



**A STUDY TO DETERMINE THE HISTOLOGICAL
PATTERN OF NON-TRAUMATIC ILEO-CECAL
PERFORATIONS AND TO EVALUATE THE XPERT
MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF
INTESTINAL TB AT THE UNIVERSITY
TEACHING HOSPITAL, LUSAKA.**

By

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**A DISSERTATION SUBMITTED TO THE UNIVERSITY OF
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REQUIREMENTS FOR THE AWARD OF MASTER OF
MEDICINE IN GENERAL SURGERY**

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SCHOOL OF MEDICINE
DEPT OF SURGERY
LUSAKA**

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DECLARATION

I hereby declare that this dissertation entitled, *A STUDY TO DETERMINE THE HISTOLOGICAL PATTERN OF NON-TRAUMATIC ILEO-CECAL PERFORATIONS AND TO EVALUATE THE XPERT MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF INTESTINAL TB AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA* represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other University elsewhere.

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CERTIFICATE OF APPROVAL

**THIS DISSERTATION BY DR. SHULA CHANDA IS APPROVED AS FULFILLING PART OF THE
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ABSTRACT

Intestinal Perforations(IP) are a common cause of generalized peritonitis, the fecal contamination and overwhelming sepsis that result lead to high morbidity and mortality. Primary infectious causes include Typhoid and Tuberculosis. A high prevalence of Tuberculosis and annual typhoid fever outbreaks in Zambia suggest that *Salmonella Typhi* and *Mycobacteria Tuberculosis* (MTB) could be a cause of IPs seen at the UTH.

Objectives:

To study the histological features of ileo-cecal perforations and their correlation with the XPERT MTB/RIF ASSAY for the rapid diagnosis of intestinal TB.

Material and Methods

The study is a descriptive cross sectional study conducted from March 2014 - March 2015. The study population were patients aged between 1-90years who presented with peritonitis and intra-operatively were found to have ileo-cecal perforations, edges of the perforation and mesenteric lymph nodes were harvested intra-operatively. Convenience sampling was used to capture the study participants. A questionnaire was administered on the 2nd postoperative day to capture demographic and clinical data. Data collected was entered in Microsoft Excel 2007 and analysed in STATA version 11.

Results

35 patients with IP were enrolled. More than half (66%, n- 23) were males while less than half (44%, n-12) were females, with a mean age of twenty-one (21) years and a mean duration of illness of four (4) days. The Terminal Ileum was the most common site of perforation and single perforations were the most common. The histological pattern were as follows more than three quarters (94.3%) of the study participants had non specific inflammation, while 5.7% (n=2) had features consistent with Mycobacterium Tuberculosis infection. DNA PCR for MTB confirmed the 2 positive samples for MTB using the MTB/RIF assay. None of the study participants had any Gastrointestinal Malignancy or a previous history of Tuberculosis.

Conclusion

The histological pattern of ileo-cecal perforations at the UTH is mostly non specific inflammation and that of MTB, the terminal ileum is the most common site for IP, with single perforations being predominant. DNA-PCR is a fast & efficient diagnostic tool for Intestinal Tuberculosis.

DEDICATION

To my dear new wife, who allowed me to be away from her whilst putting in all the hours needed to bring this work to paper, to my Mum and Dad for encouraging me to be a researcher and to my patients for challenging me to think outside the box. This work is dedicated to you.

ACKNOWLEDGEMENTS

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Definitions

1. **Perforation:** a hole or break in the containing walls or membranes of an organ or structure of the body. Perforation occurs when erosion, infection or other factors create a weak spot in the organ and internal pressure causes a rupture. It also may result from a deep penetrating wound caused by trauma. (Mosby's Medical Dictionary, 2009.)

2. **Intestinal Perforation:** a complication of ulcerative colitis, intestinal obstruction, ulceration and other disorders in which there is inflammation of the intestinal wall or obstruction of the intestinal lumen. (Mosby's Medical Dictionary, 2009)

3. **Peritonitis:** inflammation of the membrane which lines the inside of the abdomen and all of the internal organs. This membrane is called the peritoneum (Mosby's Medical Dictionary, 2009)

4. **Case Definition of Intestinal Perforation**
An Intestinal Perforation is a loss of the bowel wall integrity, resulting in escape of digestive tract contents into the peritoneal cavity inevitably leading to peritonitis (Mosby's Medical Dictionary, 2009).

5. **Ileum**
The lowest of the three portions of the small intestine. It runs from the jejunum to the ileocecal valve. (The Bantam Medical Dictionary, 2004).

6. **Cecum**
Blind-ended pouch at the junction of the small and large intestines, situated below the ileocecal valve. The upper end is continuous with the colon and the lower end bears the vermiform appendix. (The Bantam Medical Dictionary, 2004).

7. **Ileo-cecal**
Relating to the ileum and cecum. (The Bantam Medical Dictionary, 2004).

Abbreviations

AIDS - Acquired Immunodeficiency Syndrome

AFB – Acid Fast Bacilli

ATT – Anti-tuberculosis Treatment

DNA - Deoxyribonucleic acid

FBC - Full Blood Count

HAART - Highly Active AntiRetroviral Treatment

HIV - Human Immunodeficiency Virus

IP – Intestinal Perforation

IRIS - Immune Reconstitution syndrome

PCR - Polymerase Chain Reaction

MTB – Mycobacteria tuberculosis

NSAID - Non-Steroidal Anti-Inflammatory drug

TB - Tuberculosis

UCLMS – University College London Medical School

UTH - University Teaching Hospital

UNZA – University of Zambia

CHAPTER 1

1.0. Background Information

1.1. Introduction

This study focuses on intestinal perforations (IP) which are a common cause of generalized peritonitis, the resultant fecal contamination and overwhelming sepsis lead to high morbidity and mortality (Adesunkanmi et al, 2003). This study will determine the histological pattern of non-traumatic ileo-cecal perforations at the University Teaching Hospital, Lusaka, Zambia and evaluate the accuracy of the Xpert MTB/RIF assay to diagnose intestinal TB. Gastric and duodenal ulcers were excluded from the study as their etiology is widely known to be due to peptic ulcer disease and NSAID use (Anand BS et al, 2015). The knowledge gained will be used to inform on improvements to diagnostic algorithms, possibly incorporating rapid new TB diagnostics, enabling timely initiation of appropriate life-saving therapy. This chapter describes the statement of the problem, the study objectives, the research question and the study variables.

1.2. Statement of the Problem

The UTH surgical dept receives 40-50 cases of non-traumatic IPs annually. Diagnostic services to promptly identify the etiological causes are poor. These cases have a high mortality, little evidence suggests non communicable diseases and the main candidates are infectious causes namely Typhoid fever and TB. Patients generally receive broad spectrum antibiotics in the post operative period but blood and stool cultures are not routinely performed to establish a definitive diagnosis. Histology results of ulcer edge and mesenteric lymph nodes are not routinely followed up. Thus the etiologies of these perforations are generally unknown. Most terminal ileal perforations are largely assumed to be due to typhoid fever (Nthele. A, 2007) without histological evidence.

