Optimizing the recovery rate of *Mycobacterium species* from gastric lavages in children

at

an urban Zambian Hospital

by

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Submitted in complete fulfillment of the requirements

for the Magister Technologiae: Biomedical Technology

at the

Nelson Mandela Metropolitan University

December 2009

Promoter: Mrs. Esther Baxter

Co-Promoter: Dr. James C. L. Mwansa
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ABSTRACT

Tuberculosis (TB) has re-emerged as a major worldwide public health hazard with increasing incidence among adults and children. Although cases among children represent a small percentage of all TB cases, they are a reservoir from which many adult cases will arise. Estimates indicate that 9 million people develop TB annually, out of which 1 million (11%) occur in children less than 15 years old. Childhood tuberculosis is on the increase worldwide because of persisting inability to conform the diagnosis, leading to a large number of children dying of undiagnosed tuberculosis.

Diagnosis of pulmonary tuberculosis has depended on bacteriological examination of sputum. In most of the developing countries sputum smear microscopy has been used as it has been found to be cheap and relative efficient. As a result of the high TB burden, there is an urgent need for improved methods of laboratory diagnosis of TB. This is especially needed in children were diagnosis is more challenging as mycobacteria is being detected in fewer than 50% of the cases. Children cannot produce adequate sputum samples for examination. Their sputum samples, if produced, has a low bacterial yield and making detection of mycobacteria by using the smear microscopy difficult. Therefore, gastric lavages from children are being recommended as the best specimen for culture.

In this study, gastric lavages from 408 children suspected of having tuberculosis were examined for the recovery of mycobacteria. Recovery was optimized by the use of the relatively new non-radiometric fully automated BACTEC MGIT 960. BACTEC MGIT 960 produced a positivity rate of 27.2% against 17.2% that of Lowenstein-Jensen (L-J) media, which is a conventional culture method used widely. The direct microscopy which is the cheapest traditional method used in diagnosis of tuberculosis (TB) yielded a 5.6% positive rate. The BACTEC MGIT 960 had also a very high isolate detection rate of 98.2% compared to that of L-J media of 61.9%, and only 20.4% were detected with the direct microscopy. On time taken to detection or mean time to detection (TTD) of
isolates, the BACTEC MGIT 960 technique had a shorter mean time to detection, 12.5 days as compared to 34.3 days shown by the L-J media technique.

The study showed that children normally get tuberculosis from adult members of the household. A positive TB case was found in the households of 55.4% of the suspects. The study has found that 46.4% of the children below the age of 4 years developed the disease, compared to 10.5% the older children in the age group 10 to 14 years.

The study found that tuberculosis in children is mainly caused by *Mycobacterium tuberculosis*. Out of the 113 isolates detected, 110 (97.3%) were *M. tuberculosis*. The remaining 2.7% were the non-tuberculous *M. avium* complex and *M. kansasii*. It was inconclusive whether the 2.7% of other species were causing tuberculosis and this need to be studied further.
ACKNOWLEDGEMENTS

I wish to pay my sincere gratitude to the following for their support, assistance and encouragement through out the long period of the research as well as compilation of this thesis of my course.

My Promoters; Mrs. Esther Baxter and Dr. James Mwansa for all your effort in guidance, support and motivation, especially that I was away from you most of the time.

My family members, my wife (Sibeso Katiwa) and children (David Lubasi Jr, Tapelo Lubasi and Stephen Lubasi), for sacrificing to let me use most of the Family finances towards school and the research.

University Teaching Hospital, for allowing me to conduct the research in the institution, as well as in the concerned departments.

§ Management; The Managing Director, Dr. Peter Mwaba; The Deputy Managing Director; Dr. Lackson Kasonka.

§ Pathology Department; The Director; Dr. Victor Mudenda, The CMLT; Mr. Davy Nsama; and all the staff.

§ TB Laboratory; The Sectional Head; Mrs Charity Habeenzu and all the staff for their tireless effort put in the research.

§ Paediatric and child Health Department; Head Dr. Chipepo Kankasa, all the senior staff, the matron and the ward supervisors and the rest of the staff in general for their tireless effort put in the research.
My fellow M Tech students for the encouragement.

Staff of Kanye SDA Hospital and the college of nursing for spiritual support.

My friends and relatives especially my mother in law (Ms. Ireen Akalaluka) for the warm reception each time I was in Zambia for the research work.

And lastly God almighty, in whom all things are possible.
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<table>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>ADA</td>
<td>Antibody Detection Assay</td>
</tr>
<tr>
<td>AFB</td>
<td>Acid Fast Bacilli</td>
</tr>
<tr>
<td>ATS</td>
<td>American Thoracic Society</td>
</tr>
<tr>
<td>B.C.</td>
<td>Before Christ</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette-Guerin</td>
</tr>
<tr>
<td>C¹⁴</td>
<td>Radioactive carbon 14</td>
</tr>
<tr>
<td>CDL</td>
<td>Chest Diseases Laboratory</td>
</tr>
<tr>
<td>Conc</td>
<td>Concentration</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CR</td>
<td>Compliment receptor</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>DEC</td>
<td>Disease Endemic Countries</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DST</td>
<td>Direct susceptibility test</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte Sedimentation Rate</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas-liquid chromatography</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
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<tr>
<td>Hr</td>
<td>Hour</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>IUATLD</td>
<td>International Union Against Tuberculosis and Lung Disease</td>
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<tr>
<td>LAM</td>
<td>Lipoarabinomanna</td>
</tr>
<tr>
<td>L-J</td>
<td>Lowenstein-Jensen media</td>
</tr>
<tr>
<td>NaClO</td>
<td>Sodium hypochlorite</td>
</tr>
<tr>
<td>MAC</td>
<td><em>Mycobacterium avium</em> complex</td>
</tr>
<tr>
<td>MGIT</td>
<td>Mycobacteria Growth Indicator Tube</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>MOTT</td>
<td><em>Mycobacterium</em> other than tuberculosis</td>
</tr>
<tr>
<td>MTC</td>
<td><em>Mycobacterium tuberculosis</em> complex</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NTM</td>
<td>Non tuberculous mycobacteria</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PNBA</td>
<td>Para Nitrobenzoic acid</td>
</tr>
<tr>
<td>PTB</td>
<td>Pulmonary Tuberculosis</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>Sp.</td>
<td>Species</td>
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<tr>
<td>SOP</td>
<td>Standard operating procedure</td>
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<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TCH</td>
<td>Thiophen-2-carboxylic acid hydrazide</td>
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<tr>
<td>TTD</td>
<td>Time taken to detection</td>
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<tr>
<td>TST</td>
<td>Tuberculin Skin Test</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
</tr>
<tr>
<td>ZN</td>
<td>Ziehl-Neelsen</td>
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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Tuberculosis in ancient times

Tuberculosis (TB) remains one of the deadliest diseases in the world and has been with mankind all through its existence as shown by the finding of Pott’s disease in an Egyptian mummy (Verver and Veen, 2006, p.870). It is an ancient disease dating from 2000 to 8000 B.C. when evidence of human tuberculosis, most convincingly as spinal gibbus, has been unearthed in petrified bones as well as seen in the hunchbacks depicted in statuettes and figurines of ancient artworks (Verver and Veen, 2006, p.870).

In the eighteenth and nineteenth centuries, tuberculosis was known as the white plague, decimating the population of Europe (Baron, Peterson and Finegold, 1994, p.590). More recently, the finding of a TB primary complex in a Peruvian, whose mummmified remains where exhumed in 1990, demonstrated that TB existed in the Americas before Columbus (Verver and Veen, 2006, p.870). Verver and Veen, 2006, p.870 further mentions that while the existence of tuberculosis is not clear in the Peruvians, it is in the Egyptians who where a sea going nation as proved by Thor Heyerdal.

Tuberculosis “consumption”, ‘phthisis’ then, was known as a chronic debilitating disease and once refered to as the scourge of Victorian Britain, still remains a major health problem in much of the world today (Timbury, Mc Cartney, Bishan and Ward, 2003, p.222). Koch’s isolation of Mycobacterium tuberculosis in the late 19th century and proof that he could transfer infection from one animal to another and then recover the organism in pure culture (Koch’s postulates) soon put to rest theories that tuberculosis was caused by heredity, bad humors or other causes (Nardell and Fennelly, 2006, p.796).
1.2 Tuberculosis today

In the early 1600s, the incidence of tuberculosis in western Europe increased sharply, probably due to industrialization and its concomitant urbanization and overcrowding of households, and it peaked in the 18th century increasing steadily through the 19th and 20th centuries (Verver and Veen, 2006, p.870). Tuberculosis has made a dramatic comeback in industrialized countries because of the AIDS pandemic and to an increase in the number of immigrants and homeless people (Zannetti, Ardito, Sechi, Sanguinetti, Molicotti, Delogu, Pinna, Madi and Fadda, 1997).

The WHO reported 9 million new TB cases and approximately 2 million TB deaths in 2004. More than 80% of these cases were recorded in sub-Saharan Africa and Asia (WHO Report 2006b). The emergence of the human immuno deficiency virus (HIV) has changed the face of tuberculosis. In 1995, about one third of the 15 million HIV-infected people world wide were also co-infected with *M. tuberculosis* and out of these 70% live in sub-Saharan Africa, 20% in Asia and 8% in Latin America and the Caribbean (WHO 1996). A person who is infected with HIV has a 10 times increased risk of developing TB compared to an individual who is not infected with HIV. TB notifications have increased in populations where both HIV infection and tuberculosis infections are common e.g. some parts of sub-Saharan Africa have seen a tripling in the number of notifications over the past decade. The HIV seroprevalency in TB patients from these areas are up to 70% (WHO 1996).

One of the most distressing problems arising from the resurgence of tuberculosis among AIDS patients and the homeless population is the increasing incidence of multiple drug resistance among *M. tuberculosis* isolates called multidrug-resistant *M. tuberculosis* (MDR-TB) (Baron et. al., 1994, p.570). MDR-TB describes strains of TB that are resistant to at least two of the main first-line TB drugs, and estimates indicates that there are
about 425,000 cases of MDR-TB a year, mostly occurring in the former Soviet Union, China and India (WHO 2006a)

Recently South Africa has reported extensively drug resistant TB (XDR-TB) which has been seen worldwide, including the United States of America, Eastern Europe and some parts of Africa. Western Europe has reported no cases thus far. (Gandhi, Moll, Sturm, Pawinski, Govender, Lallo, Zeller, Andrews and Friedland, 2006). XDR-TB strains are those described as strains not only resistant to the front-line drugs, but also to three or more of the six classes of the second-line drugs (Gandhi et al., 2006). An analysis of sputum cultures from 1540 patients from January 2006 to March 2006, in Kwa Zulu Natal, South Africa, showed that 536 (36%) of the sputum cultures were TB positive. MDR-TB strains were detected in 221 (41%) of these cultures. Fifty-three (10%) of the cultures were XDR-TB cases. Fifty-two of the 53 cases have died and HIV infection was present in 47 of 47 who were tested (Bartlett, 2007).

Rates of MDR-TB tuberculosis among new cases of tuberculosis in sub-Saharan Africa have been low in the past, ranging from 0.8% to 2.6% in the last global drug resistance survey (1999-2002), compared with 7.8 to 14.2% in countries with the highest rates (Gandhi et al., 2006).

The incidence of tuberculosis in Zambia has risen four-fold from 100 per 100,000 people in 1980 to about 450 per 100,000 people in 1996 (NASTL, 1996) with morbidity rate standing at a staggering 88.7 per 100,000 population (Chanda and Gosnell, 2006).

In the USA, in 1986, 22,800 i.e. 9.5 per 100,000 population active clinical cases were detected (Cotran, Kumar and Robbins, 1999, p.349). More than 28,000 cases were reported in 1992, 10% increase over 1991 (Baron et al.,
Western Cape Province of South Africa had a high incidence of about 600 per 100,000 population in 1990 (Schaaf, Beyers, Smits and Donald, 1993).

