* strain as well as the acetylator status of the individual in case of INH-resistant TB. This is however, often not possible. Under these circumstances, knowing or suspecting (because of adult drug-resistant contact) that a child has primary INH- or multidrug-resistant TB, a high dose of

INH (15-20 mg/kg/d) with close supervision for side effects, may be beneficial in up to 50% of cases.

Further studies with regard to the use of INH in INH-resistant cases are needed. INH may for example improve the function of other antituberculosis drugs even though the organism is resistant to INH itself. A long-term follow-up study in adults with primary MDR TB treated with regimens with and without INH to specifically look at the speed with which sputum smears and cultures become negative, and remain negative, may resolve this problem.

Recommendation: We propose that INH at higher dosages of 15-20 mg/kg/d (maximum 500 mg) should remain part of the treatment regimen of children with MDR TB, especially in developing countries where second line drugs are less freely available. Treatment should be given under direct supervision and include observation for adverse events such as hepatotoxicity. Pyridoxine can be added specifically in older children to prevent peripheral neuropathy.

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