Hence the researcher conducted a review of the operative theatre register at the University Teaching Hospital (UTH), Lusaka, Zambia, which identified 43 cases of non-traumatic IPs operated on between sept 2011 and Aug 2012 with high mortality. Case presentation is mostly in the terminal ileum, which is typically indicative of infectious causes with tumours rarely seen. Despite the use of broad-spectrum antibiotics, mortality remains high. This disease adversely affects the quality of life of the patients. It also involves opportunity costs for seeking treatment at the hospital for the family as a whole.

Hence the need to conduct this study, as the histological pattern will inform on possible etiology thus ensuring specific care is accorded.

1.3. Study Justification

During my time working in the Dept of Surgery at the University Teaching Hospital as a surgical registrar, I have noted that diagnostic services to identify the etiology of IPs are not done promptly and so may lead to high mortality, hence the need to conduct this study that aims at determining the histological pattern of non-traumatic ileo-cecal perforations which informs on etiology and to evaluate the Xpert MTB/RIF assay for rapid diagnosis of intestinal TB at the University Teaching Hospital, Lusaka in Zambia.

According to the literature review Typhoid Fever, Tuberculosis, HIV and lymphoma's form some of the major etiological factors leading to IPs. Zambia has an adult prevalence of HIV of 14%, and 22% in Lusaka, TB prevalence of 433 per 100,000 upto 37% of newly diagnosed TB patients are HIV positive, a TB/HIV co-infection rate of 70% (CIDRZ Tuberculosis service program, 2013) and yearly typhoid outbreaks (Ministry of Health, 2013). HIV has an influence on IPs due to its association with bowel lymphoma as found by Mudasser 2012. The high mortality associated with IPs as high as 40% as found by Azer et al, 2011 warrants a search for etiological factors in order to design preventive programs and also to ensure disease appropriate postoperative therapy. This Study will provide baseline data for the histological pattern of ileo-cecal perforations seen at the University Teaching Hospital informing on etiology, and propose a diagnostic algorithm capable of enabling timely initiation of appropriate life-saving therapy.

1.4. Research Objectives

General Objective: To study the histological features of ileo-cecal perforations and their correlation with the XPERT MTB/RIF Assay for the rapid diagnosis of intestinal TB.

Specific Objectives:

1. To determine the histological features of ileo-caecal perforations
2. To identify the presence of tuberculous DNA in ileo-caecal perforations using DNA PCR via the Xpert MTB/RIF assay

1.5. Study Variables

Variable Description

1.6. Dependent Variable

1. Ileo-cecal perforations
2. Mesenteric lymph nodes
3. Specific histology

1.7. Independent Variables

1. Age
2. Sex
3. Residence
4. Water source
5. Toilet type
6. Education

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Introduction

This study dwelt on the existing knowledge of the histological patterns of IPs as well as on the diagnosis of the etiological factors. For the purpose of this study, the literature review focused on the overview of the histological patterns, causes and the diagnostic modalities of IPs as noted globally, regionally and locally here in Zambia.

2.2. Overview of Intestinal Perforations

Azer et al, (2011), reported mortality from IP to be as high as 40%. Known etiologies include both non-communicable and communicable diseases. Primary infectious causes include typhoid and tuberculosis, both highly prevalent pathogens in high HIV burden settings such as Sub-Saharan Africa where the study setting is located.

Studies to determine the etiology of IPs have been conducted in numerous countries and continents with varying profiles. Countries in the developed world tend to show a profile in which non-infectious causes predominate while those conducted in the developing world feature infectious causes predominantly Typhoid fever and Tuberculosis (TB).

Nationally, a review of the operative theatre register at the University Teaching Hospital (UTH), Lusaka, Zambia, identified 43 cases of non-traumatic IPs operated on between sept 2011 and Aug 2012 with high mortality. Case presentation is mostly in the terminal ileum, which is typically indicative of infectious causes with tumours rarely seen. Despite the use of broad-spectrum antibiotics, mortality remains high, and so we hypothesize that untreated disseminated TB could be a major cause of death.

2.3. Causes and Diagnosis of Intestinal Perforations

The causes of non-traumatic IPs are quite diverse, Azer et al 2011 mentions NSAIDs, necrotizing vasculitis, bacterial infections, intestinal ischemia, inflammatory bowel disease (ulcerative colitis, Crohns disease), intra abdominal malignancy and even some benign tumours such as desmoid tumours to be some of the causes, bacterial infections

such as typhoid fever causing about 5% of IPs. Patients with IPs usually present with abdominal pain, abdominal examination reveals generalized tenderness suggesting peritonitis, plain supine and erect radiographs of the abdomen are the most common first steps in diagnosing patients with medical history and/or clinical signs of IP, findings suggestive of perforation include free air trapped in the sub-diaphragmatic locations (Azer et al, 2011).

Some studies investigating the profile of IPs on the Asian subcontinent produced some interesting results. Jain et al, (2010) studied 192 patients in New Delhi, India and found that 46.4% of perforations were due to typhoid, 39.2% were non specific inflammation, 12.8% were due to tuberculosis and 1.6% were due to malignant neoplasm. Mahajan et al, (2011) studied 164 cases also in New Delhi, India and used histopathological examination to determine the aetiology of IPs and found that 43,3% were due to infections, 29.9% - TB, 13.4% - typhoid fever along with 3 cases due to malignancy. In neighbouring Pakistan Afridi et al, (2008) studied 300 cases of perforation peritonitis in Pakistan and found that the most common causes of perforation peritonitis were peptic ulcer disease (45%), perforated duodenal ulcer (43.6%) and gastric ulcer 1.3% followed by small bowel tuberculosis (21%) and typhoid (17%), large bowel perforation due to tuberculosis 5%, malignancy 2.6% and volvulus 0.3%. Perforation due to acute appendicitis (5%).

A much smaller study in the United Arab Emirates by Eid et al, (2008) studied 4 cases and concluded that IPs can be due to typhoid, HIV, tuberculosis, and possibly hook worms.

A study in the United States on the causes of pneumo-peritoneum in the 21st century by Kumar et al (2012) found the following; perforated viscus (41%) and postoperative (<8 days) residual air (37%). Regarding the cases of visceral perforation, only 45% had free air on imaging studies, and for these the predominant cause was peptic ulcer (16%), diverticulitis (16%), trauma (14%), malignancy (14%), bowel ischemia (10%), appendicitis (6%), and endoscopy (4%). Typhoid fever and Tuberculosis are notably absent.