![Zambia Tuberculosis Case Rate](image)

(Taken from Chanda, 2002)

Figure 1 – Zambia Tuberculosis Case Rate from 1964 to 2005

According to the WHO Report for 2006, TB notification rates for some selected African countries have risen as follows:- from about 378 per 100 000 population in 1994 to about 471 per 100 000 population in 2004 for Zambia; 206 per 100 000 population in 1994 to about 434 per 100 000 population in 2004 for Zimbabwe; 301 per 100 000 population in 1994 to about 573 per 100 000 population in 2004 for Botswana; and 221 per 100 000 population in 1994 to about 560 per 100 000 population in 2004 for South Africa.
Table1: Tuberculosis case rates for 1994 and 2004 for selected African Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>1994</th>
<th>2004</th>
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<tbody>
<tr>
<td><strong>Southern region</strong></td>
<td></td>
<td></td>
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<tr>
<td>Zambia</td>
<td>378</td>
<td>471</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>206</td>
<td>434</td>
</tr>
<tr>
<td>Botswana</td>
<td>301</td>
<td>573</td>
</tr>
<tr>
<td>South Africa</td>
<td>221</td>
<td>560</td>
</tr>
<tr>
<td><strong>Central &amp; Eastern region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.R. Congo</td>
<td>88</td>
<td>167</td>
</tr>
<tr>
<td>Tanzania</td>
<td>116</td>
<td>166</td>
</tr>
<tr>
<td>Kenya</td>
<td>87</td>
<td>304</td>
</tr>
<tr>
<td>Uganda</td>
<td>133</td>
<td>157</td>
</tr>
<tr>
<td><strong>Northern &amp; western region</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algeria</td>
<td>48</td>
<td>61</td>
</tr>
<tr>
<td>Chad</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Cameroon</td>
<td>56</td>
<td>110</td>
</tr>
<tr>
<td>Cote d'Ivoire</td>
<td>98</td>
<td>112</td>
</tr>
<tr>
<td>Nigeria</td>
<td>8</td>
<td>44</td>
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(Taken from WHO Report 2006a)

For some selected countries in the Central and East African region, the report indicates that cases have risen as follows:- 88 per 100 000 population in 1994 to about 167 per 100 000 population in 2004 for D.R. Congo; 116 per 100 000 population in 1994 to about 166 per 100 000 population for Tanzania; 87 per 100 000 population in 1994 to about 304 per 100 000 population for Kenya; and 133 per 100 000 population in 1994 to about 157 per 100 000 population for Uganda. The report also highlights the following selected countries in Northern and Western Africa has having had a steady increase in notification rates, thus; 48 per 100 000 population in 1994 to about 61 per 100 000 population for Algeria; 48 per 100 000 population in 1994 to about 52 per 100
000 population for Chad; 56 per 100,000 population in 1994 to about 110 per 100,000 population for Cameroon; 98 per 100,000 population in 1994 to about 112 per 100,000 population for Cote d'Ivoire and 8 per 100,000 population in 1994 to about 44 per 100,000 population for Nigeria. (see table 1 and figure 2)

![African Countries TB case rates for 1994 and 2004](image)

(Taken from WHO Report 2006a)

**Figure 2: Tuberculosis case rates for 1994 and 2004 for selected African Countries**

Generally in most of the industrialized countries the picture is the other way round, as can be seen in table 2 and figure 3.
### Table 2: Tuberculosis case rates for 1990 and 2004 for selected industrialized Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>1990</th>
<th>2004</th>
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<tbody>
<tr>
<td>United States of America</td>
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<tr>
<td>Canada</td>
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<td>Germany</td>
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<tr>
<td>Netherlands</td>
<td>13</td>
<td>8</td>
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<td>Italy</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Russia</td>
<td>51</td>
<td>115</td>
</tr>
</tbody>
</table>

(Taken from WHO Report 2006a)

In most industrialized Countries, TB notification or incidence rates have generally declined as follows: 10 per 100,000 population in 1990 to about 5 per 100,000 population in 2004 for the USA; 9 per 100,000 population in 1990 to about 5 per 100,000 population in 2004 for Canada; 26 per 100,000 population in 1990 to about 8 per 100,000 population in 2004 for Germany; 13 per 100,000 population in 1990 to about 8 per 100,000 population in 2004 for Netherlands; 14 per 100,000 population in 1990 to about 7 per 100,000 population in 2004 for Italy; while cases have remained static at 12 per 100,000 population both in 1990 and 2004 for the United Kingdom, and Russia recording a steady increase, thus 51 per 100,000 population in 1994 to about 115 per 100,000 population in 2004 (WHO Report 2006a). (see table 2 and Figure 3)
Figure 3: Tuberculosis case rates for 1990 and 2004 for selected Industrialised Countries

1.3 Infectious tuberculosis

Tuberculosis is an infectious disease, caused in most cases by microorganisms called *Mycobacterium tuberculosis*, which usually enter the body by inhalation through the lungs and spread from the initial location (in the lungs) to other parts of the body via the blood stream and the lymphatic system (Enarson, Rieder, Arnaqdotir and Tre'bucq, 2000, p.3). Isolation of *M. tuberculosis* or *M. bovis* is always indicative of disease, whereas isolation of other mycobacteria may or may not be clinically significant (Baron et al., 1994, p.592). The spread of tuberculosis is usually determined by the concentration of the organisms within the lungs, thus patients with numerous bacilli are the most infectious (Enarson et al., 2000, p.3). An individual’s risk of infection depends on the extent of exposure to droplet nuclei. Additional risk factors are close, prolonged, indoor exposure to a person infected with tuberculosis. Pulmonary tuberculosis (PTB) is the most infectious and frequent form of the disease, occurring in 80% of cases. Extra-pulmonary tuberculosis affects all other organs apart from the lungs. The most frequently
affected organs are the pleura, lymph nodes, spine, joints, genito-urinary tract, nervous system or abdomen (Enarson et al., 2000, p.3). The most frequent symptoms of pulmonary tuberculosis include: persistent cough for 3 weeks or more; sputum production which may be blood stained thus termed haemoptysis; shortness of breath; chest pains; loss of appetite; loss of weight; general feeling of illness (malaise); tiredness (fatigue); night sweats and fever (Enarson et al., 2000, p.6).

Respiratory infection in immunocompetent hosts is usually caused by \textit{M. tuberculosis}, but some Mycobacterium other than tuberculosis (MOTT) like \textit{M. avium-intracellulare} and \textit{M. kansasii} complex also cause tuberculosis-like diseases (Baron et al., 1994, p.592). The prevalence of disease caused by nontuberculous mycobacteria, especially \textit{M. avium-intracellulare} complex has increased dramatically because of AIDS. Disseminated infection with the \textit{M. avium - M. intracellulare} complex has frequently been observed as opportunistic infection occurring in the majority of patients with AIDS (Griethuysen, Jansz and Buiting, 1996). \textit{M. avium} complex is the most common form of tuberculosis in the United States today because of its high prevalence in patients suffering from AIDS (Baron et al., 1994, p.592).

The following four factors have an influence on the pathogenesis of the disease: (1) the virulence of the strain of \textit{M. tuberculosis}; (2) the role of induced hypersensitivity by the host; (3) the role of immunity or resistance by the host and (4) the genesis of the Granulomatous pattern of reaction so characteristic of tuberculosis (Cotran et al., 1999, p.349).

Pathogenicity of \textit{M. tuberculosis} is related to the ability to escape killing by macrophages and thereby inducing delayed type hypersensitivity (Cotran et al., 1999, p.349).
*M. tuberculosis* has several factors that aid its pathogenesis. Several components of the cell wall e.g. the cord factor: a surface glycolipid which produces granulomas and a lipoarabinomanna (LAM) - a major heteropolysaccharide which inhibits the macrophage activation. Complement plays a part as it is activated on the surface of *M. tuberculosis* and opsonizes the organism to facilitate its uptake by macrophage complement receptor CR3 (Mac-1 integrin). The heat-shock protein of *M. tuberculosis* plays a role in autoimmune reactions induced by *M. tuberculosis* (Cotran *et al.*, 1999, p.349).

### 1.4 Classification of tuberculosis

The classification of *Mycobacteria* sp. includes those that produce a spectrum of infections in humans and animals ranging from localized lesions to dissemination infection. A tentative proposed classification based on pathogenesis and the natural history of tuberculosis includes:-

1. **obligate pathogens** that cause exclusively human infections;
2. **facultative pathogens** that are found primarily in animals or the environment but produce documented human infections;
3. **potentially opportunistic pathogenic species** found in the environment but produce human infections;
4. **saprophytic species** found in the environment that do not cause human infection (Baron *et al.*, 1994, p.591).

Watt, Rayner and Harris 1999 p.329 says “it is convenient to divide mycobacteria of clinical interest into those associated with tuberculosis – the *M. tuberculosis* complex or MTC (*M. tuberculosis*, *M. bovis*, BCG, *M. africanum* and *M. microti*) – and other mycobacteria that may be associated with human disease, the latter group having been given several collective names such as atypical, anonymous, non-tuberculous, tuberculoid, opportunistic and mycobacteria other than tuberculosis (MOTT)”.
1.5 Structure of tubercle bacilli

The Mycobacteria considered the most significant in clinical disease is *Mycobacterium tuberculosis* (Tubercle bacilli). This bacillus is a thin rod, with round extremities, 2-µm long and 0.2-0.3µm thick, nonmotile, noncapsulated and nonsporing. The bacillus is classified as being Gram-positive although many species stain poorly with this stain, even after prolonged staining, because of the characteristics of the cell wall. Sixty percent of the cell wall is rich in chemically diverse lipids. (Watt, Rayner and Harris, 1999, p.329). The thickness of the cell wall is due to the presence of long-chain fatty acids (mycolic acids) which form a thick palisade (Watt *et al.*, 1999, p.329). It is called an “acid-alcohol fast bacillus” as it resists decolourization by strong mineral acids and alcohol (Munoz and Starke, 2006, p.327). The tubercle bacillus will stain with acid-fast stains like the Ziehl-Neelsen (ZN) stain or fluorescent stains like the Auramine stain (Watt *et al.*, 1999, p.333).

1.6 Tuberculosis in children

Childhood tuberculosis usually occurs as a direct consequence of the initial infection with *Mycobacterium tuberculosis* which can rapidly progress to disease in the youngest children and which tends to involve extrapulmonary sites more commonly than in adults (Munzo and Stark, 2006, p.308). Munzo and Stark (2006), p.308 further argues that because most children with tuberculosis infection and disease acquire the organism from adults in their environment, the epidemiology of childhood tuberculosis reflects that in adults.

Estimations indicate that in the past decade there were 88 million cases of tuberculosis, out of which 15 million were children and 5 million of whom have died (Schaaf *et al.*, 1993). Current estimations by WHO indicate that one third
of the world’s population is infected with *Mycobacterium tuberculosis*, and that each year about 9 million people develop TB, of whom about 2 million die. The report further indicates that of the 9 million annual TB cases, about 1 million (11%) occur in children (under 15 years of age) and of these childhood cases, 75% occur annually in 22 high-burden countries that together account for 80% of the world’s estimated incident cases (WHO Report 2006b). In Countries worldwide, the reported percentage of all TB cases occurring in children varies from 3% to more than 25% (WHO Report 2006a).

In Zambia, 21 000 new cases were notified during 1991. Approximately 2 100 were children under the age of 14 years (Osborne, 1995). Children in this age group constitute about 49% of the estimated 8.6 million people in the country. By comparison, in the USA which had an estimated population of 255 million in 1991, 23,000 new cases of tuberculosis occur every year and approximately 1,200 are children (Osborne, 1995). In England and Wales, the notification rate for children was at 294 per 100,000 population in 1988 (Davidson, 2000). In Canada, despite the availability of effective therapy, out of the over 2,000 cases reported annually, 10 to 15% occur in children (Canadian Paediatric Society, (CPS), 2002). Recent studies have indicated that childhood tuberculosis is believed to be on the rise worldwide, because of persisting inability to confirm the diagnosis, and a large number of children even die of undiagnosed tuberculosis (Saluja, Ajinkya and Khemni, 2002).

Patterns of drug resistance among children with tuberculosis tend to reflect those found among adults in the same population (Munzo and Stark, 2006, p.324). Munzo and Stark (2006) p.324 further indicates that certain epidemiological factors such as residence in a country or area with high rates of drug resistance, homelessness and previous antituberculosis therapy in a child or adult source case are clues to determining drug resistance in childhood tuberculosis. Davidson (2000) made reference to about 9 studies on anti-TB treatment in children carried out in the era of short-course
chemotherapy. It showed that the same regime used in treating TB in adults succeeded in children. It is no longer possible to be sure that the likelihood of drug resistance is low in children anywhere in the world. Primary resistance to isoniazid (i.e., resistance in previously untreated TB cases) occurs in over 5% of cases in the UK and of which 8% of the cases occur in London (Davidson, 2000). Childhood TB drug resistance is now extremely common in other parts of the world, for example, in St Petersburg over 20% of isolates are isoniazid resistant, and 7% indicate multi-drug resistant tuberculosis (Davidson, 2000).

The morbidity and mortality of tuberculosis in childhood can be attributed to the high rate of infections in infants and the frequency of disseminated forms (Schaaf et al., 1993). Diagnosis of tuberculosis in children is quite difficult especially that in the majority of instances (with the exception of disseminated tuberculosis, tuberculic meningitis, spinal tuberculosis and tuberculosis in immunosuppressed children), childhood tuberculosis is a mild disease that may heal on its own, even with minimal or no treatment. Children with tuberculosis should be treated to prevent complications and to ensure that they do not subsequently develop tuberculosis from reactivation of their infection (Enarson et al., 2000, p.9).

1.7 Diagnosist of childhood tuberculosis
Diagnosis of tuberculosis among children poses technical and operational challenges because of vague and non-specific symptomatology and the difficulty in getting sputum sample for testing (Saluja et al., 2002). Davidson (2000) reports that children are less likely to have respiratory disease and unlikely to have cavitary pulmonary disease. It is reported that children with cavities do not easily expectorate their sputum. The children, especially those under ten years of age, are not able to cough up sputum but instead they tend to swallow their sputum (Enarson et al., 2000, p.9).
Similar to adults, pulmonary tuberculosis is the most common form of tuberculosis in children (Enarson et al. 2000, p9). Childhood tuberculosis has a limited influence on the immediate epidemiology of the disease within a community because children are rarely a source of infection to contacts (Munzo and Stark 2006, p.307). Munzo and Stark (2006) p.307 go on to say the occurrence of tuberculosis in children is however a marker for ongoing transmission of infection among all age groups in a society and infected children also represent a large portion of the pool from which future tuberculosis cases will arise. Only a very small proportion of children have tuberculosis that is smear positive (Enarson et al., 2000, p.9).

