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Assessing Antibiotic-Resistance Patterns and Clinical outcomes among People-Living with Hiv/Aids with Features of Sepsis, in Northern Tanzania

Tsere, Bonphace

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ASSESSING ANTIBIOTIC-RESISTANCE PATTERNS AND CLINICAL OUTCOMES AMONG PEOPLE-LIVING WITH HIV/AIDS WITH FEATURES OF SEPSIS, IN NORTHERN TANZANIA

Donatus Bonphace Tsere

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Mortality in people living with human immunodeficiency virus (PLHIV) remains substantial in Sub-Saharan Africa. Despite sepsis being the major cause of mortality in PLHIV, its optimal management remains questionable due to poorly characterized etiological agents as well as effective antimicrobials. This study aimed to assess etiological agents in PLHIV with sepsis, antibiotic susceptibility and mortality determinants. A prospective cohort design was conducted at three referral hospitals in Kilimanjaro region from May-December 2021. Patients with sepsis were screened for Tuberculosis (TB) using urine lateral flow–lipoarabinomannan (LF-LAM) and sputum Xpert®-Mycobacterium tuberculosis (MTB)/rifampicin (RIF) assays. Microbiological diagnostic tests as well as general clinical laboratory tests were also done. Anti-TB and broad-spectrum antibiotics were initiated accordingly. Patients were followed-up for 28 days. Variables were compared using an independent Chi-Square/t-tests. 98 patients were enrolled with a mean age of 44 (SD 12.9) years old with 59 (60.2%) being females. The TB was detected in 36 (36.7%) patients, with LF-LAM detecting 12 cases missed by XpertMTB/RIF. Isolated pathogens considered also as the cause of sepsis in patients with TB and those without TB included Staphylococcus aureus (4(11.1%) vs 6(9.7%)), Streptococcus pneumonia (3(8.3%) vs 2(3.2%)), Cryptococcal Spp. (3(8.3%) vs 2(3.2%)), respectively. Abnormal CBC, CRP, INR was in 31 (31.6%), 49(50%) and 40 (40.8%), respectively. The *S. aureus* isolates demonstrated 90% resistance against cotrimoxazole and low rate (10%) against gentamicin. Mortality was (9.2%) and was associated with malnutrition (p=0.000), high MEWS scores (p=0.000), Karnofsky score<50% (p= 0.028) and higher INR values (p=0.025). Multiple pathogens contributed to sepsis in PLHIV that necessitate frequent use of antibiotics and leading to high antibiotic-resistance among bacterial isolates. Also, malnutrition and prolonged INR were considered a risk factor for mortality.

DECLARATION

I, Donatus Bonphace Tsere, do hereby declare to the Senate of the Nelson Mandela Institution of Science and Technology that this dissertation is my own original work and that it has not been, nor will it be presented to any other university for a similar or any other degree award, and is not previously or currently under copyright.

Donatus Bonphace Tsere Date

The above declaration is confirmed by:

Prof. Gabriel Mkilema Shirima Date

Prof. Stellah George Mpagama Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology the dissertation entitled; *"Assessing antibiotic-resistance patterns and clinical outcome among people-living with HIV/AIDS with features of sepsis, in Northern Tanzania".* in partial fulfillment of the requirements for the award of a Master's Degree in Life science of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

Prof. Gabriel Mkilema Shirima Date

Prof. Stellah George Mpagama Date

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DEDICATION

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LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial resistance
CBC	Complete Blood Count
CLSI	Clinical and Laboratory Standards Institute
COVID 19	Corona Virus Disease 2019
CRF	Case report form
CRP	C-Reactive protein
CrAg	Cryptococcal antigen test
DNA	Deoxyribonucleic acid
DST	Drug susceptibility test
HIV	Human immunodeficiency virus
HAART	Highly active antiretroviral therapy
INR	International normalized ratio
IQR	Interquartile range
KCMC	Kilimanjaro Christian Medical Centre
KIDH	Kibong'oto Infectious Diseases Hospital
LAM	Lipoarabinomannan Assay
MAID-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MEWS	Modified early warning signs score
MIC	Minimal inhibitory concentration
MoH	Ministry of health
MRDT	Malaria rapid diagnostic test
NM-AIST	Nelson Mandela Institution of Science and Technology
PCR	Polymerase chain reaction
PLHIV	People living with HIV/AIDS
TB	Tuberculosis
UNAIDS	United Nations Programme on HIV/AIDS
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

