ORIGINAL PAPER

Optimizing the recovery rate of Mycobacterium species from gastric lavages in children at an urban Zambian Hospital

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ABSTRACT

Tuberculosis (TB) has re-emerged as a major worldwide public health hazard with increasing incidence among adults and children, with children representing a small percentage of all TB cases and possible reservoir from which many adult cases will arise.

Objectives: To determine whether the BACTEC MGIT 960 culture system will optimize the isolation of *Mycobacterium* species and also whether different *Mycobacterium* species are the etiological agents of TB in children.

Design: Gastric lavage specimens were received from a total of 408 TB suspects from different wards of the Department of Paediatrics and Child Health wing of the University Teaching Hospital (UTH) and examined by microscopy, Lowenstein-Jensen (L-J) culture and MGIT 960 culture.

Main outcomes and results: This study analyzed gastric lavages from 408 children suspected of having TB. Recovery of *Mycobacterium spp* was optimized by the use of the relatively new non-radiometric fully automated BACTEC MGIT 960 which produced a positivity rate of 27.2% against 17.2% that of L-J media. Direct microscopy yielded a 5.6% positive rate. 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% of the older children in the age group 10 to 14 years.

Conclusion: 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.

INTRODUCTION

Tuberculosis (TB) remains one of the deadliest diseases in the world and has been with man kind all through its existence as shown by the finding of Pott's disease in an Egyptian mummy [23]. 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[26]. The emergence of the human immuno deficiency virus (HIV) has changed the face of

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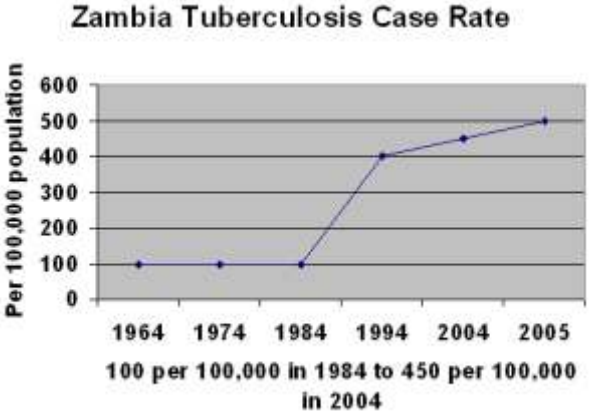
tuberculosis[24]. In 1995, about one third of the 15 million HIV-infected people world wide were also co-infected with *M. tuberculosis* and out of these 70% live in sub-Saharan Africa, 20% in Asia and 8% in Latin America and the Caribbean [24]. Resurgence of tuberculosis among AIDS patients and the homeless population has lead to increased MDR-TB cases [3]. 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 [25]

Recently South Africa has reported extensively drug resistant TB (XDR-TB) which has been seen in some areas worldwide [8]. 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 ([8].
Sputum culture analysis done on 53 cases in 2006, Kwa Zulu Natal, South Africa indicated findings of 41% MDR-TB strains, 10% XDR-TB strains, 47 tested HIV positive and 52 of the 53 cases died [8].

The incidence of TB 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 [5].

Figure 1: Zambia Tuberculosis Case Rate from 1964 to 2005)



Taken from Chanda, 2002

Childhood tuberculosis is on the rise worldwide due to persisting inability to confirm the diagnosis.[18]. Munzo and Stark (2006), p.308 indicated that most children with tuberculosis acquire the organism from adults in their environment, with the epidemiology of childhood tuberculosis reflecting that in adults. Estimations indicate that in the past decade there were 88 million cases of tuberculosis, of which 15 million were children and 5 million of whom have died [19]. 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 [26]. 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 [26]. In Countries worldwide, the reported percentage of all TB cases occurring in children varies from 3% to more than 25% [25].

In Zambia, 21 000 new cases were notified during 1991 and approximately 2 100 were children under the age of 14 years [17]. Children in this age group constitute about 49% of the estimated 8.6 million people in the country [17]. 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 [17]. In England and Wales, the notification rate for children was at 294 per 100,000 population in 1988 [6]. In Canada, despite the availability of effective therapy, out of the over 2,000 cases reported annually, 10 to 15% occur in children [4]. 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 [14]. 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 [14]. Figure 2 indicates data for the period 2000-2005 the number of positive gastric lavage smears done at UTH TB laboratory.

Figure 2: Positive TB cases diagnosed from children at UTH TB laboratory by direct microscopy technique during 2000 to 2005.

Year	Total smears	Positives smears	% Positive
2000	4435	276	6.2%
2001	4177	189	4.5%
2002	5556	280	5.0%
2003	5898	198	3.4%
2004	4245	210	4.9%
2005	4521	124	2.7%

METHODS

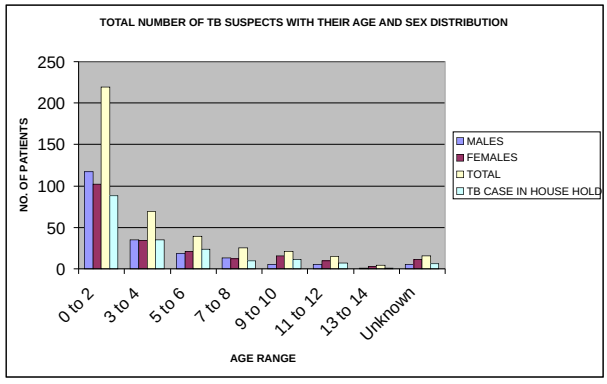
Four hundred and eight (408) early morning gastric lavage specimens were collected and processed inside certified biological safety cabinets [22]. Each specimen received was decontaminated, processed and the deposit of the sample was used to inoculate L-J culture media and the BACTEC MGIT 960 media [22]. Smears were prepared prior to processing for culture [22]. The isolates were identified by staining with Ziehl-Neelsen for acid-fastness of the bacteria as well as specific morphology in liquid media was detected [11]. *Mycobacterium tuberculosis* complex exhibited serpentine cording while dot and cross barring morphology was observed in *Mycobacterium avium* complex and *Mycobacterium kansaii* respectively [11].