On the African continent Adesunkanmi et al, (2003) studied the causes and outcomes of IPs in a semi-urban African community, the study recruited 128 patients over a six year period from 1995-2000. They found that typhoid perforation occurred in 75 (58%) patients, acute appendicitis in 21 (16.3%), peptic ulcer disease in 18 (14%), trauma in eight (6.3%), five were idiopathic and one malignant sigmoid carcinoid tumour. Also in Nigeria Iwatt et al, (1988) studied 50 patients over 44 months with free small bowel perforation and found that 46% (23) had typhoid, 22% (11) had trauma, 18% (9) had strangulated hernia, 12% (6) had duodenal ulceration and 2% (1) had non specific ileitis.

In August, 2008, Kasese district hospital in Uganda began to receive numerous cases of IPs with an undiagnosed febrile illness, the Uganda Ministry of Health suspected typhoid fever as the cause (Neil et al, 2011). The same Research team (Neil et al, 2011) investigated this outbreak of IPs, of 577 cases seen between December 2007 and July 2009, 289 were hospitalized, 249 had IPs, 47 deaths occurred, *Salmonella typhi* was isolated from 27(33%) of 81 patients, surgical specimens from 30 patients were consistent with typhoid fever.

For the developing world it can be seen that from the literature another major cause of IPs is tuberculosis. Gastrointestinal tuberculosis may result in intestinal obstruction and perforation, even after anti-tuberculosis therapy has been initiated (Lee et al, 2012). More studies have investigated the relationship between tuberculosis and IP, such as the following: Lueng et al, (2006) in Hong Kong reported a case of tuberculous IP in a 63 year old HIV negative man 3.5 months after starting anti tuberculosis therapy, highlighting the importance of prompt diagnosis and surgical management to reduce morbidity and mortality. Dogo et al, (2008), in Nigeria highlighted the underestimation of tuberculous ileal perforation as a complication of intestinal tuberculosis in HIV in a case study of one patient. Abro et al, (2010) studied 60 patients in Pakistan with histopathologically confirmed intestinal TB and discovered that IPs occurred in 10% of patients enrolled in the study he further recommended that resection and anastomosis, stricturoplasty and right hemi-colectomy be the main surgical options. In industrialized Italy, Coccolini et al, (2011) studied 119 cases of abdominal TB with IPs retrospectively analyzing the best treatment options, they concluded that resection and primary anastomosis of the affected portion offered the best results in tubercular perforations.

Elamurugan et al, (2012) in India described a case of primary appendicular tuberculosis in a patient who presented with cecal perforation citing this rare occurrence.

IPs have also been associated with HIV infection as highlighted by the following studies: Guex et al, (2002) described an unusual presentation of IP in an HIV and TB co infected patient as a result of the immune reconstitution syndrome, in this case the IRIS phenomenon occurred after a year on HAART, and 11 months of ATT, the patient presented with peritonitis resulting from two jejunal perforations and an inflamed ileo-cecal region of bowel. Michalopoulos et al, (2013), described a case of multiple small bowel perforations in a HIV infected young man secondary to cytomegalovirus ileitis, they attributed the bowel perforation as being due to muscle cell destruction as viral inclusions were present within smooth muscle fibres.

Intestinal lymphomas are also a possible cause of IPs as highlighted by the following: Yang et al, 2012 described a case of enteropathy-associated T cell lymphoma in a 39 year old African American man causing a deep necrotic ulcer of the jejunum, highlighting that diagnosis of the underlying cause is rarely made prior to pathological examination of the specimen. Tan et al, (2012) studied 7 patients with bowel perforation secondary to small bowel malignancy between 2004 and 2007 and reported that 4 cases were due to lymphoma, 1 leiomyosarcoma and 2 due to metastatic lung tumours. Tsai et al, (2013) described a case of large bowel perforation in a 55 year old male due to peripheral T-cell lymphoma a subtype of intestinal T-cell lymphoma.

Zambia is a country with high prevalence of TB (433 per 100,000), HIV (14%) , TB-HIV co-infection (70%) –(CIDRZ Tuberculosis service program, 2013) and also frequent typhoid fever outbreaks (The Ministry of Health, Lusaka 2013). But apart from this high TB burden the country also has a heavy burden of unsuspected Pulmonary TB in sputum producing adults with co-morbid non communicable diseases as high as 10% as evaluated by Bates et al, (2012). A review of 21 postmortem studies across Africa by the same author found that pulmonary and extra-pulmonary TB was detected in over 69% of HIV associated deaths indicating a very large burden of disseminated TB (Bates et al, 2013).

The most specific test for diagnosis of TB is culture of AFB. This is a slow process taking between 3-8 weeks (Herchline et al 2013). The diagnosis of extrapulmonary TB, and more specifically to this study intestinal TB is often delayed due to lack of specific symptoms (Tan et al, 2009). Further compounding the delays in diagnosis are frequent delays in their reporting experienced in Laboratories.

But a rapid method for the diagnosis of TB exists, Sarrami et al 2010, noted that DNA PCR detection of mycobacteria is faster than culture (48 hours instead of weeks) and is useful for specimens which are not appropriate for culture. Also Yonal et al, (2010) noted that its sensitivity and specificity may be up to 82.6% and 95%, respectively in the detection of *mycobateria tuberculosis*.

The WHO in December 2010 endorsed the use of DNA PCR for the rapid diagnosis of TB by rolling out the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), which is capable of detecting MTB and also Rifampicin resistant strains (World Health Organisation, 2013). This method will significantly reduce the time of diagnosis from weeks to days thus having a significant impact on the reduction of morbidity and mortality rates. Trials of the Xpert MTB/RIF assay at the UTH have shown it to be very sensitive and specific for rapid diagnosis of pulmonary TB in both adults and children (O'grady et al 2012, & Bates et al 2012)

In the diagnosis of IPs, biopsy of ulcer edges is useful in determining their etiology (mahajan et al 2011). Mahajan et al, (2011) described the histological appearance of IPs due to typhoid as having erythrophagocytosis in the region of the ulcer associated with histiocytic granulomas. Bruschi et al, (2012) also described the same features referring to them as 'typhoid nodules' macrophages that contain erythrocytes, bacteria, and degenerated lymphocytes. However Murinello et al 2008, in a case report of a single patient, endoscopically biopsied intestinal ulcers that showed features of non-specific inflammation i.e polymorphic inflammatory infiltrate with predominance of swollen histiocytes and polymorphonuclear leucocytes, this patient had a positive blood culture for *Salmonella typhi*. This leads one to question whether non-specific inflammation as a histological finding may in deed be due to Typhoid fever.

Mahajan et al, (2011) also described the histopathological appearance of IPs due to tuberculosis as having epithelioid cell granulomas & caseous necrosis in most cases.