While human immunodeficiency virus (HIV) infection and mortality are significantly reduced globally, Eastern and Southern Africa region still suffer a disproportionate high burden of people living with HIV/AIDS (PLHIV) and HIV related mortality. In 2019, 20.7 million PLHIV were accounting for 54.4% of the global burden and 300 000 HIV related death which is 43.5% of global morality (Chihana *et al*., 2019; Gona *et al*., 2020; Kharsany & Karim, 2016; Pandey & Galvani, 2019; UNAIDS, 2020). The use of combined antiretroviral therapy (ART) and antimicrobial prophylaxis have significantly reduced opportunistic infections, however, PLHIV with advanced immune suppression (CD4+ T cells < 200 cells/µL) yet develop severe-disseminated infection including sepsis (Montaner *et al.*, 2014; Taiwo & Hassan, 2010). The global burden of sepsis is alarming, with estimated incidences of 31.5 million cases and potentially 5.3 million deaths annually. Studies have shown that PLHIV constitutes a high proportion of sepsis case and a high mortality rate as compared to those with HIV-negative status (Gingo & Morris, 2015; Moreira, 2015; Rudd *et al*., 2020; WHO, 2020). Etiological pathogens of sepsis include bacteria, fungi, parasites and viruses. As a consequence of altered immune mechanisms in advanced PLHIV all these microbes may contribute to causing sepsis in either in solitary or polymicrobial patterns (Justiz & Naik, 2019; Moore *et al*., 2019; Taramasso *et al*., 2016; Widjaja *et al*., 2018).

Etiological pathogens of sepsis are poorly characterized in sub-Saharan Africa. One contributing factor is a lack of diagnostic tests in most health facilities. Currently in Tanzania as a representative of many regions in sub-Saharan Africa, blood culture, which is a gold standard diagnostic test in sepsis, is only available in tertiary hospitals. In contrast, novel rapid diagnostic tests are more widely adopted for the detection of *Mycobacterium tuberculosis* (MTB) only (Elbireer *et al*., 2011; Jacobs *et al*., 2019; Opota *et al*., 2015). The use of Xpert® MTB/RIF Assay and Mycobacterium Determine™ TB Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) have significantly improved TB detection (Barr *et al*., 2020; Bedell *et al*., 2012; Jacob *et al*., 2013; Kerkhoff *et al.*, 2017; Mbelele *et al*., 2017; Meremo *et al*., 2012). The MTB has been recovered in up to 38% of PLHIV with sepsis. Despite a diagnosis of tuberculosis (TB) sepsis in PLHIV and initiation of appropriate therapy, mortality has remained substantial (Barr *et al*., 2020; Bedell *et al*., 2012; Byashalira *et al*., 2019; Gingo & Morris, 2015; Jacob *et al*., 2013; Meremo *et al*., 2012) While factors such as delay in treatment initiation can contribute to mortality, polymicrobial infections that may go undiagnosed can

also play a part (Dolin *et al*., 2019). However, the constrained ability to confirm the etiological agent has promoted widely adoption of clinical diagnosis of sepsis as well as empirical antimicrobial therapy. Clinical diagnosis involves the use of standardized universal scoring systems such as Modified early warning signs (MEWS), Universal assessment of vital signs (UVA), Sequential Organ Failure Assessment (SOFA) and Quick SOFA (Evans, 2018; McLymont & Glover, 2016; Moore *et al*., 2017; Pairattanakorn *et al*., 2021; Thodphetch *et al*., 2021). These scoring systems grade body temperature, respiratory rate, systolic blood pressure, oxygen saturation and level of consciousness not only to earmark the presence of sepsis but also to predict mortality. The rapid empirical antimicrobial administration is widely implemented as a lifesaving approach. However, in the absence of adequate characterization of sepsis etiological agents, an assortment of effective antimicrobial chemotherapy remains an open question in sub–Saharan Africa. This causes optimal clinical management of sepsis to remain unknown (Andrews, 2010; Lewis *et al*., 2019; Martin-Loeches *et al*., 2015; Noémie *et al.,* 2015; Schorr *et al*., 2014). The downfall of empirical antibiotic therapy is not only doubtful efficacy but also promotes irrational use of antibiotics which is among the major mainspring of antibiotic resistance.

Antibiotic-resistance is currently a growing global public health concern by turning common infections untreatable and increasing the cost of treatments, duration of hospitalization as well as unfavorable treatment outcomes (Habyarimana *et al*., 2021; Seboxa *et al.*, 2015). The actual global burden of antibiotic resistance is underestimated, whereas in resource-limited sub-Saharan Africa the situation is speculated to be worse due to weak/absence of antibiotic stewardship as well as limited drug susceptibility testing in clinical settings (Collaborators, 2022; Naylor *et al.*, 2018; Toy *et al*., 2019; Tula *et al.*, 2015). The PLHIV are among the high-risk population for developing and spreading antibiotic resistances due to their frequent and prolonged exposure to empirical antibiotics for both prophylaxis and treatment of mild/severe opportunistic infections (Ragavan & Arunagirinathan, 2018). Despite this high-risk, there are insufficient studies done in Tanzania to analyse the existing patterns and burden of antibiotic-resistance among PLHIV with sepsis.