Recommendations confirming childhood tuberculosis have been as follows :-

- A history of contact with a case of infectious tuberculosis, particularly in the same household;
- An abnormal chest radiograph showing unilateral lymphadenopathy and/or shadows in the lung field indicating infiltration;
- A positive tuberculin skin test, where such a test is available.

In the absence of all the above, it is highly unlikely that the child has tuberculosis and any child whose tuberculin skin test remains consistently negative over some months of observation, while the clinical condition is good or shows improvement is not considered as having tuberculosis (Enarson et al., 2000, p.9). However, any child under 5 years of age and in contact with a smear positive case and with signs or symptoms suggesting tuberculosis, should be regarded as having active tuberculosis, thus this child should be given a full course of treatment. Although an abnormal chest radiograph has been recommended as one of the considerations for confirming tuberculosis in children, the guidelines by the International Union Against Tuberculosis and Lung Disease indicate that diagnosis by means of radiographic examination in suspected TB patients is unreliable (Enarson et al., 2000, p.9). Abnormalities identified on a chest radiograph may be due to
tuberculosis or to a variety of other conditions, therefore the appearance on the radiograph is not specific for tuberculosis (Enarson et al., 2000, p.8).

In Zambia, in the Department of Paediatrics and Child Health wing of the University Teaching Hospital (UTH), tuberculosis is usually suspected when a child has a history of chronic illness that includes a cough and/or fever, weight loss or failure to thrive, an inability to return to normal health after measles or whooping cough and history of contact with an adult case of pulmonary tuberculosis (Osborne, 1995). As much as diagnosing and treating tuberculosis in symptomatic children can decrease mortality, earlier intervention in the asymptomatic stage can further decrease morbidity (CPS 2002); and since children almost always acquire tuberculosis from an infectious adult, the most efficient method of finding infected children is through contact investigations of adults with infectious PTB (CPS 2002). Since the emergence of HIV, the interaction of HIV and tuberculosis is becoming evident in childhood because of HIV co-infected mothers who are capable of passing tuberculosis to their children congenitally (Saluja et al., 2002). Therefore, the rise of tuberculosis cases in adults as a direct result of the HIV epidemic is expected to be paralleled by a similar rise of tuberculosis cases in children. At Lusaka’s University Teaching Hospital, HIV sero prevalence rates in children with probable tuberculosis have risen from 24% in 1989 to over 70% in 1993 (Osborne, 1995). The occurrence of other infections in children with tuberculosis is not uncommon and should not deter the clinician from starting or continuing anti-tuberculous treatment in suspicious circumstances (Schaaf et al., 1993).

In Zambia the laboratory diagnosis of tuberculosis relies on Ziehl-Neelsen staining microscopy technique for most districts and peripheral health centres, and fluorochrome microscopy technique for the National Reference Centre (Chest Disease Laboratory) and the University Teaching Hospital. Most peripheral health centres are not able to carry out gastric lavage aspiration
and therefore the children requiring gastric lavage aspiration are referred to University Teaching Hospital.

Table 3 and figure 4 indicates data for the period 2000-2005 showing the number of positives gastric lavage smears done at UTH TB laboratory.

**Table 3: Positive TB cases diagnosed from children at UTH TB laboratory by direct microscopy technique during 2000 to 2005.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Total smears</th>
<th>Positives smears</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>4435</td>
<td>276</td>
<td>6.2%</td>
</tr>
<tr>
<td>2001</td>
<td>4177</td>
<td>189</td>
<td>4.5%</td>
</tr>
<tr>
<td>2002</td>
<td>5556</td>
<td>280</td>
<td>5.0%</td>
</tr>
<tr>
<td>2003</td>
<td>5898</td>
<td>198</td>
<td>3.4%</td>
</tr>
<tr>
<td>2004</td>
<td>4245</td>
<td>210</td>
<td>4.9%</td>
</tr>
<tr>
<td>2005</td>
<td>4521</td>
<td>124</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

(Taken from UTH TB laboratory annual reports 2000 – 2005)

Direct microscopy smear, although cheaper, has a very low sensitivity even after concentration techniques to recover all the tuberculosis bacilli present in the sample (Habeenzu, Lubasi and Fleming, 1998). Conventional cultures, using Lowenstein-Jensen and Ogawa culture media, are more specific but require 4 – 8 weeks incubation time before any results are available (Lubasi, Habeenzu and Mitarai, 2004).
Radiometric BACTEC 460 and the non-radiometric BACTEC 9000 MB have demonstrated a high efficiency in recovery of mycobacteria (Zannetti et al., 1997). These methods are expensive for developing countries but are essential for the recovery of mycobacteria especially in gastric lavages from children aged 0-10 years.

The diagnosis of childhood tuberculosis poses a challenge and is difficult to establish even in Countries with modern clinical and laboratory facilities, because of the clinical presentation being variable and non specific often mimicking other common pediatric diseases (Munzo and Starke, 2006, p.308)
Chapter 2

LABORATORY DIAGNOSTIC PROCEDURES FOR TUBERCULOSIS

Acid-fast bacilli may infect almost any tissue or organ of the body, and the successful isolation of the organism depends on the quality of the specimens and the appropriate processing and culture techniques employed by the mycobacteriology laboratory (Baron et al., 1994, p.594). As mycobacterial disease may occur in almost any site in the body a variety of clinical materials may be submitted to the laboratory for examination. The most common specimens are sputum (natural or induced) and gastric aspirates. Other specimens include: urine, cerebral spinal fluid, pleural fluid, bronchial washings, material from abscesses, endometrial scrapings, bone marrow and other biopsy specimens (American Thoracic Society (ATS). 2000). In the setting of disseminated or miliary disease, multiple organs may be seeded with tubercle bacilli, and hence tissue examination may be very helpful, e.g. liver and bone marrow biopsies. These specimens have provided important diagnostic opportunities and have yielded information suggesting tuberculosis in about 40-90% of patients (Munzo and Starke, 2006, p.327). The need for invasive procedures to obtain specimens is inversely related to the ease of obtaining specimens through less aggressive means. Pulmonary and genitourinary tuberculosis, situations in which tubercle bacilli are frequently shed into easily sampled body fluids, generally do not require biopsies for diagnosis (Munzo and Starke, 2006, p.327).

Although paediatric tuberculosis has a propensity for extra-pulmonary disease, pulmonary tuberculosis is the most common manifestation in non-HIV infected children. Therefore sputum is the specimen most commonly required for the diagnosis of PTB. Unfortunately younger children rarely expectorate sputum and
even when obtained it is frequently negative on smear and/or culture (conventional L-J) (Osborne, 1995). Gastric aspiration is necessary for those patients, particularly children, who cannot produce sputum even with aerosol inhalation (ATS 1999). Obtaining 3 consecutive early morning gastric aspirates for culture increases sensitivity up to 70% in children with tuberculosis (Munzo and Starke, 2006, p.327). About 50ml of gastric contents should be aspirated early in the morning, after the patient has fasted for about 8 to 10 hours, and preferably while the patient is still in bed. Gastric aspiration is best performed with hospitalized patients (ATS 1999). The procedure is limited to senile, non ambulatory children, most desirably collected at the child’s bedside before the child arises and before exertion empties the stomach. Collection of gastric lavage cannot be performed as an office or clinical procedure (Baron et al., 1994).

The diagnosis of tuberculosis has used a variety of clinical and microbiological methods. Recent developments have made diagnosing this disease even more challenging and urgent. Technological developments have created new tools for meeting this diagnostic challenge (Boulahbal and Heifets, 2006, p.33).

2.1 Microscopic examination

2.1.1 Staining methods

Traditionally the acid-fast stains have been used in the early diagnosis of mycobacterial infections. Watt et al., 1999, p.332 mentions that in 1882 Ehrlich discovered that mycobacteria stained with fuchs in and resisted decolourization by mineral acids. In the same year Ziehl continued developing the stain and he changed the mordant to carbolic acid and he also increased the concentration of the carbolic acid. He incorporated this with the dye to form carbol-fuchsin which
is the primary stain in the standard stain for demonstrating acid-fastness (Watt et al., 1999, p.332).

Acid-fast bacilli are difficult to stain because of the lipid content of the cell wall. The phenol in the carbolfuchsin of the Ziehl–Neelson (ZN) stain dissolves the lipid sufficiently to allow penetration of the primary stain. The cell wall retains the primary stain even after exposure to the decolorizing agent, acid-alcohol, hence the term acid-fast bacillus (AFB) (Isenberg, 1995, p.3.5.1).

The stains for acid-fast bacilli (AFB) include fluorochrome (e.g. auramine/rhodamine) and traditional (e.g. Ziehl-Neelsen) which share the advantages of being relatively rapid and inexpensive to perform with a 10 fold increased sensitivity with concentrated fluorescent methods, allow the microscopists to quickly screen slides for the presence of AFB (Boulahbal and Heifets, 2006, p.33). Today, the primary laboratory tool supporting case detection in the vast majority of cases in disease endemic countries (DECs) remains the microscopic examination. However, shortcomings of AFB microscopy seriously limit both the extent and quality of its application, and ultimately, the impact on TB control (Perkins, 2000). Shortcomings include its low sensitivity, the problem made more critical by the rising incidence of smear–negative tuberculosis in countries where HIV infection is prevalent such as in sub-Saharan Africa (Perkins, 2000). Results show that ZN smear examination has a sensitivity of 33.79% and a specificity of 100% compared to Polymerase chain reaction (PCR) that has a much higher sensitivity of 74.4%. Unfortunately, techniques like PCR are expensive and not available in the majority of African countries (Negi, Khan, Gupta, Pasha, Khare and Lal, 2005)

It has been estimated that for the acid fast stains to reliably identify AFB, at least 10,000 AFB must be present per milliliter of specimen, also variations depend on the skill of the technologist (Boulahbal and Heifets, 2006, p.33). The stains do not speciate organisms and are therefore less specific than other technologies for
identification. Unfortunately, the AFB stains do not distinguish between dead and live mycobacteria and thus are a particular limitation when evaluating patients who are receiving antimycobacterial therapy and also early identification of any drug resistance (Boulahbal and Heifets, 2006, p.33).

Although acid-fast stains are not truly confirmatory of tuberculosis in that other species of mycobacteria as well as unrelated organisms, such as the weakly acid-fast Nocardia, may be positive, they however provide a result that substantially narrows the differential diagnosis and allows the clinician to focus on a rather circumscribed set of options and remains the only affordable tool in low-income Countries (Boulahbal and Heifets, 2006, p.33).

2.2 Culture methods

2.2.1 Conventional culture methods

Today, the “gold standard” for diagnosis of tuberculosis is still detection of M. tuberculosis by culture (Zannetti et al., 1997). Culture is more sensitive than AFB staining and can reliably find mycobacteria even when they are present in a concentration of about 10 organisms/ml of specimen and is a “gold standard” for the definitive diagnosis of tuberculosis (Boulahbal and Heifets, 2006, p.33).

One of the major drawbacks of conventional culture is that they are slow, requiring weeks before a positive culture for M. tuberculosis can be identified. The other disadvantage of conventional cultures is that they require at least a moderately well-equipped laboratory (Boulahbal and Heifets, 2006, p.33)

Three different types of traditional culture media are available; egg based (Lowenstein-Jensen), agar based (Middle-brook 7H10 or 7H11), and liquid (Middlebrook 7H12 and other commercially available broths). These can be made
into selective media by adding antibiotics. The growth of mycobacteria tends to be slightly better on the Lowenstein-Jensen (L-J) but more rapid on the Middlebrook media and even more rapid on liquid media (ATS 1999).

All clinical specimens suspected of containing mycobacteria should be inoculated onto culture media for four reasons:

§ culture is much more sensitive than microscopy, being able to detect as few as 10 bacteria/ml of material.
§ growth of the organisms is necessary for precise species identification.
§ drug susceptibility testing can be performed.
§ genotyping of different species can be done.

Generally the sensitivity of mycobacterial culture is 80-85% and the specificity about 98% (ATS 1999).

The conventional culture process usually involves the use of an egg-potato-base (e.g. L-J) and/or an agar (e.g. Middlebrook 7H-11) medium. The specimens that are not sterile (e.g. sputum) and/or combined with other cellular material or debris must first be subjected to a digestion and decontamination process and when properly done, this process prevents the overgrowth of more rapidly growing bacteria (Boulahbal and Heifet, 2006, p.33).