Sharma et al (2009) found that biopsy of perforation edges was useful in a third of the cases they studied, but that diagnostic rate could be improved by taking additional biopsy material. Regarding the diagnosis of typhoid fever, Ukwenya et al (2012) found Polymerase Chain Reaction detecting salmonella DNA to be 100% specific and 100% sensitive and in areas where affordable is set to become the gold standard for the diagnosis of typhoid fever. The study will employ histological analysis of IPs to possibly inform on their etiology and the MTB/RIF assay using DNA-PCR to establish/confirm the presence of MTB DNA. The study will employ DNA-PCR to screen for MTB as this particular pathogen requires specific Anti Tuberculous treatment in the postoperative period, whereas Salmonella Typhi would be susceptible to the broad spectrum antibiotics patients are placed on routinely postoperatively.

After a review of the literature the causes of IP are diverse, this study would significantly influence management of these patients at the UTH and provide baseline data as to the histological pattern of IPs at the UTH which may inform on etiology and propose a diagnostic algorithm capable of enabling timely initiation of appropriate life-saving therapy.

CHAPTER THREE

3.0. RESEARCH METHODOLOGY

RESEARCH DESIGN

The study was a descriptive cross sectional study conducted at the Department of Surgery of the University Teaching Hospital, Lusaka. It ran for a period of 12 months from February 2014 to February 2015. All Adults and Children between the ages of 1 – 90 years with an intra operative finding of IP without a history of trauma were recruited using convenience sampling. A total number of 35 patients were recruited. Intraoperatively a biopsy of the perforation edges was harvested along with mesenteric lymph nodes if present and stored in 4% formaldehyde as is standard surgical practice.

The author administered a standardized questionnaire on the 2nd postoperative day and obtained consent to draw a blood sample for Blood Culture, and have the harvested intraoperative samples tested for Mycobacteria Tuberculosis using DNA-PCR and capture the results of the histopathological analysis of their specimens.

Tissue sections for histopathology were examined after initial staining with haematoxylin and eosin, Ziehl-Neelsen stains were applied where necessary. Tissue for the GeneXpert MTB/RIF assay was processed in a category 3 biosafety cabinet. 1 mL of distilled deionised RNase and DNase-free water was added and the sample was macerated, transferred to a 10 mL glass screw cap tube containing glass beads, and topped up with 2 mL of deionised water. After vortexing for 5 min, the tube was left to stand for 30 min. 1 mL of supernatant was transferred into a 50 mL Falcon tube containing 3 mL of GeneXpert reaction mix, vortexed, and then left to stand for 20 min. 2 mL of the clear reactant–sample mixture was added to the GeneXpert MTB/RIF cartridge and processed through the GeneXpert machine.

3.1.1 Inclusion and Exclusion Criteria

3.1.2 Inclusion Criteria

- I. Intraoperative finding of IP without a history of trauma
- II. Age between 1 – 90 years

3.1.3 Exclusion Criteria

- I. Refused Consent
- II. IP with a history of trauma
- III. Gastric and duodenal perforations

3.2 Sample Size

Sample size was calculated using the prevalence formula below taking a Confidence Interval of 95% . Azer, et al 2011 estimated that bacterial infections such as typhoid fever cause 5% of IPs, therefore P was set at 0,05.

$$N = \frac{Z^2 \times P(1-P)}{(E)^2}$$

N – sample size

Z – 1,96 – Z Statistic

P – 0,05 expected prevalence

E – 0,05 accepted accuracy

$$N = \frac{3,8416 \times 0,05(0,95)}{0,0025}$$

$$N = 73$$

However it must be noted that the University Teaching Hospital receives a yearly average of 40 cases of Intestinal Perforations. The study captured a cohort close to this number and was limited by the 1 year period for Data collection allocated to research by UNZABREC.

3.3. Data Management

The author used a data collecting tool to collect demographic and clinical data as well as record results of DNA PCR and histopathology. Participants personal details did not appear on the Data collection tool, identification numbers were used for data entry and collection. Data was entered in Microsoft Office Excel 2007 and analysed using STATA version 11.

3.4. Statistical Analysis

Data was exported from Microsoft Excel to Stata for statistical analysis. Frequency tables and graphs were used to describe socio-demographic and etiological factors.

3.5. Ethical Considerations

Ethical approval was sought from the University of Zambia Biomedical Research Ethics Committee. Permission was obtained from UTH Management and the Department of Surgery. All participant records were kept secure and electronic files were password protected.

CHAPTER FOUR

4.0: DATA ANALYSIS AND PRESENTATION OF FINDINGS

4.1. Introduction:

This chapter describes how the data collected data was analysed. The findings are presented. This study determined the histological pattern of non-traumatic Ileo-cecal perforations. It also evaluated the Xpert MTB/RIF ASSAY for rapid diagnosis of intestinal TB at the University Teaching Hospital in Lusaka.

The study enrolled 35 participants over the study period from March 2014 to March 2015. This fell short of the calculated sample size of 73 participants but accurately reflected the yearly number of cases the institution receives on average. Due to the 1 year time limit the study could not be extended to achieve the targeted sample size. This has been highlighted under the study limitations. The study results were based on all the study participants and were analysed using STATA version 11.

4.2: Data Presentation of Findings

The study findings have been presented in sections as follows: Section A is the socio-demographic data of the study participants, section B is the descriptive data of the participants' clinical characteristics, section C is the results of the participant's microbiological findings, Section D is the participant's histological findings and Section E is the results of DNA PCR.

4.2.1: Section A: Socio-demographic Characteristics

Figure 1: Age distribution of Intestinal Perforation patients

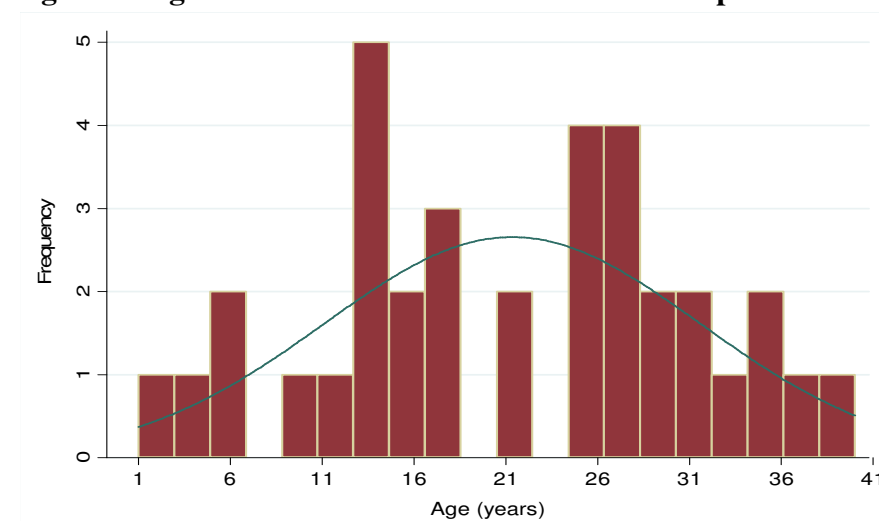


Figure 1 illustrates the age distribution of the study participants. Their ages ranged from 1 – 40 years, with a mean age of 21.3 years and a Mode age of 25 years.