Despite higher mortality rate of sepsis among people living with HIV/AIDS, little is known on factors that predicts or/and contribute to mortality due to scare studies. This situation is worse in sub–Saharan Africa and in Tanzania as well as well where clinical assessment and follow ups are not properly done in most of health care facilities. In studies done in south India and at University of Maryland-USA, shows that deaths among septic PLHIV were significantly contributed by higher scores of sepsis grade (APACHE II, MEWS and SIRS), presence of AIDS defining diseases and multiorgan failure including renal, liver and heart (Chowdhury &

Chakraborty, 2017; Sowah *et al*., 2022).

Hence this prospective cohort study deployed conventional microbiological tests (blood culture, serology, malaria rapid test) to determine etiological agents of sepsis among PLHIV in northern Tanzania and Agar disk diffusion method to assess antibiotic-susceptibility profile among isolated bacteria. Clinical outcome and factors associated with mortality were also assessed.

1.2 Statement of Problem

Sepsis accounted for high mortality rate among PLHIV(Gingo & Morris, 2015). Its etiological agents are poorly analyzed in resource limited sub–Saharan Africa, with the prevalence of potential pathogens remaining to be conceptualized (Lewis *et al.*, 2019). Constrained microbiological diagnostic capacity in most health facility in sub-Saharan Africa being among factors for this under characterization of etiological microbes (Hunsperger *et al*., 2019; Jacobs *et al*., 2019; Vincent, 2016). Inadequate evidence on the local etiological patterns of sepsis among PLHIV has led to the wide use of less effective empirical treatment and prophylaxis. This not only affects treatment outcomes but may also promote a catastrophic burden of antibiotic-resistances (Martin-Loeches *et al*., 2015; Morton *et al*., 2018).

Antibiotic-resistance is currently a public health threat and global agenda. Currently, little is known about the burden and existing antibiotic-resistance patterns among PLHIV with sepsis in Tanzania and other Sub-Saharan African countries (Collaborators, 2022; Jemal *et al*., 2020). This is due to inadequate/lack of antibiotic stewardship as well as routine antibiotic-susceptibility Testing. The underestimated burden of antibiotic resistance may provide a comforting situation while slowly turning commonly treatable diseases untreatable. Hence increasing treatment costs, prolonged hospitalization, unfavorable outcomes and eventually heavily burdened health systems (Naylor *et al*., 2018; Seboxa *et al*., 2015; Tula *et al*., 2015).

Addressing these problems will be of practical benefits for people living with HIV as well as expanding understanding of this widely-spreading phenomenon. Therefore, this study aims to characterize etiological agents for sepsis and antibiotic susceptibility patterns of isolated bacteria as the appropriate approach to improve clinical outcomes among PLHIV.

1.3 Rationale of the Study

The purpose of this study is to fill the knowledge and policy gap, on the spectrum of etiological pathogens of sepsis and patterns of bacterial antibiotic resistance among PLHIV. Findings from the study will inform on wide-scope of a spectrum of etiologies of sepsis among PLHIV and

the existing burden of antibiotic resistance, which will also serve as progress status for national strategic plans on supporting the National Antimicrobial resistance strategy launched in 2017.

The findings gained through this study will guide health care providers in an assortment of effective antibiotics to improve clinical management of sepsis in PLHIV as well as outcomes, also will be shared with the Ministry of Health (MoH) and other stakeholders, to strengthen stewardship programs for antibiotic resistance.

1.4 Research Objectives

1.4.1 Broad Objective

To determine the etiological agents and phenotypic antibiotic-resistance patterns and clinical outcome among people living with HIV/AIDS in Northern Tanzania for better management of sepsis.

1.4.2 Specific Objectives

(i) To determine etiological pathogens among PLHIV with features of sepsis.

(ii) To determine and characterize antibiotic-resistance patterns of isolated bacteria from people living with HIV/AIDS.

(iii) To assess factors associated with clinical outcomes among PLHIV with sepsis.

1.5 Research Questions

(i) What are the etiological pathogens of sepsis among People living with HIV/AIDS?

(ii) What are antibiotic-resistance patterns among isolated bacteria from People living with HIV/AIDS?

(iii) What are the factors associated with clinical outcomes among PLHIV with sepsis?

1.6 Significance of the Study

This will help to improve the current management of sepsis in people living with HIV/AIDS and related guidelines and policy implementation in improving wellbeing of PLHIV and population health in general. The findings from this study will contribute to informing the finetuning of the current sepsis management approach towards improving the health services in the country.