2.3 Commercial systems

A major improvement in diagnosis of tuberculosis has been the development of automated commercial broth systems. The instruments (BACTEC 460 and BACTEC 960) reduces the time for culturing *M. tuberculosis* by several weeks and sensitivity results can be obtained within 1 – 2 weeks rather than in 4 – 5 weeks when using the conventional culture methods. (Watt et al., 1999). Currently, this method of culture, using the half-automated radiometric BACTEC
460 and the fully automated BACTEC 960, are widely accepted as reference standards. (Cruciani, Scarparo, Malena, Bosco, Serpelloni and Mengoli, 2004)

2.3.1 BACTEC 460 system

The Bactec 460 system (Becton-Dickinson Microbiology system, Sparks, Md.) (see figure 5) was the first semi-automated liquid medium system to be introduced for the rapid detection of mycobacterial growth. (Heifets, Linder, Sanchez, Spenser and Brennan, 2000). This is a semi-automated instrument which can test up to 60 vials at a time at a rate of 82+/−2 s per vial (Isenberg, 1995). In this system growth medium for culturing mycobacteria is supplemented with a substrate, labeled with radioactive carbon (C\textsuperscript{14}), which is utilized by mycobacteria. During bacterial metabolism carbon dioxide, labeled with C\textsuperscript{14} is produced from the substrate. The C\textsuperscript{14} is being detected quantitatively by measuring the radioactivity with the BACTEC 460 system. The rate and amount of C\textsuperscript{14} CO\textsubscript{2} produced is proportional to the rate and amount of growth occurring in the medium (Isenberg, 1995). This system can indicate the presence of mycobacteria weeks before conventional culture systems, usually within one week or less with drug susceptibility tests requiring an average of about 9.3 days (Boulahbal and Heifets, 2006, p.33). After about 20 years of successful use in many clinical laboratories it can be considered the one most frequently in used among such systems (Heifets et al., 2000).

This system can be used for the rapid identification of bacteria other than mycobacteria and it is appropriate for large clinical microbiology laboratories because of their cost, technical sophistication and use of radioisotopes. (Boulahbal and Heifets, 2006, p.33)
However, BACTEC 460 system has some disadvantages. These include the use of radioactive material, the cumbersome manual loading and unloading, the potential hazard of needle stick injury, the risk of cross contamination and the lack of computerized data management (Cruciani et al., 2004).

A new automated culture system has been developed, BACTEC MGIT 960, which does not use any radioactive materials (Griethuysen et al., 1996).
2.3.2 BACTEC MGIT 960

The BACTEC MGIT 960 is a newly developed non-radiometric, fully automated, continuously monitoring system and was introduced as an alternative to the radiometric BACTEC 460 for growth and detection of mycobacteria (Cruciani et al., 2004). (See figure 6) The use of non-radioactive indicators is one of the attractions of this rapid culture system (Boulahbal and Heifets, 2006, p.33)
FIGURE 6: BACTEC MGIT 960 (Becton-Dickinson Microbiology system, Sparks, Md.)
The BACTEC MGIT 960 system can test up to 960 7-ml MGIT vials for the presence of mycobacteria using non-radiometric fluorescent sensor that responds to the concentration of oxygen in the culture medium (Akos Somoskovi, Csaba Kodmon, Akos Lantos, Zoton Bartfai, Lilla Tamasi, Judit Fuzy and Pal Magyar, 2000). These fluorescent systems use an oxygen-quenching fluorescent indicator, where the presence of fluorescence indicates the growth of microorganisms. This has reduced culture time to less than 2 weeks (Zannetti et al., 1997). The results of several comparative studies show that BACTEC MGIT 960 is a suitable tool for the detection of *Mycobacterium tuberculosis* and other mycobacterial species, though rather wide variations in the diagnostic performance have been reported (Cruciani et al., 2004).

### 2.3.3 Comparison of BACTEC Systems

Apart from the systems discussed similar systems exists namely BACTEC MGIT 9000MB. A study by Zanetti et al., 1997 reviewed different systems, and found the following: out of the total of 325 sputum samples cultured, 41 (12.6%) were positive for TB by BACTEC MGIT 9000MB and 38 (11.7%) were positive for TB by BACTEC 460. Zanetti et al 1997 made an observation that the fluorescent BACTEC MGIT 9000MB system is as rapid and sensitive for recovery of mycobacteria as the radiometric BACTEC 460 system and was more sensitive than conventional culture in L-J medium. Zanetti et al., 1997 concluded their findings by highlighting practical advantages offered by the BACTEC MGIT 9000MB, among several others, such as complete automation, continuous monitoring, data management and most importantly it is non radiometric.

A study by Fernando et al., 2000 revealed the following findings: that out of the total 120 isolates of mycobacteria, 99 (82.5%) were detected by the BACTEC MGIT 960 and 96 (80.0%) by the BACTEC 460. Fernando et al., 2000 concluded their study stating that the BACTEC MGIT 960 may be considered a substitute
for the radiometric BACTEC 460 system. The system is automated, sensitive, rapid, less labor-intensive and non-radiometric compared to BACTEC 460.

Cruciani et al., 2004 analyzed several studies that compared the results of BACTEC MGIT 960, BACTEC 460 and conventional solid media. The data provided additional evidence of the value of the BACTEC MGIT 960. The shorter detection time of acid-fast bacilli, the more convenient technology and an elevated diagnostic accuracy of almost all the clinically important mycobacteria species make the BACTEC MGIT 960 system, a valuable alternative to the radiometric system (Cruciani et al., 2004).

2.4 Other methods

Other diagnostic methods for the identification of mycobacteria include Nucleic Acid Probes which involves use of complementary DNA or RNA probes that are highly specific for nucleic acid with certain species of mycobacteria. If the appropriate nucleic acid sequence is present in sufficient amounts within the specimen being probed, the cDNA or cRNA will hybridize with them, producing a radio isotopic or other signal, depending on the system in use. Thus the major advantage of such probes is their high degree of specificity (Boulahbal and Heifets, 2006, p.38)

Chromatographic techniques can be used to detect certain forms of lipids found in the cell wall of mycobacteria. Gas-liquid chromatography (GLC) coupled with a mass spectrometer has been used to detect minute amounts of indolic-type compounds in CSF from patients with tuberculous meningitis, thereby distinguishing them from patients with other forms of lymphocytic meningeal disease (Boulahbal and Heifets, 2006, p.38).

Methods, other than culture, which aids the diagnosis of tuberculosis include the tuberculin skin tests, which just excludes tuberculosis, Imaging (Chest Radiograph), which may show abnormalities but does not prove the presence of
active disease and Computed Tomography, an elegant means of assessing the lung parenchyma, mediastinum and pleura (Boulahbal and Heifets, 2006, p.35).

2.5 Aims and objectives of research

The aim of the research project is to optimize the isolation rate of *Mycobacterium* species from gastric lavages in children.

The following objectives will be addressed in this research project.

- Determine whether the BACTEC MGIT 960 culture system will optimize the isolation of *Mycobacterium* species.
- Determine whether different *Mycobacterium* species are the etiological agents of tuberculosis in children.
Chapter 3

Materials and Methods

This study was carried out at the University Teaching Hospital in Zambia. The study was approved by the Ethics Committee of Nelson Mandela Metropolitan University (NMMU) in Port Elizabeth, South Africa (see Appendix 1 and 2). Permission for the study was granted by the Medical Superintendent of the UTH through the University of Zambia School of medicine Ethical committee (see appendix 3). Consent forms (Appendix 5) and introductory letters (Appendix 6) were given to the parents involved. A questionnaire was complete by the hospital staff collecting the gastric lavage specimens (see Appendix 4).

Four hundred and eight (408) early morning gastric lavage specimens were collected and processed inside certified biological safety cabinets. Each specimen received was decontaminated, processed and the deposit of the sample was used to inoculate L-J culture media (conventional method) and the BACTEC MGIT 960 media. Smears were prepared prior to processing for culture.

3.1 Staining methods

The prepared smears from the gastric lavages were stained using the phenol-auramine method which is a fluorescent staining method.

2X3 cm size smears were made on clean grease free slides. The slides were treated as follows:

- Air dried and flooded entirely with auramine-phenol stain.
- The smears were allowed to stain for 15 minutes, then rinsed with water
Slides were flooded with 0.5% acid-alcohol, and allowed to decolorize for 2 minutes. Then rinsed with water.

Slides were flooded with the counterstain, potassium permanganate, and left for 2 minutes.

Slides were rinsed with water and allowed to air dry.

Screening of stained smears was done using 20X and 40X objective lenses. The 100X objective lens was used to confirm the characteristic morphology of the fluorescing tuberculosis bacilli. (Isenberg, 1995, p.3.5.8). Results were recorded using the WHO and IUATLD reporting scale below:

### Table 4: The Reporting scale for Acid Fast Bacilli (AFB) recommended by WHO and IUATLD.

<table>
<thead>
<tr>
<th>No. of AFB bacilli</th>
<th>Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB in at least 100 fields</td>
<td>Negative / No acid-fast bacilli observed</td>
</tr>
<tr>
<td>1-9 AFB per 100 fields</td>
<td>Record exact figure</td>
</tr>
<tr>
<td>10-99 AFB per 100 fields</td>
<td>1+</td>
</tr>
<tr>
<td>1-10 AFB per field in at least 50 fields</td>
<td>2+</td>
</tr>
<tr>
<td>&gt; 10 AFB per field in at least 20 fields</td>
<td>3+</td>
</tr>
</tbody>
</table>

(Taken from National Guidelines for Quality Assurance of Sputum smear Microscopy in Tuberculosis control; Ministry of Health (2005))

### 3.2 Culturing procedure

After the decontamination procedure of the gastric lavage, inoculation was done onto L-J media and BACTEC MGIT 960 media. The BACTEC MGIT 960 was commercially prepared.
3.2.1 Lowenstein-Jensen (L-J) culture method

Decontamination of gastric lavages was done as follows:

- A volume of 2% NaOH, equal to the volume of specimen, was added to the gastric lavages in a sterile 50-ml conical screw cap centrifuge tube.
- Specimen was agitated using a votex mixer and then allowed to stand for 15 minutes at room temperature.
- Sterile distilled water was added to the 50ml mark on the tube to dilute the effect of the NaOH.
- Tubes were mixed and centrifuged at 3,000 rpm for 15 minutes.
- The supernatant was poured off and the deposits inoculated onto L-J slants.
- Inoculated media were incubated at 37°C for up to 8 weeks.
- Positive cultures were confirmed by Ziehl-Neelsen staining and further identification of different species was done (Isenberg, 1995, p.3.5.8).

3.2.2 BACTEC MGIT 960

Decontamination of gastric lavages was done as for the L-J culture method. Inoculation of MGIT tubes proceeded as follows:

- A lyophilized vial of BBL MGIT PANTA Antibiotic Mixture was reconstituted with 15 ml of BACTEC MGIT Growth Supplement.
- 0.8ml of Growth Supplement/MGIT PANTA Antibiotic Mixture was added to the MGIT tube prior to inoculating the specimen.
- 0.5ml of the decontaminated concentrated specimen suspension was added to the MGIT tube.
- The MGIT tube was tightly recapped and mixed well.
The tube was placed in the MGIT 960 instrument which automatically tested the sample for the duration of the recommended 42 day testing or until positives were detected.

Positive cultures were confirmed by Ziehl-Neelson staining and further identification was done.

3.3 Identification and Typing

A culture of mycobacteria can often be presumptively differentiated into either the *M. tuberculosis* complex (MTC) or *Mycobacterium* other than tuberculosis (MOTT) group by experienced workers on the evidence of a ZN smear and the colonial morphology (Watt *et al.*, 1999, p.330).

The isolates were stained with Ziehl-Neelsen staining. The acid-fastness of the bacteria as well as specific morphology in liquid media was detected. *Mycobacterium tuberculosis* complex exhibited serpentine cording while dot and cross barring morphology was observed in *Mycobacterium avium* complex and *Mycobacterium kansaii* respectively.

Differentiation within the MOTT group can be done by including additional test. The following tests were included to either confirm *M. tuberculosis* and/or differentiate the MOTT group.

3.3.1 Niacin accumulation

Mycobacteria produce nicotinic acid during growth, but *M. tuberculosis*, does thereby excreting it into the agar from which it is extracted and detected. *M. tuberculosis* normally gives a positive niacin result. The test was done as follows:
$ 2$ ml of sterile hot water was added to about $3$ weeks old subcultures slants with heavy/confluent growth.

$ 2$ ml of liquid from slant was transferred to a clean test tube.

$ 2$ ml of $4\%$ aniline was added to the tube.

About $2$ ml of Cyanogen bromide was also added to the same tube, and the tube was left for $5$ minutes. The tubes were examined for presence of a yellow colour, which indicated a positive result and no colour change indicated a negative result (Isenberg, 1995).

### 3.3.2 P – Nitrobenzoic acid (PNBA) sensitivity

This test differentiates various *Mycobacterium* species depending on sensitivity to PNBA. *M. tuberculosis* does not grow on media containing PNBA, but most other *Mycobacterium* spp do.

$ PNBA$ was incorporated in the L-J media before coagulation at a concentration of $26$ ml in $500$ ml, and was labeled with a circled black cross.