Table 1: Intestinal Perforation Patients Demographic Characteristics contd.

Patient characteristics	No.	Proportion (%)
Sex		
Female	12	34.3
Male	23	65.7
Education level		
None	4	11.4
Primary	5	14.3
Secondary	26	74.3
Marital status		
Single	21	60.0
Married	14	40.0
Area of residence		
Rural	2	5.7
(High Density)Urban	33	94.3
HIV status		
Positive	1	2.8
Negative	31	88.6
Uncertain	3	8.6
Water source		
Borehole	5	14.3
Communal Tap	30	85.7
Toilet type		
Pit	26	74.3
Flush	9	25.7

Table 1 above summarizes the demographic characteristics of the participants below.

More than half of the study participants (66% n=23) were male while less than half (44%, n= 12) were female, thus giving a Male : Female ratio of 1.9 : 1

More than three-quarters (94.3%, n= 33) of the participants were urban dwellers residing in high density areas while 2 (5,7%) were from rural areas.

Regarding water source more than three –quarters of the study participants (85.7%, n=30) used Communal tap water while (14.3%, n=5) used Borehole water. Despite the majority of participants residing in urban areas only a quarter of the participants (25.7%, n=9) used flush toilets while almost three quarters of the participants (74.3%, n=26) used pit

latrines. Regarding education level, (11.4%, n=4) participants were younger than school going age, 14.3%, n= 5 had primary education and almost three quarters (74.3%, n=26) had secondary level of education.

4.2.2: Section B: Descriptive Data of Clinical Characteristics

Regarding HIV status (2.9%, n=1) participant was HIV positive and on HAART for a period of 3 months. More than half of the study participants (88.6%, n=31) were HIV negative and 3 (8.6%) were of unknown status. None of the participants had Tuberculosis or a history of previous Tuberculosis treatment. None of the participants had a histologically confirmed malignancy. None of the participants had a history of treatment for Typhoid fever.

The mean duration of illness was 4 days, the most common combination of gastrointestinal symptoms present were vomiting, abdominal pain and diarrhea present in 5 (14.3%) participants, along with vomiting and abdominal pain also present in 5 (14.3%) participants, all these patients also had peritonitis necessitating exploratory laparotomy. See Table 2.

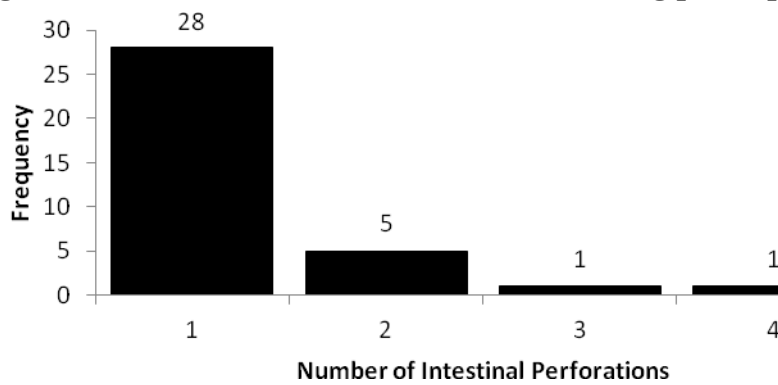
Table 2: Gastrointestinal Symptoms Present

Symptoms	Frequency	Percentage %
Vomiting	1	2.9
Abdominal pain	2	5.7
Diarrhoea - non blood stained	1	2.9
Nausea, Vomiting and Fever	1	2.9
Nausea, Vomiting, Fever and Abdominal pain	4	11.4
Vomiting and Abdominal pain	5	14.3
Diarrhoea and Abdominal pain	3	8.6
Abdominal pain, Vomiting and Diarrhoea	5	14.3
Vomiting, Diarrhoea and Fever	2	5.7
Vomiting, Fever and Constipation	1	2.9
Diarrhoea and Vomiting	2	5.7

Abdominal pain & Fever	3	8.6
Diarrhoea – blood stained and abdominal pain	1	2.9
Diarrhoea-blood stained & Vomiting	1	2.9
Abdominal pain, Fever and Diarrhoea	3	8.6
Total	35	100

The most common number of perforations encountered intraoperatively was 1 present in 28 participants (80%), the highest number of perforations encountered in a single participant was 5, this is illustrated in Figure 2.

Figure 2: Number of Intestinal Perforations among participants



The most common site of IP was the terminal ileum 30 (85.7%) followed by the ileum 3 (8.6%) and lastly the cecum 2 (5.7%) as illustrated in Table 3.

Table 3: Location of Intestinal Perforations among participants

Location of Intestinal Perforations	No.	Prevalence (%)
Ileum	3	8.6
Terminal Ileum	30	85.7
Cecum	2	5.7

15 (42.9%) of 35 participants had mesenteric lymphadenopathy present intraoperatively thus this group had both IP edges and mesenteric lymph nodes submitted for analysis, 20

(57.1%) participants did not have mesenteric lymphadenopathy and thus only IP edges were submitted for analysis.

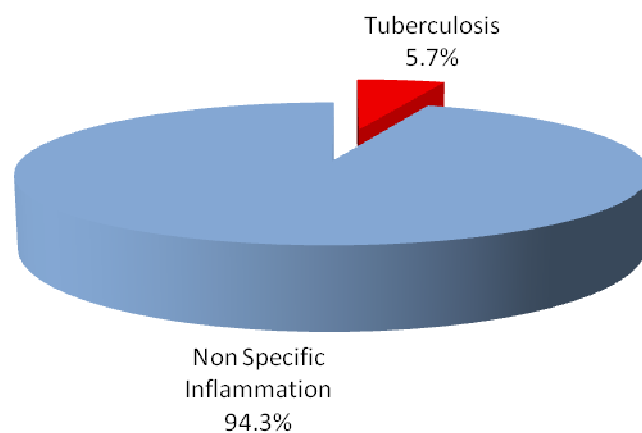
4.2.3: Section C: Results of Microbiological Findings

Blood cultures were collected on all 35 participants but results were only available from 33 participants with 2 samples untraceable in Laboratory records. Coagulase negative Staphylococcus was isolated on 2 (5.7%) of 33 samples both showing identical antibiotic sensitivity to Chloramphenicol and resistance to Oxacillin, Penicillin, Erythromycin and Ceftriaxone, with the remaining samples 31 (88.6%) showing no growth.