1.7 Delineation of the Study

This study was conducted in Kilimanjaro region in Tanzania. The study used quantitative methods. The quantitative data include age of patients, number of patients in each gender, nutritional category, karnofsky score, MEWSS, laboratory results and outcomes. The descriptive analysis was conducted and compered between patients with Tuberculosis (TB) and those who had no TB. A couple of limitations of this study are worthy highlighting. Blood culture for *Mycobacterium tuberculosis* was not done in which it may have increased recovery of TB among septic PLHIV. Molecular Antibiotic susceptibility test was not which could have further described gene involved in producing identified antibiotic resistances. Also, microbiological tests were not repeated during follow up hence no evidence for microbiological response. However, the study was representative of various health facility levels in Tanzania, hence providing a holistic picture of the management of sepsis in PLHIV.

CHAPTER TWO

LITERATURE REVIEW

2.1 Human Immunodeficiency Virus infection (HIV) and Advanced Acquired Immunodeficiency Syndrome (AIDS)

There are 1.7 million new cases of HIV infection annually. Globally in 2019, there were 38 million PLHIV. East and southern Africa regions had highest proportion of 20.7 million whereas 1.6 million cases being in Tanzania (Frank *et al*., 2019; Kharsany & Karim, 2016; Pandey & Galvani, 2019; UNAIDS, 2020). Human Immunodeficiency virus (HIV) attacks human immune system leading to the depletion of CD4+T cells, which the compromises body ability to clear invading pathogens (Huson *et al.*, 2015; Okoye, 2013). At the early stage most, patients are asymptomatic or have mild flu-like symptoms, while at the advanced stage (CD4+T cells counts< 200 cells/µL) patients present with WHO-defined danger signs including a body temperature <36°C or ≥39°C, breathing rate > 30 breath/min, heart rate >120 beat/min, inability to walk unaided as features of severe forms of opportunistic diseases such as Tuberculosis, cryptococcal meningitis as well as end organ damages such as renal failure, central nervous system disorders or/and respiratory insufficiency. In advanced immune suppression, pathogen invade the blood stream and trigger body response (sepsis) which ultimately worsens clinical conditions predisposing the patient to high morbidity and mortality (Justiz & Naik, 2019; Naif, 2013). The use of ART has improved the general health of people living with HIV by preventing severe immune suppression (Montaner *et al.*, 2014; Taiwo & Hassan, 2010). However, in low- and middle-income countries, 35% of PLHIV present with advance immunodeficiency syndrome (Chihana *et al*., 2019; Lifson *et al*., 2019).

2.2 Sepsis and Clinical Assessment

Sepsis is a clinical syndrome defined as a bodily response to blood stream infections (Singer *et al*., 2016). It presents with features of inflammation as well as end-organ damage, which includes fever, tachycardia/bradycardia, hypotension, tachypneic, leukocytosis/leucopenia (Morton *et al.*, 2018). Globally, it is estimated that in 2017 there were 48.9 million sepsis cases and 11 million sepsis-related deaths worldwide, which accounted for almost 20% of all-cause deaths globally (Fleischmann *et al.*, 2016; Frank *et al*., 2019; WHO, 2020). Sepsis is more prevalent among PLHIV (65%) (Gingo & Morris, 2015; Moreira, 2015; Taramasso *et al*., 2016). Etiology of sepsis includes bacterial (*Mycobacteria tuberculosis* complex, *Salmonella enterica, Neisseria meningitidis, Escherichia coli, Streptococcus pneumoniae, Staphylococcus aureus, Leptospira spp., Rickettsia spp., Salmonella enterica serovar Typhi, Pseudomonas*

aeruginosa, Bartonella spp., Klebsiella pneumoniae, Acinetobacter baumannii, Brucella spp., Group B Streptococcus, Hemophilus influenza, Listeria monocytogenes, Coxiella burnetii,.), parasitic (*Plasmodium spp. and Toxoplasma gondii*), fungi (*Histoplasma capsulatum, Pneumocystis jirovecii and Cryptococcus neoformans*) or viral (*Cytomegalovirus, Chikungunya virus, Dengue virus and Yellow fever virus*) (Dolin *et al.*, 2019; Justiz & Naik, 2019; Lewis *et al.*, 2019; Moore *et al.*, 2019).