Two drops of the mycobacteria suspension (one colony in $1$ ml of sterile distilled water) were inoculated on the surface of the PNBA slants and incubated until growth was observed on the control or up to the maximum time $4$ weeks (Isenberg, 1995).

### 3.3.3 Thiophen-2-carboxylic acid hydrazide (TCH)

This test differentiates various *Mycobacterium* species depending on sensitivity to TCH. *M. tuberculosis* grows on media containing TCH whilst most other species do not.

$ TCH$ was incorporated in the L-J media before coagulation at a concentration of $210\mu$l in $500$ ml and was labeled with a circled red cross.
Two drops of the mycobacteria suspension (one colony in 1ml of sterile distilled water) were inoculated on the surface of the TCH slants and incubated until growth was observed on the control or for a maximum of 4 weeks (Isenberg, 1995).

3.3.4 Sodium Chloride (NaCl) Tolerance
This test differentiates *Mycobacterium tuberculosis* from MOTT's e.g. *M. triviale* and others do not grow on this media.

- Sodium Chloride (NaCl) was incorporated in the L-J media before coagulation at a concentration of 5% and was labeled with a circled blue cross.
- Two drops of the mycobacteria suspension (one colony in 1ml of sterile distilled water) were inoculated on the surface of the NaCl slants and incubated until growth was observed in the control or for a maximum of 4 weeks (Isenberg, 1995).

3.3.5 Catalase production
The catalase enzyme splits hydrogen peroxide into water and oxygen. A positive test is indicated by bubbles formed by the oxygen. All *Mycobacterium* species except *M. bovis* display catalase activity.

- A colony or a loopful of growth was emulsified in 2 ml of sterile distilled water in a tube containing glass beads.
- The tube was vibrated on a Voltex machine to break the bacterial mass and left for 10 – 15 min.
- About 1ml of bacteria suspension was added to 2 ml of catalase reagent in a test tube using a Pasteur pipette.
- Bubble formation indicated a positive result and no bubbles indicated a negative result (Isenberg, 1995).
3.3.6 Nitrate reduction

Mycobacteria differ in their abilities to reduce nitrate, thus this characteristic can be used to distinguish various *Mycobacterium* species. *M. tuberculosis* is positive for this test while other *Mycobacterium* species are negative.

- The following were added to the tubes.
- 25 µl of concentrated hydrochloric acid (HCl), 50 µl of 0.2% sulfanilamide, and 50 µl of 0.1% *N*-1-naphthyl-ethylenediamine dihydrochloride.
- The resulting color changes were read visually after 5 min.
- Pink to red for positives and no colour change for negatives (Isenberg, 1995).

3.3.7 Growth at 25°C and 42°C

*M. tuberculosis* and most human pathogens grow at 37°C, some species like *M. marinum, M. ulcerans* grow at 25°C while others like *M. phlei* and *M. xenopi* grow at temperatures of 42°C and more.

- Bacterial cultures were inoculated onto plain L-J slants and were incubated at 25°C and 42°C, apart from the usual 37°C.
- After 3-4 weeks of incubation the slants, incubated at the various temperatures, were checked for growth. (Isenberg 1995).

3.3.8 Urease Production

This test differentiates mycobacteria on the basis of their abilities to hydrolyze urea. *M. bovis, M. africanum* and some species of *M. tuberculosis* are positive for this test while most other species are negative.

- A loopful of growth was inoculated into a urea broth.
- Incubated at 37°C for 5 days.
Examined for dark pink to red colour for a positive result and no colour change indicated a negative result (Isenberg 1995).
Chapter 4

Results

4.1 Tuberculosis suspects from the Paediatrics Departmental wards

Gastric lavage specimens were received from a total of 408 tuberculosis suspects from different wards of the Department of Paediatrics and Child Health wing of the University Teaching Hospital (UTH), as shown in Table 5 and figure 7.

The different wards function as follows: AO1 is Outpatient, accidents and emergency as well as admission ward, AO2 is a high cost general medical as well as for Anti-retroviral treatment ward, AO3 is Haemato-oncology ward, AO4 is the low cost general medical ward, AO5 is an isolation ward partitioned in different highly communicable diseases which require isolation, TB and measles being some of them. AO6 is mainly for diarrheal cases, AO7 is mainly a nutritional ward for malnourished children and finally AO8 being another general medical ward which functions similar to AO4 or more less like reinforcement of AO4. These wards tends to overlap once in a while due to congestion.

The majority of the gastric lavages were received from wards A04, A05 and A07. The number of specimens from these 3 wards were as follows: 23.3% (95/408) from ward AO4, 20.6% (84/408) from ward AO5 and 19.1% (78/408) from ward AO7.
TABLE 5: Total number of gastric lavages received from suspected tuberculosis patients in different wards of the UTH.

Total specimens per ward ($N=408$)

<table>
<thead>
<tr>
<th>Ward</th>
<th>Total</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>56</td>
<td>(13.7)</td>
</tr>
<tr>
<td>A02</td>
<td>5</td>
<td>(12.3)</td>
</tr>
<tr>
<td>A03</td>
<td>48</td>
<td>(11.8)</td>
</tr>
<tr>
<td>A04</td>
<td>95</td>
<td>(23.3)</td>
</tr>
<tr>
<td>A05</td>
<td>84</td>
<td>(20.6)</td>
</tr>
<tr>
<td>A06</td>
<td>15</td>
<td>(3.7)</td>
</tr>
<tr>
<td>A07</td>
<td>78</td>
<td>(19.1)</td>
</tr>
<tr>
<td>A08</td>
<td>24</td>
<td>(5.9)</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>3</td>
<td>(0.7)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>408</td>
<td>(100.0)</td>
</tr>
</tbody>
</table>

The number of gastric lavages received from the other wards with suspected cases of tuberculosis were follows: 13.7% (56/408) for A01, 12.3% (5/408) for A02, 11.8% (48/408) for A03, 3.7% (15/408) for A06, 5.9% (24/408) for A08, and lastly 0.7% (3/408) for those with no ward indicated. Figure 7 shows a graphical presentation of the wards with total specimens received from each ward.
4.1.1 Age and sex distribution of the suspected TB cases

Out of the 408 gastric lavages received from tuberculosis suspects, 48.8% (199/408) of the suspects were males and 51.2% (209/408) were females. There was no statistical difference between the males and females although females were slightly more than males thus 51.2% (209/408) and 48.8% (199/408) respectively (Table 6).

The ages ranged from 0 to 14 years with a mean age of 3.3 years (1347/408). Months in terms of the ages of suspects were converted to the nearest whole number. All the TB suspects were divided into different age range groups as follows:

**Group 1:**

![Figure 7: Total number of gastric lavages received from suspected tuberculosis patients in different wards of the UTH.](chart)
0 – 2 year group: includes all suspects with age less than 1 year and up to 2 year and 6 months.

**Group 2:**
3 – 4 year group: includes all suspects with age above 2 year, 6 months up to 4 years and 6 months.

**Group 3:**
5 – 6 year group: includes all suspects four years, 6 months and above, up to 6 years and 6 months.

**Group 4:**
7 – 8 year group: includes all suspects six years, 6 months and above, up to 8 years and 6 months.

**Group 5:**
9 – 10 year group: includes all suspects 8 years, 6 months and above, up to 10 years, 6 months.

**Group 6:**
11 -12 year group: includes all suspects 10 years, 6 months and above, up to 12 years, 6 months.

**Group 7:**
13 – 14 year group: includes all suspects 12 years, 6 months and above, up to and including 14 years, 6 months.

**Group 8:**
Unknown age group: included all suspects where no age where filled in on the questionnaire.

See Table 6.

The rest of the analysis of these TB suspects was based on these age classes hence forth referred to as age groups or age brackets.
TABLE 6: The age and sex distribution of the suspected tuberculosis patients.

<table>
<thead>
<tr>
<th>Age range in years</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>117 (53.4)</td>
<td>102 (46.6)</td>
<td>219 (53.7)</td>
</tr>
<tr>
<td>3-4</td>
<td>35 (50.7)</td>
<td>34 (49.3)</td>
<td>69 (16.9)</td>
</tr>
<tr>
<td>5-6</td>
<td>18 (46.2)</td>
<td>21 (53.4)</td>
<td>39 (9.6)</td>
</tr>
<tr>
<td>7-8</td>
<td>13 (52.0)</td>
<td>12 (48.0)</td>
<td>25 (61.3)</td>
</tr>
<tr>
<td>9-10</td>
<td>5 (23.8)</td>
<td>16 (76.2)</td>
<td>21 (5.1)</td>
</tr>
<tr>
<td>11-12</td>
<td>5 (33.3)</td>
<td>10 (66.6)</td>
<td>15 (3.7)</td>
</tr>
<tr>
<td>13-14</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
<td>4 (1.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (32.3)</td>
<td>11 (68.7)</td>
<td>16 (3.9)</td>
</tr>
<tr>
<td>Total</td>
<td>199 (48.8)</td>
<td>209 (51.2)</td>
<td>408 (100.0)</td>
</tr>
</tbody>
</table>

The age group from 0 to 2 years had more suspected cases of tuberculosis, followed by the 3 to 4 year age group, thus 53.7% (219/408) and 16.9% (69/408) respectively (Table 6). In the age group 0 to 2 years, 53.4% (117/219) were male and 46.6% (102/219) were females, whilst 50.7% (35/69) were male and 49.3% (34/69) were female in the group 3 to 4 years. The rest of the age groups had the TB suspects distributed as follows; 9.6% (39/408) for 5 to 6 year age group with the percentage of males to female being 46.2% (18/39) to 53.4% (21/39) respectively, 61.3% (25/408) for 7 to 8 year group with a percentage of males to females being 52.0% (13/25) and 48.0% (12/25) respectively, 5.1% (21/408) for 9 to 10 year group with a percentage of males to females being 23.8% (5/21) and 76.2% (16/21) respectively. In the age group 11 to 12 years the percentage of males were 33.3% (5/15) and the females 66.6%. In the last age group, 13 to 14 years, only 1.0% (4/408) of the children was suspected of having tuberculosis. The ratio of male to female were 25.0% (1/4) and 75.0% (3/4) respectively. In 3.9% (16/408) of the cases the age was not indicated, but the specimens were submitted from the paediatric wards with completed questionnaires. The percentage of male to female being 32.3% and 68.7% respectively for this group (Table 6). Table 7B shows a representation of the age groups and sex distribution as a percentage out of the total suspects received (408).
In the ages groups 0-2 years up to and including 7-8 years there is not a noticeable difference between the numbers of male and female suspects. However, in the older groups 9-10 up to and including 13-14 years the numbers of suspects were less and the majority of the cases were female. (Table 6). Figure 8 shows a graphical presentation of the age groups calculated out of the total 408 TB suspects.

4.1.2 TB Cases in the household of the Suspects
On average about half of the suspects had a positive case of TB in their households (Table 7A).
A total of 408 gastric lavages were received from patients suspected of having tuberculosis. It was found that in 44.6% (182/408) of the suspected cases a case of tuberculosis was confirmed in the household (Table 7A).

The distribution of the positive TB cases in the household of the TB suspects was as follows: 40.2% (88/219) of the cases were in the 0 to 2 year age group, 50.7% (35/69) in the 3 to 4 year age group and 61.5% (24/39) in the 5 to 6 year group. In the other ages groups the numbers of confirmed cases in the households showed a decline. Only 40.0% (10/25) in the 7 to 8 year group, 52.4% (11/21) in the 9 to 10 year age group, 46.7% (7/15) in 11 to 12 year group, 25.0% (1/4) in the age group 13 to 14 years and lastly 37.5% (6/16) for those whose age was not indicated (Table 7A).

**TABLE 7A: The age distribution of TB suspects with a confirmed case tuberculosis in the household**

<table>
<thead>
<tr>
<th>Age range</th>
<th>Total (%)</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>219 (53.7)</td>
<td>88 (40.2)</td>
<td>131 (59.8)</td>
</tr>
<tr>
<td>3-4</td>
<td>69 (16.9)</td>
<td>35 (50.7)</td>
<td>34 (49.3)</td>
</tr>
<tr>
<td>5-6</td>
<td>39 (9.6)</td>
<td>24 (61.5)</td>
<td>15 (38.5)</td>
</tr>
<tr>
<td>7-8</td>
<td>25 (6.1)</td>
<td>10 (40.0)</td>
<td>15 (60.0)</td>
</tr>
<tr>
<td>9-10</td>
<td>21 (5.1)</td>
<td>11 (52.4)</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>11-12</td>
<td>15 (3.7)</td>
<td>7 (46.7)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>13-14</td>
<td>4 (1.0)</td>
<td>1 (25.0)</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>16 (3.9)</td>
<td>6 (37.5)</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>Total</td>
<td>408 (100.0)</td>
<td>182 (44.6)</td>
<td>226 (55.4)</td>
</tr>
</tbody>
</table>

To get a true representation of positive TB cases in each household further analysis was done on all the suspects. Table 7B shows the analysis, the total number of suspects, their age ranges, their sex distribution and the total cases of TB in their households together with the percentages calculated from the total suspects.
Overall, the total TB suspects with a positive TB case in the household was 44.6% (182/408) (Table 7B).