4.2.4: Section D: Results of Histological Findings

35 IP samples were submitted for histological analysis, 33 (94.3%) showed non-specific inflammation and 2 (5.7%) showed features of tuberculosis. In the 33 specimens microscopy showed mostly ulcerated small bowel with underlying granulation tissue with infiltration of neutrophils & lymphocytes, which are features of non-specific inflammation. Of the 15 mesenteric lymph nodes accompanying the IPs 2 (5.7%) showed granulomatous inflammation with caseating necrosis and characteristic Langhans type Giant cells consistent with Tuberculous lymphadenitis confirmed by ZN stains. The remaining 14 specimens showed follicular hyperplasia and sinus histiocytosis features of reactive lymph nodes. See Figure 3

Figure 3: Histological features of Intestinal Perforations



4.2.5: Section E: Results of DNA PCR

The Xpert MTB/RIF assay confirmed the histopathological diagnosis of MTB in the 2 specimens, also confirming negative results in the remaining 33 specimens. The assay tested positive only on the lymph node specimens and not their partnered intestinal perforations. See Table 4 and 5.

Table 4: 2x2 Contingency table for the evaluation of MTB DNA PCR against Histology

DNA PCR	Histology		Total
	Positive	Negative	
Positive	2	0	2
Negative	0	33	33
Total	2	33	35

Table 5: MTB DNA PCR Test parameters when evaluated against Histology

Parameter	Estimate (%)	95% CI
Sensitivity	100	15.8– 100
Specificity	100	89.4 – 100
Positive predictive value	100	15.8– 100
Negative predictive value	100	89.4 – 100

CHAPTER FIVE

5.0 DISCUSSION OF FINDINGS

5.1: Introduction

The purpose of the study was to determine the histological pattern of non-traumatic ileocecal perforations and their correlation with the Xpert MTB/RIF ASSAY for rapid diagnosis of intestinal TB at the University Teaching Hospital in Lusaka. The discussion has focused on the socio demographic characteristics of the study participants, the histology, microbiology and DNA PCR results.

5.2: Socio-demographic Characteristics

The study enrolled more males (66%, n=23) than females (44%, n=12), with a mean age of 21 years, a demographic similar to reports by Butler T et al, 1985. The mean duration of illness of 4 days was similar to findings by Adesunkami A, et al 2003.

The majority of participants (60%) were single, had secondary level of education (74.3%) resided in urban areas (33%) used communal tap water as a water source (85.7%) and the pit latrine was the most common type of toilet (74.3%). It can be theorized that a single individual, without running water in his/her home and without a flush toilet may be more likely to drink untreated water and have difficulties in practicing good hygiene, and thus be more likely to suffer from gastrointestinal infections.

The most common combination of gastrointestinal symptoms present were vomiting, abdominal pain and diarrhea present in 5 (14.3%) participants, along with vomiting and abdominal pain also present in 5 (14.3%) participants, all these patients also had peritonitis necessitating exploratory laparotomy.

5.3: Histology Results

Histological analysis of the 35 IPs showed granulation tissue with infiltration of neutrophils & lymphocytes, suggesting non – specific inflammation as a cause. Mahajan et al 2011, used findings of histological analysis of lymph nodes to supplement those of IPs when IP analysis yielded non-specific findings. This approach has also been adopted in this study. 2 (5.7%) of analysed lymph nodes showed features of tuberculosis, granulomatous inflammation, central caseous necrosis, epithelioid cells rimmed by mononuclear cells and Langhans type giant cells. Ziehl-Neelsen Stain was positive for

both specimens. Thus based on histological analysis the pattern of non-traumatic ileo-cecal perforation at the UTH is as follows Non-specific inflammation 94.3%, Tuberculosis 5.7%. Notably absent from this spectrum is Typhoid, which is largely assumed to be responsible for IPs at the UTH as mentioned by Nthele et al 2007.

The absence of Typhoid and malignancy from the IP spectrum is in contrast with findings by Mahajan et al, (2011), who found 43.3% non specific inflammation, 29.9% Tuberculosis, 13.4% Typhoid and 13.4% due to malignancy. Several studies quoted in Literature review such as those by Afridi et al (2008) and Jain et al, (2010) all found typhoid and TB to be represented in the spectra of causes of IP. A possible explanation for this finding could be the small sample size of the study due to its time limitations, the aforementioned studies in the literature review recruited larger amounts of patients and ran for much longer time periods. However, the presence of a different pathogen that causes gastrointestinal symptoms and IPs must also be sought. One such group of organisms is *campylobacter species* that produces gastrointestinal symptoms and cases of IP have been documented in literature (Jassim et al, 2011).

Another possible reason for the lack of typhoid in this spectrum may be the limited amount of tissue submitted for histological analysis, as these cases were largely assumed to be typhoid the surgical management has been liberal peritoneal lavage and simple closure of the perforation as described by Wani et al, (2006). Thus only ulcer edges are submitted for histology, limiting the amount of tissue available for examination, large segments of ileum are only submitted when multiple perforations are present necessitating ileal resection and anastomosis.

The study also found a much lower rate of TB (5.7%) as compared with other studies cited in the review of literature such as Mahajan et al, (2011) in India who found 29% of IPs were caused by Tuberculosis. But Adesunkanmi et al, (2003) and Iwatt et al (1988) in Nigeria did not find any Tuberculosis in their studies on IP despite Nigeria's TB prevalence (570 per 100,000 [World Health Organisation, 2014]) being higher than that of Zambia (433 per 100,000 (CIDRZ Tuberculosis Service Program 2013) . A possible reason for this could be that India's TB prevalence (2600 per 100,000 [World Health Organisation, 2014]) is six (6) times higher than that of Zambia.

5.4: Microbiology Results

Blood cultures did not yield any growth of *Salmonella Typhi* in this study. Despite the average duration of illness being 4 days. Blood cultures for *Salmonella Typhi* are usually positive in the 1st week of illness in 85%-90% of cases as noted by Bruschi et al 2012. Coagulase Negative Staphylococcus was cultured from 2 (5.7%) blood samples with sensitivity to Chloramphenicol. A possible explanation for this is contamination from skin during phlebotomy for blood culture. This organism is an avirulent skin commensal as stated by Tufariello et al 2014, but can also be responsible for sepsis in the presence of immune compromise and indwelling devices.

5.5: DNA PCR Results

The MTB/RIF assay produced test results promptly within 48hrs of the specimens being submitted and produced results consistent with histological findings. MTB DNA was present in 2 (5.7%) of the 35 participants.