People living with HIV/AIDS are prone to a wider spectrum of etiology in either solitary or polymicrobial. Diagnosis of sepsis includes clinical and microbiological approaches. Microbiological diagnostic tests include conventional blood culture, Serum Cryptococcal antigen test (CrAg), Malaria Rapid Diagnostic Test (MRDT), TB urine LAM, sputum Xpert® MTB/RIF and polymerase chain reaction (PCR). In the clinical approach, various quick assessment scoring system has been developed. They include Sequential Organ Failure Assessment (SOFA), Quick SOFA (qSOFA), Modified early warning signs (MEWS) and Universal assessment of vital signs (UVA). These scoring systems grades body temperature, respiratory rate, systolic blood pressure, oxygen saturation and level of consciousness not only to identify presence of sepsis but also predicting mortality. Additional laboratory tests such as C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), White blood cells (WBC) as well as coagulation profile, appends on sepsis severity grading (Evans, 2018; Singer *et al.*, 2016; Vincent, 2016). Identification of etiological agents is key for proper management of sepsis; this is achieved through microbiological tests.

2.3 Microbiological Diagnosis Tests

2.3.1 Blood Culture

Blood culture remains to be the gold standard test for recovering invading pathogens from the blood stream. The procedure involves aseptic collection of and adequate volume of blood sample (10 mLs) into both anaerobic and aerobic blood culture bottles, then incubated into either a fully automated system or using a conventional incubator for 5 to 7 days (Opota *et al.*, 2015). For a fully automated system, a positive culture bottle will be detected as color change at the bottom due to a change in pH as a result of carbon dioxide generation. Positive culture bottles are subjected to identification procedures including sub-culturing in solid media, gram staining, conventional biochemical tests, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and/or nucleic-acid based methods such as PCR and gene sequencing (Perry *et al.*, 2017) (Fig. 1).

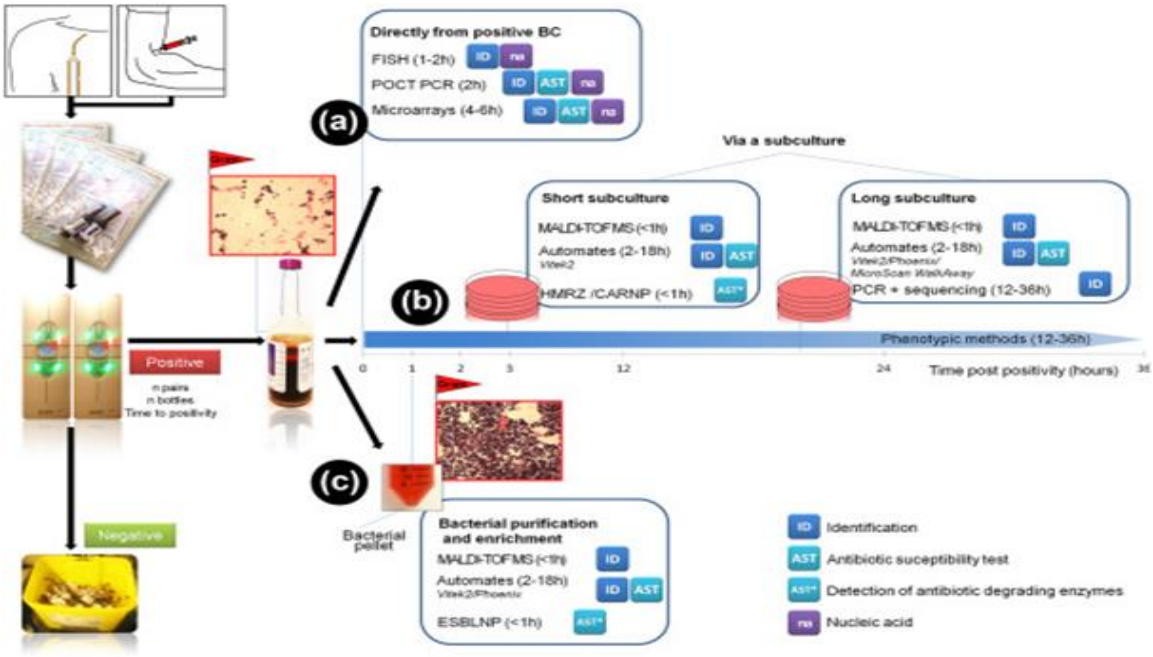

Figure 1: Procedure for blood culture and Methods to identify microorganisms from positive blood cultures (Opota *et al*., 2015)

2.3.2 Drug Susceptibility Test /Antibiotic Susceptibility Testing

Antibiotic susceptibility testing is the laboratory procedure to determine effectiveness of specific antibiotic against target bacteria. The procedure can be phenotypic (disk-agar diffusion method and Minimal inhibitory concentration (MIC)) and molecular technique (Polymerize chain reaction (PCR) and DNA sequencing) (Benkova *et al*., 2020; Khan *et al*., 2019; Vasala *et al*., 2020). Phenotypic antibiotic susceptibility testing, utilizes isolates from culture, where they are inoculated in a particular growth medium (e.g., Mueller Hinton Agar, MHA for disk diffusion) or an MIC panel, followed by addition of antibiotic disks (for disk diffusion), then incubation of plates (disk diffusion) or panels (MIC), measuring the zone of inhibition or reading MIC panel and lastly interpretation of AST results. Antibiotic susceptibility testing by employing molecular technique involves analyzing of DNA sequence for detection of mutations (resistance gene). An example includes Xpert® MTB/Rif assay which detects rifampicin resistance in *Mycobacterium tuberculosis*.