The age groups 0-2 and 3-4 years had more TB positive cases in their households, thus 21.6% (88/408) and 8.6% (35/408) respectively as compared to the other age groups as shown in Table 7B and Figure 9.

**TABLE 7B: The age and sex distribution of the total TB suspects with a confirmed case of tuberculosis in the household**

<table>
<thead>
<tr>
<th>Age range</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Total (%)</th>
<th>Yes (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>117 (28.7)</td>
<td>102 (25.0)</td>
<td>219 (53.7)</td>
<td>88 (21.6)</td>
<td>131 (32.1)</td>
</tr>
<tr>
<td>3-4</td>
<td>35 (8.6)</td>
<td>34 (8.3)</td>
<td>69 (16.9)</td>
<td>35 (8.6)</td>
<td>34 (8.3)</td>
</tr>
<tr>
<td>5-6</td>
<td>18 (4.4)</td>
<td>21 (5.1)</td>
<td>39 (9.6)</td>
<td>24 (5.9)</td>
<td>15 (3.7)</td>
</tr>
<tr>
<td>7-8</td>
<td>13 (3.2)</td>
<td>12 (2.9)</td>
<td>25 (6.1)</td>
<td>10 (2.5)</td>
<td>15 (3.7)</td>
</tr>
<tr>
<td>9-10</td>
<td>5 (1.2)</td>
<td>16 (3.9)</td>
<td>21 (5.1)</td>
<td>11 (2.7)</td>
<td>10 (2.5)</td>
</tr>
<tr>
<td>11-12</td>
<td>5 (1.2)</td>
<td>10 (2.5)</td>
<td>15 (3.7)</td>
<td>7 (1.7)</td>
<td>8 (2.0)</td>
</tr>
<tr>
<td>13-14</td>
<td>1 (0.2)</td>
<td>3 (0.7)</td>
<td>4 (1.0)</td>
<td>1 (0.2)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (1.2)</td>
<td>11 (2.7)</td>
<td>16 (3.9)</td>
<td>6 (1.5)</td>
<td>10 (2.5)</td>
</tr>
<tr>
<td>Total</td>
<td>199 (48.8)</td>
<td>209 (51.2)</td>
<td>408 (100.0)</td>
<td>182 (44.6)</td>
<td>226 (55.4)</td>
</tr>
</tbody>
</table>

The rest of the age groups had a positive TB case in the household as follows; 5.9% (24/408) for 5 to 6 years, 2.5% (10/408) for 7 to 8 years, 2.7% (11/408) for 9 to 10 years, 1.7% (7/408) for 11 to 12 years and lastly 0.2% (1/408) for 13 to 14 years (Table 7B). Figure 9 shows the graphical presentation of the age group and sex distribution, with a case of TB in the household.
4.2 Detection of *Mycobacterium* sp utilizing MGIT 960, L-J culture and microscopy techniques

Gastric lavages were received from 408 patients suspected of having tuberculosis. Using the MGIT 960 technique 27.2% (111/408) were confirmed positive and 17.2% (70/408) were confirmed positive using L-J media culture. Only 5.6% (23/408) were confirmed positive by using direct microscopy (Table 8 and Figure 10).
Totals\( (n=408)\)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Positive</th>
<th>(%)</th>
<th>Negative</th>
<th>(%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT 960</td>
<td>111</td>
<td>(27.2)</td>
<td>297</td>
<td>(72.8)</td>
<td>408</td>
</tr>
<tr>
<td>L-J Media</td>
<td>70</td>
<td>(17.2)</td>
<td>338</td>
<td>(82.8)</td>
<td>408</td>
</tr>
<tr>
<td>Direct Microscopy</td>
<td>23</td>
<td>(5.6 )</td>
<td>385</td>
<td>(94.4)</td>
<td>408</td>
</tr>
</tbody>
</table>

Figure 10 shows the graphical presentation of the total positives and negatives
samples detected by each of the techniques employed in the research.

![Graphical representation of total positives and negatives by different techniques](image)

**FIGURE 10**: Total positive and negative cases detected by MGIT 960, L-J media and direct microscopy

4.2.1 Isolates detection by MGIT 960, L-J media and direct microscopy
A total of 27.7% (113/408) mycobacteria isolates were obtained by combining the MGIT 960 and L-J media culture methods. Out of these isolates, 98.2% (111/113) cases were detected using MGIT 960, 61.9% (70/113) were grown on L-J media and the direct microscopy was only positive for 20.4% (23/113) of the cases. (Table 9).

**TABLE 9: Number of isolates detected by MGIT 960 and L-J Media**

<table>
<thead>
<tr>
<th>Culture Technique</th>
<th>Positive</th>
<th>(%)</th>
<th>Negative</th>
<th>(%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT 960</td>
<td>111</td>
<td>(98.2)</td>
<td>2</td>
<td>(1.8)</td>
<td>113</td>
</tr>
<tr>
<td>L-J Media</td>
<td>70</td>
<td>(61.9)</td>
<td>43</td>
<td>(38.0)</td>
<td>113</td>
</tr>
</tbody>
</table>

Figure 11 shows the graphical presentation of the positive isolates with the different culture techniques employed in the research.
4.3 Total number of isolates in each age group with a confirmed case of TB in the household.

The age group 0 to 2 had the highest number of isolates thus 46.0% (52/113), while the age group 13 to 14 years had the lowest number of isolates thus 2.7% (3/113) (Table 10). Distribution of isolates in the rest of the age groups as shown in table 10 was as follows; 20.4% (23/113) in the group 3 to 4 years, 9.7% (11/113) in the group 5 to 6 years, 5.3% (6/113) in the group 7 to 8 years, 7.0% (8/113) in the group 9 to 10 years, 3.5% (4/113) in the group 11 to 12 years. The last group where age was not indicated had 5.3% (6/113) of the isolates.
TABLE 10: Distribution of age groups with positive isolate and a confirmed case of tuberculosis in the household

<table>
<thead>
<tr>
<th>Age range</th>
<th>Total Isolates (%)</th>
<th>TB case in Household (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>0-2</td>
<td>52 (46.0)</td>
<td>35 (32.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 (15.0)</td>
</tr>
<tr>
<td>3-4</td>
<td>23 (20.4)</td>
<td>14 (12.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 (10.0)</td>
</tr>
<tr>
<td>5-6</td>
<td>11 (9.7)</td>
<td>10 (8.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>7-8</td>
<td>6 (5.3)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>9-10</td>
<td>8 (7.0)</td>
<td>6 (5.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>11-12</td>
<td>4 (3.5)</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>13-14</td>
<td>3 (2.7)</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (5.3)</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (4.4)</td>
</tr>
<tr>
<td>Total</td>
<td>113 (100.0)</td>
<td>75 (66.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38 (33.6)</td>
</tr>
</tbody>
</table>

Figure 12 shows a graphical presentation of the different age groups with the totals isolates and indicating whether there is a confirmed TB case in the household of the children.
The age group 0 to 2 years had the highest number of isolates, 32.8% (35/113) as well as the highest number of TB cases in their households. The lowest number of isolates, 0.9% (1/113), with a positive case of TB in their household were detected in the 13 to 14 year age group (Table 10 & figure 12). The remainder of the cases were distributed as follows; 12.4% (14/113) for the age group 3 to 4 years, 8.8% (10/113) for the age group 5 to 6 years, 3.5% (4/113) for the age group 7 to 8 years, 5.3% (6/113) for the age group 9 to 10 years, 2.7% (3/113) for the age group 11 to 12 years and lastly 1.8% (2/113) where age was not indicated (Table 10 and Figure 12).
4.4 Positivity rate and detection time for MGIT 960 and L-J media techniques.

The following were the positivity rate of the culture techniques employed in this research. MGIT 960 alone was 38.9% (44/113), and that of L-J media alone was 1.8% (2/113) (Table 11).

The mean time taken to detection (TTD) of growth and range for each technique was as follows; 12.49 (1387/111) days and range 5 to 26 days for MGIT 960 and 34.25 (2398/70) days and range 20 to 45 days for L-J media (Table 11). The highest number of isolates for MGIT 960 were detected on day 12 and these were 37, followed by day 10 yielding 16 isolates. The rest for MGIT 960 were as follows; 14 isolates on day16, 10 isolates on day 13, 8 on day 15, 7 on day 14, 6 on day 17, 3 on day 11, 2 each on days 5, 6, 19 and 20 respectively and finally 1 each on days 8 and 26 respectively (Figure 13).
L-J media technique had the highest number of isolates, eight, on day 30. The rest of the isolates were yielded as follows; 7 isolates on days 28 and 40 respectively, 5 on day 31, 4 on days 36, 37, and 42 respectively, 3 on days 29, 32, 33, 34, and 39 respectively, 2 on days 20, 27, 38, 41, 44, and 46 respectively, and finally 1 isolate on days 26, 43, 45, and 50 (Figure 13).

4.5 *Mycobacterium* species isolated

The total number of *Mycobacterium* sp, 113 isolates, we further identified to species level. A total of three different *Mycobacterium* sp were detected using biochemical tests.

*Mycobacterium tuberculosis* was identified in 110 of 113 (97.3%) isolates, *Mycobacterium avium complex* in two of the 113 isolates (1.8%) and one of the isolates proved to be *Mycobacterium kansaii* (0.9%). The different *Mycobacterium* species isolated are shown in table 11.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>No. of Isolates(%)</th>
<th>Both</th>
<th>MGIT only</th>
<th>L-J only</th>
<th>Positive in</th>
<th>TTD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>110 (97.3)</td>
<td>65</td>
<td>44</td>
<td>1</td>
<td>MGIT</td>
<td>L-J</td>
</tr>
<tr>
<td><em>M. avium complex</em></td>
<td>2 (1.8)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. kansaii</em></td>
<td>1 (0.9)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>113 (100.0)</td>
<td>67</td>
<td>44</td>
<td>2</td>
<td>12.5</td>
<td>34.2</td>
</tr>
</tbody>
</table>

The identification of the different *Mycobacterium* sp was done according to the biochemical tests previously described under Materials and Methods on pages 33 to 37.
4.6 Contamination rates of the different culture techniques.

Out of the total of 408 gastric lavages received, 1.2% (5/408) were contaminated by using the MGIT 960 technique and 0.2% (1/408) by using the L-J media technique (Table 12).
TABLE 12: Contamination rates for MGIT 960 and L-J media techniques.

<table>
<thead>
<tr>
<th>Technique</th>
<th>No. contaminated</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGIT 960</td>
<td>5</td>
<td>(1.2)</td>
</tr>
<tr>
<td>L-J Media</td>
<td>1</td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

Contamination rate was is slightly higher in the BACTEC MGIT 960 technique as compared to the L-J media technique (Table 12).

4.7 Culture identification

All positive cultures were examined macroscopically for typical colonial characteristics shown in figures 15, 16 and 17. Only three species of mycobacteria were isolated: 97.3% (110/113) *M. tuberculosis* (Figure 15), 1.8% (2/113) *M. avium complex* (Figure 16) and 0.9% (1/113) *M. kansasii* (Figure 17) as shown in table 11.

A culture of mycobacteria can often be presumptively differentiated into either the *M. tuberculosis* complex (MTC) or *Mycobacterium* other than tuberculosis (MOTT) group by experienced workers on the evidence of a ZN smear and colonial morphology (Watt et al., 1996).

The following were the mycobacteria species isolated with their characteristic colonial morphological appearance.

*M. tuberculosis* colonies were whitish or buffy coloured, dry, friable and rough on L-J media (Figure 15).
FIGURE 15: Colonies of *M. tuberculosis* isolated

*M. avium complex* colonies were glossy, whitish, smooth and slightly translucent on L-J media (Figure 16).

FIGURE 16: Colonies of *M. avium complex* isolated
*M. kansaii* colonies were yellowish orange, smooth and opaque on L-J media (Figure 17).

![Image of* M. kansaii* colonies](image)

**FIGURE 17: Colonies of* M. kansaii* isolated**

The isolates were then stained with Ziehl-Neelsen staining. The acid fastness of the bacteria as well as specific morphology in liquid media was detected. *Mycobacterium tuberculosis* complex exhibited serpentine cording while dot and cross barring morphology was observed in *Mycobacterium avium* complex and *Mycobacterium kansaii* respectively.

The following tests were used to either confirm *M. tuberculosis* and/or differentiate the MOTT group:

Niacin accumulation test; P – Nitrobenzoic acid (PNBA) sensitivity test;
Thiophen-2-carboxylic acid hydrazide (TCH) test; Catalase production test;
Nitrate reduction test; Growth at 25°C and 42°C and Urease Production test.

A summary of the biochemical tests and the results for the identification of the different mycobacteria can be seen in table 13.