Against Histology, the Sensitivity and Specificity of DNA PCR was estimated at 100%. This entails that among IP patients DNA PCR will correctly identify 100% of histologically confirmed TB infected patients and also correctly identify 100% of histologically negative patients. Therefore, the test will not produce any false positives or negatives. This data indicate that as a diagnostic tool, DNA PCR is a viable and rapid diagnostic tool for TB. See Tables 4 and 5. This was in keeping with the WHO policy statement in December 2010 regarding the roll out of the Xpert MTB/RIF assay (World Health Organisation 2013)

CHAPTER SIX

6.1 CONCLUSION

1. The study found that more males were affected by IPs, their mean age was 21 years, they had secondary level education, were single, resided in urban areas, were HIV negative, and used tap water as a water source but Pit latrines as toilets.
2. The mean duration of illness was 4 days, with abdominal pain, vomiting and diarrhea being the most prevalent symptoms. The most common site of IP was the terminal ileum with a single perforation predominating the perforation spectrum.
3. The exact role of Typhoid as an etiological factor in the study is difficult to determine, as histological features of typhoid nodules (i.e Tissue infiltrated with macrophages that contain bacteria, erythrocytes or degenerated lymphocytes, Bruschi et al 2012) were not identified and *S.Typhi* was not obtained in Blood cultures. However Histological findings revealed non-specific inflammation that stood at 94.3% and TB 5.7% as etiological factors. Some schools of thought do advocate that non-specific inflammation is equal to typhoid.
4. The Xpert MTB/RIF assay proved to be 100% specific and Sensitive in diagnosing TB, providing quick and reliable results.

6.2 STUDY STRENGTHS

1. The study objectives were met.
2. The study employed a modern technique for the rapid diagnosis of intestinal TB - the MTB/RIF ASSAY.
3. Study participants benefitted from timely initiation of life saving therapy.
4. The study has provided new insight into the possible etiology of IPs at the UTH.

6.3 STUDY LIMITATIONS

1. The study was hampered by time limitations preventing it from reaching the calculated sample size, and thus its findings cannot be generalized to the population.
2. The biological specimens were immediately placed in 4% Formaldehyde for preservation thus rendering culture impossible.
3. DNA PCR to detect *Salmonella Typhi* was not possible due to non delivery of PCR primers necessary from suppliers.
4. The study was descriptive and cross sectional and thus did not have a control group, preventing inferences about cause being made.

6.3 RECOMMENDATIONS

1. A further study to be done, of longer duration with a control group without IP to enable inferences on cause to be made.
2. The Xpert MTB/RIF assay be included in the diagnostic algorithm of patients presenting with intestinal perforation enabling timely initiation of life saving Anti-Tuberculosis Therapy.
3. Clinicians to be made aware of other pathogens that may cause IP such as *campylobacter* through dissemination of study findings.
4. DNA PCR studies specific for *Salmonella Typhi*, *Campylobacter* and *Cytomegalovirus* be done on IP samples.
5. Blood and stool cultures to be done in all patients routinely.

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APPENDIX 2: PARTICIPANT INFORMATION SHEET

Introduction

My name is Dr. Shula Chanda, a Registrar in the Department of Surgery at the University Teaching Hospital. You had an operation to treat the cause of your abdominal pain. During the operation you were found to have a perforation(s) on your intestines. I am conducting a study that will determine the cause of these intestinal perforations, using an accurate scientific test. The test will be done on the perforation sample that has been removed from your intestines and the results will help ensure you get the correct treatment for your condition.

Procedure

I am requesting you to participate in the study by

- I. Giving approximately 10mls of blood.
- II. Giving blood on voluntary basis for an HIV test if your HIV status is not known. You will be counseled by a trained counselor prior to and after HIV testing. If it is known, just the record will be taken note of.
- III. Allowing one of your biopsy samples (half of the Intestinal perforation sample) to be submitted for a DNA PCR test.

Foreseeable Risk

During the collection of blood, you may experience some discomfort or bruising at the site of collection. To minimize this, trained personnel will collect blood using the smallest needle, which is sterile, not used before, and free of germs and aseptic technique will be employed.

Benefits

You will benefit directly in that we will be sure of the cause of your intestinal perforation. We will inform both you and your attending surgeon of the results. This will mean that the treatment you will get will be specific for your condition. Secondly, you will make a

major contribution to the information known about the causes of Intestinal Perforations in UTH. The study will neither delay your treatment nor prolong your stay in the hospital.

Confidentiality

The researcher will keep the records and results of your blood, as well as the result of the biopsy locked in a cabinet and the keys will be kept by the researcher. The results will not be disclosed to other people, neither will other people be told of your participation in the study.

Voluntariness

If you feel that you have been injured or inconvenienced as a direct consequence of participation in the study, you are at liberty to withdraw from the study at any time without any penalty or loss of benefits.

Contact Details

In the case where you have any questions or seek clarification, please contact me Dr. Shula Chanda on 0978661987, Department of Surgery, University Teaching Hospital, P/B RW1X, Lusaka.

You may also contact the Chairperson of the University of Zambia Biomedical and Research Ethics Committee. Ridgeway Campus. P.O Box 50110, Lusaka, Zambia, telephone 0211-256067 if you would like to know your rights as a research participant.

APPENDIX 3: CONSENT FORM

Your signing this form means that you understand the information presented and that you want to participate in the study. You understand that participation is voluntary and you may withdraw from the study at any time. If you agree to participate in the study, kindly sign the consent form that follows.

I.....

On this day ofmonth of of the year..... do understand the nature of this study and the risks of participating in this study have been explained to me. I have read the foregoing information, or it has been read to me. I have had an opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this research and agree to the terms of the study as laid out by the researcher.

Signature or Thumb print of participant

Name of the participant

Date..... (Day / month / year)

Statement by a Witness

I have witnessed the accurate reading of the consent form to the participant, and the individual has had an opportunity to ask questions. I confirm that the participant has given consent freely

Name of witness :

Signature of witness:

Date : (Day / month / year)

Statement by the Researcher

I have accurately read out the information to the participant and to the best of my ability made sure that the participant understands that the following will be done:

1. Collection of sample for Blood culture
2. Collection of blood sample for HIV test

I confirm that the participant was given an opportunity to ask questions and all the questions have been answered correctly and to the best of my ability. I confirm that the participant was not coerced into giving consent and consent has been given freely and voluntarily.

Name of researcher:

Signature of Researcher:.....

Date:..... (Date / Month /Year)

APPENDIX 4: ASSENT FORM

Hello, I am a doctor here conducting a research study. A research study is something we do to try and find out why children get ill. This study is about finding what made your tummy hurt. We want to find out what made your tummy start paining, and what caused a hole on your bowels. The things we learn from this study will help the doctors know what medicine to use for each patient, to treat them better, so that they get well soon and can go home quicker. We now need to ask you if it's O.K to use a special test on a small piece of the hole in your bowels, and after you are better take a little of your blood. You can agree to participate in this study by signing on the form below, but only if you want to.