2.3.3 Cryptococcal Antigen Lateral Flow Assay

Cryptococcal Antigen Lateral Flow Assay is an immunochromatographic test system for the qualitative or semi-quantitative detection of the capsular polysaccharide antigens of Cryptococcus species complex (*Cryptococcus neoformans* and *Cryptococcus gattii*) in serum, plasma, whole blood and cerebral spinal fluid (CSF) (Nalintya *et al*., 2017; Rajasingham *et al*., 2019). The CrAg LFA utilizes highly sensitive and specific anti-cryptococcal mouse

monoclonal (GXM) as the primary antigen shed by the organism. To perform the procedure, specimens and specimen diluent are added into an appropriate test tube, and the lateral flow device is placed into a test tube. If CrAg is present in the specimen, then it binds to the anti-CrAg antibodies forming the antibody-antigen complex a sandwich at the test line resulting into a visible line and immobilized antibodies at the control line will bind to the control antibody and form a visible control line. Results are interpreted after 10 minutes where positive test results create two lines (test and control) and negative test results form only one line (control). If a control line fails to develop then the test is not valid (Fig. 2).

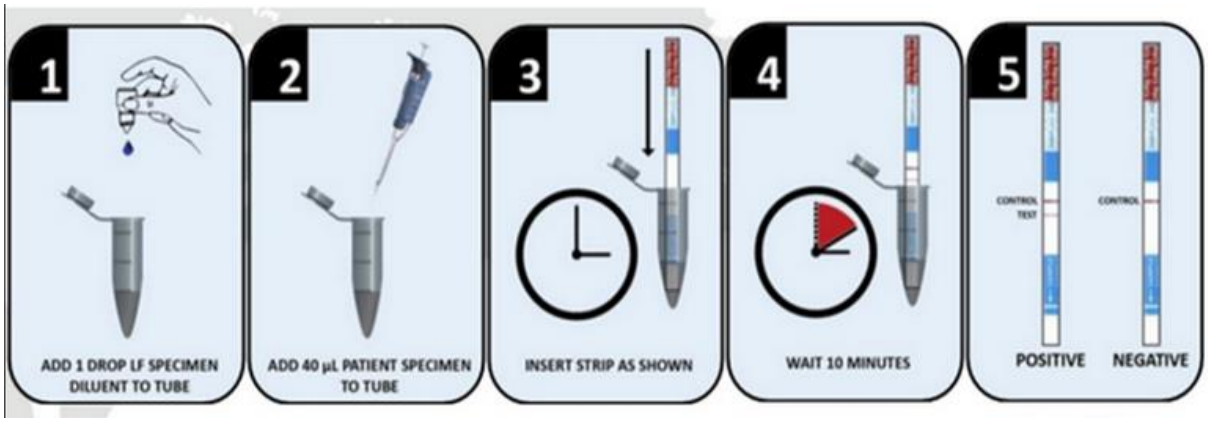

Figure 2: Five easy steps to perform the detection of cryptococcal antigen using lateral flow assay (Pelfrey & Bauman, 2012)

2.3.4 Mycobacterium Determine™ TB Lateral Flow Urine Lipoarabinomannan Assay

Mycobacterium Determine™ TB Lateral Flow Urine Lipoarabinomannan Assay (LF-LAM) is an immunochromatographic test for the qualitative detection of lipoarabinomannan (LAM) antigen of Mycobacteria in human urine where a lipopolysaccharide present in mycobacterial cell walls is released from metabolically active or degenerating bacterial cells. The LAM is predominately present in people with active TB disease. The test is performed manually by applying 60 µL of urine to the Determine™ TB LAM Ag test strip and incubating at room temperature for 25 minutes (Fig. 3). The strip is then visually inspected by eye for the visible band on the test strip at patient and control bars (Bulterys *et al*., 2020; Byashalira *et al*., 2019; WHO, 2019).