**TABLE 13: Results of tests used to identify the Mycobacterium species**

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>M. tuberculosis</th>
<th>M. avium complex</th>
<th>M. kansaii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P-Nitrobenzoic acid (PNBA)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TCH</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sodium Chloride (5%)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 25°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 42°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Chapter 5

Discussion and Conclusion

Diagnosis of tuberculosis in children has posed one of the biggest challenges in the health sector especially in African countries with limited resources. Confirmation of the disease by using the laboratory for the diagnosis is very difficult. This can be seen by the low positivity rate of microscopy tests in the TB laboratory of the University Teaching Hospital in Zambia (Table 1). Certain researchers have identified some of the reasons for the biggest challenges in the diagnosis of tuberculosis. The symptoms of tuberculosis are sometimes non-specific and the difficulty to interpret the skin test and chest radiographs were mentioned as some of the challenges. The routine laboratory tests are also not always helpful in the diagnosis (Khan and Starke, 1995). Engelbrecht, Marais, Donald and Schaaf, (2006) in their study added on to say that the diagnosis of childhood tuberculosis is often problematic and depends on a combination of epidemiological factors, clinical and radiological features, and/or bacteriological confirmation. The diagnosis of tuberculosis in children is not usually attempted routinely as the children must be admitted to the hospital and this adds to the problem. The diagnosis of tuberculosis is traditionally based on chest radiography, tuberculin skin testing, and mycobacterial staining and/or culture although these investigations may not always be positive in children with tuberculosis (Shingadia and Novelli, 2003).

In Zambia, 99% of the Health Services in the country use direct microscopy for diagnosis of tuberculosis, a scenario which exists in most of the developing countries due to a lack of resources (Lubasi et al., 2004). The basic diagnostic methods for pulmonary tuberculosis in developing countries are still sputum microscopy although it is less sensitive than culture (Bahador, Etemadi, Kazemi, Hajabdolbaghi, Ghorbanzadeh and Pajand, 2006). Studies by Habeenzu et al., (1998) and Miornier, H., Ganlov, G., Yohannes, Z., and Adane Y. (1996), have
highlighted a few advantages of microscopy. Microscopy is a fast and cheap method for the diagnosis of tuberculosis. The sensitivity of microscopy has been improved by employing concentration techniques. Habeenzu et al., (1998) improved direct microscopy from 43.4% to 76.3% after using a NaClO concentration method. Aung et al., (2001) improved their sensitivity of direct microscopy from 26.2% to 30.9% also after treatment with NaClO and a concentration technique. These improvements may have a bearing on the diagnosis and the confirmation of tuberculosis in adults, but may have very little impact on the diagnosis in children.

There is an urgent need to improve the diagnosis and treatment of TB in children. The critical areas for further research should include a better understanding of the epidemiology of childhood TB, vaccine development, and most of all the development of better diagnostic techniques (WHO, 2006a). It is estimated that less than 20% of approximately 8 million predicted annual cases of TB worldwide are identified by smear positive. Therefore the targets of a 70% case detection rate and 85% treatment success are not likely to be achieved with the existing methods of smear microscopy (Chakravorty, Dudeja, Hanif and Tyagi, 2005).

Culture still represents the cornerstone for a definitive diagnosis of tuberculosis and other mycobacterioses (Enrico, Paola, Claudia, Tullias, Giampietro and Domenico, 1999). In recent years the development of rapid and reliable culture methods for detecting acid-fast bacilli has been regarded as an absolute priority. The reasons for this renewed concern include the serious public health risk due to the re-emergence of tuberculosis and the appearance of multidrug-resistant strains of \textit{M. tuberculosis}. The high incidence of \textit{Mycobacterium avium} complex disease in patients with AIDS adds to the health risk (Enrico \textit{et al.}, 1999). Currently, mycobacterial culture can be performed with conventional solid media and by one of the available broth-based methods. The radiometric semi-automated BACTEC 460 was one of the first broth-based system to permit the significantly earlier detection of mycobacteria. The introduction of the new
BACTEC MGIT 960 has overcome draw backs of the BACTEC 460 and are now widely accepted as the “gold standard” (Enrico et al., 1999).

The use of the radiometric BACTEC 460 considerably improved the recovery of mycobacteria and decreased the time to detect the growth of mycobacteria. This procedure is still labor-intensive and requires special safety methods as radio-isotopes are used in this system. The problems of the use of radio-isotopes has been overcome by the introduction of a non-radiometric, fully automated system, the BACTEC MGIT 960 (Somoskovi, et al., 2000). A combination of conventional solid media together with a broth-based method is accepted as a reference standard for the diagnosis of mycobacterial infection (Cruciani et al., 2004). The automation process for the cultivation of mycobacteria species should be high on the list of priorities for laboratories dealing with large specimen loads (Somoskovi et al., 2000). This is especially relevant in most of the developing countries where a rise in the notification of tuberculosis cases are seen as shown in Table 1 and Figure 2.

Although AFB microscopy and conventional L-J media culture remain the cornerstone for the diagnosis of TB, the sensitivity of these traditional methods is quite low. There is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens especially those specimens containing small numbers of organisms. The detection of smaller numbers of mycobacteria and earlier detection of the organisms will hasten the treatment with appropriate anti-mycobacterial therapy and prevent the spread of infection in the community (Rishi, Sinha, Malhotra and Pal, 2007).

The aim of this study was to determine whether employing the BACTEC MGIT 960 would optimize isolation of Mycobacterium species in gastric lavages, and determine whether different Mycobacterium species are the etiological agents of tuberculosis in children. This in turn would lead to the improvement of the laboratory diagnosis of tuberculosis in children in Lusaka urban district of
Zambia. Although the bacteriological confirmation of TB in children is not always possible, it should be sought whenever possible (WHO, 2006a)

Results of this study have shown that recovery of *Mycobacterium* spp. from gastric lavages in children was optimized by use of the relatively new non-radiometric fully automated BACTEC MGIT 960. The MGIT 960 had a positivity rate of 27.2% compared to 17.2% of L-J culture media, which is the conventional culture method widely used. The direct microscopy, which is the cheapest traditional method widely used in diagnosis of tuberculosis, had a positivity rate of 5.6% (Table 8).

The results have also shown a very high isolate detection rate by the MGIT 960. It was 98.2% compared to the L-J media of 61.9%, and only 20.4% for direct microscopy (Table 9 & 11). On time taken to detection of growth or mean time to detection (TTD), the MGIT 960 technique had a shorter mean time to detection, 12.5 days as compared to the 34.3 days shown by the L-J media technique (Table 11).

The results in this study compared to some previous studies. Zannetti *et al.*, (1997) compared the recovery rates of BACTEC 460, BACTEC 9000 MB and L-J media. The BACTEC 460 and BACTEC 9000 MB showed no significant difference in the recovery rate being 91.9% and 95.1% respectively. A significant difference was detected between the BACTEC 9000 MB and the L-J media. The BACTEC 9000 MB recovered 37% more than the L-J media. Similary, in this study the BACTEC MGIT 960 recovered more isolates, 98.2% (111/113), compared to the L-J media with 61.9% (70/113) (Table 9). On time taken to detection Zannetti *et al.*, (1997) in their study found that the BACTEC 9000 MB had a mean time to detection of 10.3 days and the L-J media was 27.3 days. In this study, the mean time to detection for MGIT 960 was 12.5 days and that of the L-J media was 34.3 days (Table 10).
Fernando et al., (2000) in their study had an isolate recovery rate of 82.5% (99/120) for MGIT 960 and 70.0% (84/120) for L-J media. The mean time to detection of growth was 13.2 days for MGIT 960 and 22.2 days for L-J media. Somoskovi et al., (2000) in their study had an isolate recovery rate of 96.5% (55/57) for MGIT 960 and 80.7% (46/57) for L-J media and the mean time to detection of growth was 14.3 days and 35.8 days for MGIT 960 and L-J media respectively. Griethuysen et al., (1996) had a recovery rate of 95.9% for BACTEC 9000 MB system and 79.9% for the L-J media and the mean time to detection of growth was 17.6 days for the BACTEC system and 29.4 days for the L-J media. The isolate recovery rate was less in this study when compared to above mentioned studies. It might be attributed to the fact that this study only cultured gastric lavages and not sputum specimens. The numbers of mycobacteria in gastric lavages is usually less than the numbers found in sputum samples. However, the detection of smaller numbers of mycobacteria and the earlier detection of the organisms will hasten the treatment with appropriate anti-mycobacterial therapy and thus prevent the spread of infection in the community (Rishi et al., 2007). Saluja et al., 2002 suggests that more and more gastric lavage specimens be collected to rule out tuberculosis, since the sputum yield from children of various groups are difficult.

In this study the contamination rate for the BACTEC MGIT 960 was slightly higher compared to that of L-J media (Table 12). Other studies have shown a similar trend in terms of contamination rates. A study by Somoskovi et al (2000) also had a higher contamination rate with the MGIT 960 when compared to the L-J media, 3.7% and 1.2% respectively. Zannetti et al., (1997) showed no contamination with the L-J media and a 4.1% contamination rate in the BACTEC 9000 MB. In studies by Griethuysen et al., (1996) and Fernando et al., (2000) the trends were different. They had a slightly higher contamination rate in L-J media when compared to the BACTEC 9000 MB and BACTEC MGIT 960. In Griethuysen et al., the contamination rates were 6.5% and 6.0% for L-J Media.
and BACTEC 9000 MB respectively and Fernando et al., had a contamination rate of 4.1% and 3.3% for L-J media and BACTEC MGIT 960 respectively.

The majority of the TB suspects came from wards A04, A05 and A07 (78/408), while ward A02 had the least number of suspects (Table 5 & figure 7). It was expected that wards AO4 will have more patients than the other wards as AO4 is a general medical ward. Ward AO5 is a TB isolation ward and slightly less specimens were received from this ward. Ward AO7 is a nutritional ward for malnourished patients. Most of the sick children admitted in this ward suffer from malnutrition. There was no statistical difference between the male and female TB suspects, although females were slightly more than males (Table 6). In terms of the total of TB suspects in the various age groups, the age group from 0 to 2 years had more TB suspects followed by the 3 to 4 year old age group (Table 6). The other TB suspects were distributed as shown in Table 6. This pattern was consistent with observations made in the research by Khan et al., (1995). Their studies have shown that in 40 to 50% of the infants with untreated TB infection, the disease developed within 1 to 2 years, and that the risk decreased to 15% among older children. In children that are immune compromised the risk of TB disease progression is also increased especially those below 3 years (WHO 2006a). In a study done in Kampala Uganda, Guwatudde, Nakakeeto, Jones-Lopez, Maganda, Chiunda, Mugerwa, Ellner, Bukenya and Whalen, (2003) have shown that children aged 5 years or less and have contact with HIV-seropositive people carries the highest risk for active tuberculosis. A study done in Cape Town in South Africa, Hatherill, Hawkridge, Whitelaw, Tameris, Mohamed, Moyo, Hanekom and Hussey (2006), found that they isolated NTM from older children and more M. tuberculosis from younger children. The reason that was suggested is that older ambulant toddlers are more exposed to environmental soil where they easily pick up these NTM.

In this study, the age group and sex distribution totals of suspects, this time with a case of Tuberculosis in the household, showed the age groups 0-2 and 3-4
years having more TB positive cases in their households see Table 7A & 7B and figure 9. The total TB suspects with a positive TB case in the household was 44.6% (182/408) (Table 7A&B). The rest of the age groups had a positive TB case in the household as shown in Table 7B. These findings compares with conclusions made by Guwatudde et al., 2003 that tuberculosis is common among household contacts of index cases in Africa, especially among young children and those with HIV-infected contacts. Children younger than 5 years, despite being vaccinated with BCG and with a positive skin test, but in close contact with infected adults are at high risk to developed TB infections due to *Mycobacterium tuberculosis* (Lienhardt, Bennett, Del Prete, Bah-Sow, Newport, Gustafson, Manneh, Gomes, Hill and MacAdam, 2002).

In the results of this study, the isolation of *Mycobacterium tuberculosis* is similar to that presented by the suspects pattern shown in table 6 (Table 10), that is, age group 0 to 2 having the highest number of isolates thus 46.0% (52/113), while the age group 13 to 14 years had the lowest number of isolates thus 2.7% (3/113) (Table 10). Distribution of isolates in the rest of the age groups was as shown in table 10 and figure 12. The age group 0 to 2 years apart from having the highest number of isolates also had the highest number of positives cases of TB suspects in their house holds, thus 32.8% (35/113), with the lowest number of isolates with positive cases of TB in their house hold being the age group 13 to 14 years, thus 0.9% (1/113) (Table 10 & Figure12). The rest of the distribution of total isolates and a positive TB case in the household was as shown in Table 10.

These findings agree with the research findings by Khan et al., (1995) and Davidson et al., (2000) who stated that “TB diagnosis in children usually follows a discovery of a case in an adult” (Khan et al., 1995), “Children with TB have acquired the disease from infective adults and not from other children” (Davidson et al., 2000). Shingadia and Novelli (2003) also observed that childhood tuberculosis represents a sentinel event in the community suggesting recent transmission from an infectious adult.
The need for rapid, specific and inexpensive detection of *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex and *Mycobacterium kansaii* from clinical specimens is a great concern. This is especially true for the developing countries where there is a high incidence of mycobacterial disease and a lack of economical resources (Tu, Chang, Huaug, Huaug, Liu, Shin-Jung Lee, 2003). This observation is very true and needs serious consideration, given the tuberculosis notification cases which are continuously on the increase in most of the developing countries (Table 1 & Figure 2).