BENEFITS TO YOU:

The study is helping provide supplies for the test to be done, and for those children in whom we find holes in their bowels, we will make sure the doctor puts you on the right medicine to make you better.

BENEFITS TO THE COMMUNITY:

The study is helping all children at UTH who might have holes in their bowels. The results of the research study will help us treat all such cases better in future

DISADVANTAGES TO PARTICIPANTS:

You and your mum/guardian have to sit and answer a few questions a day or two after your operation when you are feeling better and we will need to take a sample of your blood. It should not take more than 10 minutes

For further information about the study, you are free to contact the following on their mobile numbers:

Principal Investigator- Dr Shula Chanda Mobile: 0978661987, UNZA SOM Ethics Committee Chairperson 260-1-256067

I have read, and understand the study and agree to take part.

	Signature	Date	Thumb Print
Child Participant	_____	__/__/__	
Witness	_____	__/__/__	

Children under 7 years of age

Your signing this form means that you understand the information presented and that you want your child to participate in the study. You understand that participation is voluntary and you may withdraw your child from the study at any time. If you agree to participate in the study, kindly sign the consent form that follows.

I.....

On this day ofmonth of of the year..... do understand the nature of this study and the risks of my child participating in this study have been explained to me. I have read the foregoing information, or it has been read to me. I have had an opportunity to ask questions about it and any questions that I have asked have been answered to my **satisfaction**. I consent voluntarily for my child to be a participant in this research and agree to the terms of the study as laid out by the researcher.

Signature or Thumb print of Guardian

Relationship of Guardian to participant.....

Name of the participant

Date..... (Day / month / year)

Statement by a Witness

I have witnessed the accurate reading of the consent form to the participant, and the individual has had an opportunity to ask questions. I confirm that the participant has given consent freely

Name of witness :

Signature of witness:

Date : (Day / month / year)

Statement by the Researcher

I have accurately read out the information to the guardian of the participant and to the best of my ability made sure that he/she understands that the following will be done:

1. Collection of sample for Blood culture
2. Collection of blood sample for HIV test

I confirm that the guardian was given an opportunity to ask questions and all the questions have been answered correctly and to the best of my ability. I confirm that the guardian of the participant was not coerced into giving assent and assent has been given freely and voluntarily.

Name of researcher:

Signature of Researcher:.....

Date:..... (Date / Month /Year)

APPENDIX 5: METHOD FOR COLLECTION OF BLOOD CULTURE

Blood will be collected from two sites distal to any venous lines present on a limb but preferably from a limb without a line.

16-20mls of blood will be collected from site #1, with 8-10mls inoculated into an aerobic blood culture bottle and another 8-10mls into an anaerobic blood culture bottle.

10mls of blood will be collected from site #2 and inoculated into an aerobic blood culture bottle.

Site Preparation

Vigorously cleanse the skin over the venipuncture site in a circle approximately 5cm in diameter with methylated spirit. Scrubbing should continue for 30 seconds.

Starting in the centre of the circle, apply 10% povidone iodine in ever widening circles until the entire circle is saturated with iodine.

Leave the iodine on the venipuncture site to act for 60 seconds

Do not touch the venipuncture site after preparation and prior to phlebotomy

Mark a level 10mls above the fluid level on the blood culture bottle

Remove the cap and disinfect the septum with a methylated spirit swab and allow it to dry

Venipuncture

Perform venipuncture with needle and syringe and draw 10mls of blood

Innoculate the blood into the blood culture vial, do not change needles before the inoculation

After collection mix the blood culture vial gently by inversion

Label the blood culture vial and fill out the laboratory request form.

APPENDIX 6: DATA CAPTURE SHEET

Title: A STUDY TO DETERMINE THE ETIOLOGY OF NON-TRAUMATIC INTESTINAL PERFORATIONS AND TO EVALUATE THE XPERT MTB/RIF ASSAY FOR RAPID DIAGNOSIS OF GASTROINTESTINAL TB AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA.

Researcher: Dr Shula Chanda.

1) DEMOGRAPHIC DATA

i. Participant Number.....

ii. Age.....

iii. Sex : Male Female

iv. Marital Status
a. Single

b. Married

c. Divorced

d. Widowed

v. Residence
a. Urban

b. Rural

vi. Water Source
a. Tap Water

b. Well

vii. Toilet type
a. Flush toilet

b. Pit Latrine

viii. Education

a. None

b. Primary

c. Secondary

d. Tertiary

2) CLINICAL

i. HIV status

1. Positive

2. Negative

3. If positive, is patient on HAART

a) Yes

b) No

4. Duration of HAART

a. 0-3months

b. 4-6months

c. 6-12months

d. 1-3years

e. 4-5years

f. >6years

ii. Is Patient suffering from TB?

a) Yes

Site a. Lung

b. Abdomen

c. CNS

d. Bone

e. Other

i. On ATT? Yes No
ii. If Yes, Duration a. Initiation phase

b. Continuation phase

b) No

iii. Is Patient suffering from a Histologically confirmed Malignancy?

a) Yes Diagnosis.....

i. On treatment? Yes No

ii. If Yes duration

A . 0-3months

a. 4-6months

b. 6-12months

2. No

iv. Has Patient ever been treated for Typhoid Fever?

a. Yes

i. Diagnosis was Clinical Serological

b. No

iv. Duration of Gastrointestinal symptoms

- a. 24 hrs
- b. 1-3days
- c. 3-5 days
- d. 5-7 days
- e. 1-2 weeks

v. Gastrointestinal Symptoms present

- a. Nausea
- b. Vomiting
- c. Abdominal pain
- d. Blood stained diarrhea
- e. Diarrhoea non blood stained

3) SURGICAL

i. Number of Intestinal Perforations present

a. Anatomical location

- A. Terminal ileum
- B. Cecum
- C. Ascending colon
- D. Transverse colon
- E. Descending colon
- F. Sigmoid colon

ii. Mesenteric Lymphadenopathy present? Yes No

iii. Samples submitted for histopathology

1. Intestinal Perforation edges

2. Mesenteric lymph node

3. Both

4) HISTOPATHOLOGY

i. HISTOLOGY RESULTS OF INTESTINAL PERFORATION

ii. Histological

features:.....

.....
.....
.....
.....

iii. Final Diagnosis.....

iv. HISTOLOGY RESULTS OF LYMPH NODE

v. Histological features.....

.....
.....
.....

vi. Final Diagnosis.....

.....
.....

5) BLOOD CULTURE RESULTS

I. Gram Stain

II. Bacteria.....

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.....
.....

III. Sensitivity.....

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