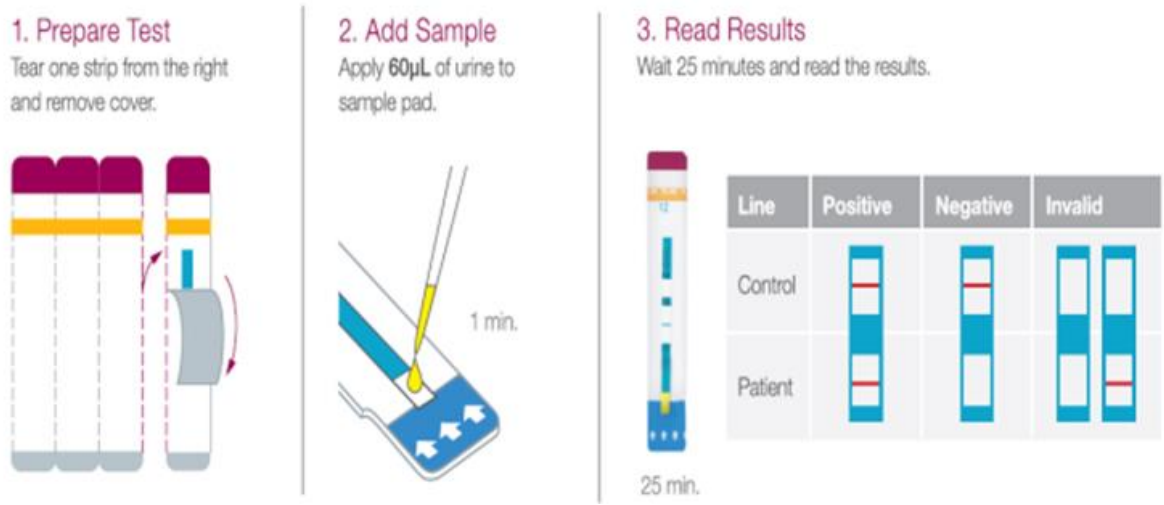

Figure 3: The TB LAM Ag testing procedure; (Determine TB-LAM Alere, Waltham, MA, USA)

2.3.5 Malaria Rapid Diagnostic Test

Malaria Rapid Diagnostic Test (RDTs) are immunochromatographic tests that identify specific antigens of malaria parasites in whole or peripheral blood (Cunningham *et al*., 2019; Mouatcho & Goldring, 2013). Currently targeted antigens are Histidine-rich protein II of Plasmodium falciparum (PfHRPII), plasmodium aldolase and parasite lactate dehydrogenase (pLDH). Malaria RDT kit consists of a test cassette, buffer, and blood collecting device. Sample for malaria RDT is obtained by gentle prick of 4th finger or other pre-collected whole blood by venipuncture. The required volume of collected blood is transferred by either pipette, inverted cup or capillary tube using to the specimen well on RDT cassette followed by the addition of recommended drops of buffer to the buffer well. The test is timed as per the manufactures recommendation before viewing the results window for visible bands. Positive results are indicated by the presence of both a control band and a test band whereas, negative results are indicated by presence of only a control band and Invalid results are when, the test does not show the control band, even if there is a test band.

Figure 4: The pictorial job aide shows the procedure for performing RDT for malaria (Kyabayinze *et al*., 2012)

2.4 Antibiotic/Antimicrobial Resistance

Antibiotic-resistance is the phenomenon in which microbes develop the ability to resist the effect of drugs which was previously effective. Mechanism of emergence and spread involves gene mutation or/and horizontal gene transfer which alters metabolic processes leading to either enzymatic modification/ degradation of antibiotic molecules, decreased penetration or enhanced efflux, protection/change of targets and other cell adaptive processes (Munita *et al*., 2016). Although antibiotic resistance is a natural phenomenon, human factors play a crucial role in its emergence and spread. This includes irrational use, misuse and improper disposal of antibiotics (Akbar *et al*., 2020; Ilić *et al.*, 2012; Munita *et al*., 2016; Tula *et al.*, 2015). In sub–Saharan Africa these are common practices since in most setting antibiotics are cheap and available over the counter even without a prescription and they are easily administered orally. Inappropriate antibiotic prescriptions includes for non-bacterial diseases, improper dosing and

extensive use in the livestock sector (Elton *et al.*, 2020; Olesen *et al*., 2018). People living with HIV/AIDS are among the high-risk populations for developing and spreading antibiotic resistance due to their frequent and prolonged exposure to empirical antibiotics as preventive therapy or treatment for frequent mild/severe opportunistic infections (Ragavan & Arunagirinathan, 2018). Antibiotic-resistance confirmations is provided through laboratory drug susceptibility testing (DST), phenotypically or using molecular assays (Cirillo *et al*., 2017; Skodvin *et al*., 2019). The actual global burden of antibiotic resistance is yet unclear, however in resource limited sub-Saharan Africa the situation is speculated to be worse due to inadequate/lack of legal framework and guidelines, weak/absence of antibiotic stewardship as well as limited drug susceptibility testing in clinical settings, and lack of research agendas on antibiotic resistance (Collaborators, 2022; Naylor *et al*., 2018; Toy *et al*., 2019; Tula *et al.*, 2015). Antibiotic-resistance turns commonly easily treatable disease incurable as well as increasing cost of treatments, duration of hospitalization as well as bad treatment outcomes such as morbidity and mortality (Habyarimana *et al*., 2021; Seboxa *et al*., 2015). A significant prevalence of antibiotic resistance among commonly isolated pathogens to both potential broad-spectrum and specific antibiotics has been demonstrated. These include the isolation of mono/multidrug resistant mycobacteria tuberculous complex, Streptococcal and enteric pathogens resistant to cephalosporins or/and sulfamethoxazole/Trimethoprim (Manyahi *et al*., 2020; Marwa *et al*., 2015; Moyo *et al*., 2010; Saldanha *et al*., 2019).