This study showed that *Mycobacterium tuberculosis* was the major causative agent of tuberculosis in children admitted in the Department of Paediatrics in the Child Health wing of the University Teaching Hospital (Table 11).

A total of three species of mycobacteria were isolated and identified by the biochemical tests thus *M. tuberculosis*, *M. avium* and *M. kansasii*, with highest number of isolates being *M. tuberculosis* (Table 13). The findings from this study has a very similar pattern with other studies from various parts of the world. They all have a mycobacterium isolation rate of 50% or above for *M. tuberculosis* and about 10% or less for the non-tuberculous Mycobacterium.

In a study done in Spain by Fernando et al., (2000), they isolated 120 mycobacterial species from 1,068 specimens of different origins. The isolate distribution were as follows; 80% (96/120) of the isolates were *M. tuberculosis*, 11.7% (14/120) were identified as *M. kansasii*, 4.2% (5/120) was *M. xenopi*, 1.7% (2/120) proved to be *M. avium* complex, 1.7% (2/120) was *M. gordonae*, and 0.8% (1/120) was *M. fortuitum*. Zanetti et al., (1997), in their study done in Italy, isolated 62 mycobacteria from 779 different respiratory specimens. The identification of the different mycobacteria isolated were as follows; 54.8% (34/62) were identified *M.tuberculosis*, 16.1% (10/62) were *M. avium*, and 29.0% (18/62) were other non-tuberculous mycobacteria. In a study done in Hungary by
Somoskovi et al., (2000), a total of 57 isolates were recovered from 377 different respiratory specimens and showed an isolate distribution as follows; 96.5% (55/57) were *M. tuberculosis*, 1.8% (1/57) were *M. avium* complex, and 1.8% (1/57) were *M. xenopi*.

Griethuysen et al., (1996) in their study done in the Netherlands had a total of 202 mycobacterium isolates from a total of 2,005 specimens. Out of this total number of isolates, 155 (76.7%) were *M. tuberculosis* complex, 15 (7.4%) were *M. avium*- *M. intracellulare* complex, 7 (3.5%) were *M. kansasii*, 3 (1.5%) were *M. malmoense*, 2 (1.0%) were *M. terrae*, 1 (0.5%) was *M. xenopi* and 19 (9.4%) were others. In a study by Hatherill et al., (2006), they isolated 301 mycobacteria from a total of 1732 samples from children. Out of the total isolates, 187 (62.8%) were *M. tuberculosis* and 114 (36.6%) were both identifiable and non-identifiable NTMs. It was found that 72 (63%) were identifiable and 37 (32%) was non-identifiable. The distribution of their identifiable non-tuberculous mycobacterium isolates were as follows; 41% were *M. intracellulare*, 6% of the isolates proved to be *M. gastri*, 4% were *M. avium* and *M. gordonae*, 3% for *M. flavescens* and *M. scrofulaceum*, and 1% for both *M. szulcii* and *M. kansasii*. As seen from all the research findings the isolation of *M. tuberculosis* is well over 50%, but the isolation of other species cannot be ignored.

*M. tuberculosis* complex and *M. leprae* are incapable of replicating in the inanimate environment, but the non-tuberculous mycobacteria, *M. avium complex* and *M. kansasii* inclusive are free-living and are usually found in association with watery habitats such as lakes, rivers and wet soil (Pfyffer, Brown-Elliot and Wallace 2003). It is therefore possible that the two non-tuberculous mycobacteria isolated in this study could be the cause of the tuberculosis in the children where they were isolated. Although the NTM are not obligate pathogens they share the features of hardiness, hydrophobicity, aerosolization and intracellular pathogenicity with *M. tuberculosis* (Hatherill et al., 2006). *M. avium complex* (MAC) has also been found to be the leading cause of
localized mycobacterial lymphadenitis in children aged 1 to 5 years and also in immunocompromised children (Pfyffer et al., 2003).

Recovery of non-tuberculous mycobacteria needs further consideration and especially due to their ubiquitous nature. The question of their clinical significance is therefore very important (Pfyffer et al., 2003). The probability of NTM TB disease is likely to increase with the extent of environmental exposure, particularly in farming communities. People with immune susceptibility to opportunistic infection and others with co-existent chronic lung disease such as cystic fibrosis are at a higher risk of contracting mycobacterial infections (Hatherill et al., 2006). Fernando et al., 2000 mention in their study the importance of isolating NTM and stating that _M. kansasii_ (genotype I) is the most frequently isolated and the most virulent species of the isolates.

In the last decade tuberculosis has re-emerged as a major worldwide public health hazard with increasing incidence among adults and children. Although cases among children represent a small percentage of all TB cases, they are a reservoir from which many adult cases will arise (Khan et al., 1995). The most of the children acquire TB infection from adults with whom they come in contact with and thus represent a large proportion of the pool from which cases will arise in the future. The distribution of TB infection in children can be considered a marker of recent ongoing transmission in the communities (Lienhardt et al., 2003). It is estimated that 9 million people will develop TB annually. Out of these 1 million (11%) will occur in children less than 15 years old (WHO Stop TB Partnership Childhood TB Subgroup 2006a). Saluja et al., (2003), highlights that childhood tuberculosis is on the increase worldwide because of persisting inability to conform the diagnosis, leading to a large number of children dying of undiagnosed tuberculosis. In their study, they showed a statistical significance of gastric lavage AFB positive of 70.3% of patients over ESR test, TB IgG test and ADA test, thereby suggesting that gastric lavage specimen be considered as one of the diagnostic tests for suspected cases of tuberculosis (Saluja et al., 2003).
Children rarely produce sputum samples adequate for examination and typically have low bacterial loads. This makes smear identification very difficult even if early morning gastric lavages, which contains aspirated respiratory secretions, is used (Munzo and Starke, 2000). Khan et al., 1995 in their analysis also indicated that the best specimen for culture from children with suspected pulmonary TB is the early morning gastric aspirate. There is therefore an urgent need to improve the diagnosis and treatment of TB in children and especially to develop better diagnostic techniques. The article, “Stop TB Partnership Childhood TB Subgroup” WHO 2006, supports the development of better diagnostic techniques. Despite showing a slightly higher contamination rate by the BACTEC MGIT 960 in this study and a few others which have been cited, it still remains an ideal method for optimizing recovery of tuberculosis especially from gastric lavage of children below 15 years and suspected of having tuberculosis.

Results of this study have shown optimized recovery of mycobacteria from gastric lavage from children suspected of having TB and that children tend to acquire pulmonary tuberculosis from an adult members of the family within the household. TB diagnosis in children usually follows a discovery of a case in an adult (Khan et al., 1995). Children with TB have acquired the disease from infective adults and not from other children (Davidson et al., 2000). A need for improved, reliable and more sensitive methods can not be over emphasized especially to avoid resistant strains of TB. An extensively drug resistant TB (XDR-TB) has been reported in South Africa and has been seen in other places, including the United States of America, Eastern Europe and some parts of Africa (Gandhi et al., 2006). Although uncommon in children, multidrug-resistant tuberculosis is also increasing and treatment will often involve longer courses of therapy with second-line antituberculosis drugs (Shingadia and Novelli, 2003).
“Because we haven’t put things right in certain sectors of our environment to combat diseases supposedly ancient like tuberculosis, we will fail to combat the deadly emerging newer ones” David Lubasi.
References


Verver, S., and Veen, J. (2006). History of Migration of TB. In Raviglione C. M. Reichman, B. L. and Hershfield, S. E. (Eds.). Reichman and Hershfield's


Appendix 1: Research project approval

Ref: 20144011

Contact person: Ms G Bhbel

Date: 28 March 2006

Address:

Mr D Lubasi
University Teaching Hospital
Pathology Department
TB Laboratory
Lusaka
ZAMBIA

Dear Mr Lubasi

FINAL RESEARCH PROPOSAL: M Tech Biomedical Technology

Please be advised that your final research proposal was approved by Faculty Management subject to the following recommendations to be made to the satisfaction of your Supervisor:

(i) That it was advised that the final copy of the research report was to be language edited.

Yours sincerely

OFFICE OF THE DEAN
FACULTY OF HEALTH SCIENCES
Appendix 2: Ethical approval from Nelson Mandela Metropolitan University

Dear Mr. Lubasi,

TO IMPROVE THE DIAGNOSIS OF TUBERCULOSIS IN CHILDREN IN ZAMBIA

Your above-entitled application for ethics approval served at the May 2006 ordinary meeting of the Research Ethics Committee (Human). The Committee approved the above-mentioned application on condition that the following matters be addressed:

(a) That the consent form be adapted so that it is addressed to the hospital, requesting permission to conduct the research in a particular hospital.
(b) That the applicant indicates the potential liability if anything goes wrong during the research study.
(c) That the applicant submits proof of a letter requesting permission from the authorities to proceed with the research study.

Please inform your co-investigators of the outcome. We wish you well with the project.

Yours sincerely,

[Signature]

Prof R du Randt
Chairperson: Research Ethics Committee (Human)

cc: Department of Research Management
    Faculty Officer, Faculty of Health Sciences
Appendix 3: Ethical approval from The University of Zambia

THE UNIVERSITY OF ZAMBIA

RESEARCH ETHICS COMMITTEE

Telephone: 260-1-250667
Telex: UNZALU-ZA 44376
Fax: +260-1-250751
E-mail: uszaecc@zamtel.zm

Assurance No. FWA00000338
IRB00001131 of IORG0000774

28 August, 2006
Ref.: 005-07-06

Mr David Lubasi
C/O Mr Davy Naama
Department of Pathology and Microbiology
University Teaching Hospital
P/B RWIX
LUSAKA

Dear Mr Lubasi,

RE: RESEARCH PROPOSAL ENTITLED: "OPTIMIZING THE RECOVERY RATE OF MYCOBACTERIUM SPECIES FROM GASTRIC LAVAGES IN CHILDREN AT AN URBAN ZAMBIAN HOSPITALS"

The above research proposal was presented to the Research Ethics Committee meeting on 26 July, 2006 where changes were recommended. We would like to acknowledge receipt of the corrected version with clarifications. The proposal has now been approved, and the need to obtain informed consent has been waived. Congratulations!

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit copy of your final report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.

Yours sincerely,

Prof. J. T. Karashani, MB, ChB, PhD
CHAIRMAN
Appendix 4: Research questionnaire

**QUESTIONNAIRE**

**RESEARCH TITLE:** Optimizing the recovery rate of *Mycobacterium* species from gastric lavages in children at an urban Zambian Hospital.

Name of patient ________________________________

Residential Address ____________________________

Age ___________ Gender _________________________

Ward __________________________

**HISTORY OF ILLNESS:**

How long has the patient been ill? Days _______ Weeks _______

Months __________________________

How long has the patient been coughing? Days _______

Weeks _______ Months _______

Has the patient been on TB treatment before? __________________________

If YES, state how long ago and the outcome of the treatment.

______________________________

Any cases of TB in the household? __________________________

If YES, state the age of patient and its relation to the patient eg 32, mother

______________________________

Number of people in the household:

Adults ___________________ Children ___________________

Name and title of specimen collector ________________ Sign: __________
Appendix 5: Research Consent Form

RESEARCH TITLE: Optimizing the recovery rate of *Mycobacterium* species from gastric lavages in children at an urban Zambian Hospital.

BACKGROUND
Diagnosis of tuberculosis among children poses technical operational challenges because of vague and non-specific symptomatology and difficulty in getting sputum samples from children.

This study aims to enhance laboratory diagnosis of tuberculosis through improved recovery of mycobacteria from gastric lavage collected from children who can not cough out sputum.

Tuberculosis is very common in Zambia, with incidence rate of about over 500 per 100,000 population. In children prevalence is about over 21,000 new cases per year at the University Teaching Hospital. So it is important for guardians/parent to enroll your child(ren) in this study once they are suspected of having TB.

BENEFITS: - Children will benefit in that those suspected of having TB will be diagnosed rapidly, and the *Mycobacteria spp* (if any) identified, thereby reducing the morbidity and mortality rate.

Name of Guardian __________________________ Sign: __________________________
Appendix 6: Research cover letter to Parent/ Guardian

Dear Parent or Guardian,

I understand that the Clinic has sent your child to the University Teaching Hospital for tests to see if your child has tuberculosis. Tuberculosis is a sickness that makes the child cough and loose weight.

When the child coughs it brings up the spit and most of the time it swallows the spit again. If they want to see if the child has the TB germs they have to look at the spit. The doctor will get the spit from the stomach of the child but it will not hurt the child. The doctor will then send it to the laboratory so that they can look for the TB germs.

Sometimes in the laboratory they cannot see the TB germs even if they are there. I am doing a research project using another way to look for the TB germs. This way is very good and very quick. I just need some of the spit the doctor takes for the laboratory.

I just want to ask your permission so that I can use some of the spit for the test. If I can it will be much better for the child, as by using this way I can see the TB germs much better than the other ways. You will know if your child has the TB germs and then the doctor can give him/her the correct medicine.

I hope you understand that I don't want anything extra than what they are taking from your child.

Thank you

David Lubasi (Research Principal Investigator)