Differentiation within the MOTT group was done using additional test which included Niacin accumulation, Nitrobenzoic acid (PNBA) sensitivity, Thiophen-2-carboxylic acid hydrazide (TCH) sensitivity, Sodium Chloride (NaCl) Tolerance, Catalase production, Nitrate reduction, Growth at 25°C and 42°C**,** as shown in figure 3 to confirm *M. tuberculosis* and/or differentiate the MOTT group, following laboratory procedures [22]**.**

RESULTS

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 (Figure 4). 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. 44.6% (182/408) of the suspected cases had a case of tuberculosis confirmed in the household (Figure 4).

Figure 4: The age and sex distribution of the total TB suspects with a confirmed case of tuberculosis in the house hold



Biochemical test	Result for the *Mycobacterium* species isolated		
	M. tuberculosis	*M. avium complex*	*M. kansaii*
Niacin	+	-	-
P-Nitrobenzoic acid (PNBA)	-	+	+
TCH	+	-	-
Sodium Chloride (5%)	+	-	-
Catalase	-	+	+
Nitrate	+	-	+
Urease	+	-	-
Growth at 25°C	-	-	-
Growth at 37°C	+	+	+
Growth at 42°C	-	-	-

Figure 3: Results of tests used to identify the *Mycobacteria species.*

In terms of positivity rates for the methods used, MGIT 960 technique had 27.2% (111/408) 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 (Figure 5).

Figure 5: Detection of *Mycobacteria sp.* Utilizing MGIT 960, L-J media and direct microscopy

Totals (*n=408*)

Technique	Positive	*(%)*	Negative	*(%)*	Total
MGIT 960	111	*(27.2)*	297	*(72.8)*	408
L-J Media	70	*(17.2)*	338	*(82.8)*	408
Direct Microscopy	23	*(5.6)*	385	*(94.4)*	408

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%). These different *Mycobacterium* species were identified using biochemical tests shown in figure 3. On time taken to detection (TTD), MGIT 960 had a TTD of 12.5 days while for L-J media it was 34.3 days. In terms of contamination rates, of the total 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.

DISCUSSION

Diagnosis of tuberculosis in children has posed one of the biggest challenges in the health sector especially in African countries with limited resources. Diagnostic confirmation of the disease by using the laboratory for the diagnosis is very difficult has can be seen by the low positivity rate of microscopy tests in the TB laboratory of the University Teaching Hospital in Zambia (Figure 2). 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 [14]. The basic diagnostic methods for pulmonary tuberculosis in developing countries are still sputum microscopy although it is less sensitive than culture [2]. Studies by Habeenzu *et al.*, (1998) and Miorner, *et al*, (1996), have highlighted a few advantages of microscopy 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.

The use of the radiometric BACTEC 460 considerably improved the recovery of mycobacteria and decreased the time to detect the growth of mycobacteria, although the 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 [20]. The automation process for the cultivation of mycobacteria species should be high on the list of priorities for laboratories dealing with large specimen loads [20]. This is especially relevant in most of the developing countries where there is a rise in the notification of tuberculosis cases for example as shown in Zambia (Figure 1).

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% (Figure 5). Compared to other studies, the recovery of *Mycobacterium* spp trend was very similar, e.g. Zannetti *et al*., (1997) had a recovery rate of 95.1% by BACTEC 9000MB and 58% by L-J media. 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. 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 Griethuysen *et al*., (1996) had a recovery rate of 95.9% for BACTEC 9000 MB system and 79.9% for the L-J media.

On time taken to detection (TTD) of growth of isolates, the MIGT 960 technique had a shorter mean time to detection, a trend very similar to other studies. In this study MGIT 960 had a TTD of 12.5 days while for L-J media it was 34.3 days. Zannetti *et*

al (1997) had a TTD of 10.3 days and 27.3 days respectively for BACTEC 9000MB and L-J media respectively. Fernando *et al*., (2000) had a TTD of 13.2 days and 22.2 days for MGIT 960 and L-J media respectively. Somoskovi *et al*., (2000) had that of 14.3 days and 35.8 days for MIGT 960 and L-J media respectively, while Griethuysen *et al*., (1996) showed a TTD of 17.6 days for the BACTEC system and 29.4 days for L-J media.

Contamination rates for the BACTEC MGIT 960 was slightly higher compared to that of L-J media. This study had contamination rates of 1.2% for MIGT 960 and 0.2% for L-J media. 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. Studies by Griethuysen *et al.*, (1996) and Fernando *et al.*, (2000) had different trends indicating a slightly higher contamination rate in L-J media 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.

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 [12]. Most children acquire TB infection from adults with whom they come in contact and thus represent a large proportion of the pool from which cases will arise in the future [12]. The distribution of TB infection in children can be considered a marker of recent ongoing transmission in the communities [13]. Children with TB have acquired the disease from infective adults and not from other children [6]. A need for improved, reliable and more sensitive methods be emphasized especially to avoid resistant strains of TB.

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