2.5 Current Situation of Sepsis in People Living with HIV/AIDS

In low-resource settings including sub-Saharan Africa, data on the burden, etiology and outcome of sepsis are limited but some studies have identified high burden and mortality. In systematic review done by Lewis *et al*. (2019) showed median of 70% (IQR 42.5-82.5%) in HIV patients among septic cases. Most studies to determine etiologies of sepsis among PLHIV in sub-Saharan African including northern Tanzania, through primary conventional diagnostic tests such as blood culture, serological test and microscopic examinations, have identified pathogens including: *Mycobacteria tuberculosis* (10-22%), *Staphylococcus aureus* (0.6-25%), *Streptococcus pneumoniae* (10-21.4%), *Escherichia coli* (6-8.7%), *Salmonella typhi* (23.2%), non-Salmonella typhi (1.2-42.6%), *Klebsiella pneumoniae* (0.6 -3%), Plasmodia spp. (6-7.1%) *and Cryptococcus neoformans* (10-15.2%) as commonly isolated etiologies (Chimese *et al*., 2012; Crump *et al*., 2011; Jacob *et al*., 2009).

Very scarce studies have utilized molecular approaches such as PCR. For example, Moore *et al*. (2019), identified a significant proportion of viral causes as well as fastidious bacteria and parasites. The author reported high virulence and mortality from Cytomegalovirus (41%),

Dengue virus (6%), Rickettsia spp. (1%), Acinetobacter spp. (1%) and Toxoplasma gondii (0.7%). This is evident that PCR improves capacity to diagnose most etiologies, although may not be feasible to use in every health facility as a primary diagnosis but can be utilized to study local broad spectrum of etiologies.

In sub-Saharan Africa there are limited data on antibiotic resistance among etiological pathogens of sepsis in PLHIV. Toy *et al.* (2019) in the study assessing multi-country distribution and characterizing, confirms the presence of extended-spectrum beta lactamase (ESBL) production among pathogens causing bloodstream infections in sub-Saharan Africa (Toy *et al*., 2019). Studies have isolated a significant proportion of *Mycobacterium tuberculosis*-resistant strains to mono/multi-anti TB (12.5%), Salmonella spp. resistant to chloramphenicol (84.5%) ampicillin (69.2%), cotrimoxazole (38.5%), gentamicin (23%) and ciprofloxacin (7.6%); *Escherichia coli* demonstrated multiple resistances to many commonly used antibiotics including ampicillin (75-82.5%), co-trimoxazole (75-84.4%), amoxicillin/clavulanate (43.6-62.5%), gentamicin (62.5%), and ciprofloxacin (25%) (Crump *et al*., 2011; Manyahi *et al*., 2020; Marwa *et al*., 2015; Mwambete & Eulambius, 2018).

Outcome for sepsis management in sub-Saharan Africa including Tanzania is heterogenous between studies with high 28-days pooled mortality rate of 54% among PLHIV (Lewis *et al*., 2019). Mortality rate of sepsis in PLHIV was 34.8% compared to 14.8% of HIV negative patients. In addition based on severity of sepsis, severe sepsis shows high mortality of 39% over normal sepsis 19% (Jacob *et al.*, 2009; Lewis *et al.*, 2019; Marwa *et al*., 2015; Morpeth *et al*., 2008). In comparing the mortality rate between the approach of antibiotic therapy, those patients treated with pathogen-specific antibiotics had low morality rate of 18% as compared to 50% of those treated with an empirical broad-spectrum antibiotics (Jacob *et al*., 2009), this finding provides hope of further reducing the mortality rate by not only treating sepsis with the specific antibiotic but also to which pathogen is susceptible.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Kilimanjaro region located in northern Tanzania, at three tertiary/ referral hospitals which were Kibong'oto Infectious disease Hospital (KIDH), Kilimanjaro Christian Medical Centre (KCMC) and Mawenzi Regional Referral Hospital (MRRH). This study was designed to find answers to questions raised by previous study by Byasharila *et al.* (2019), in Kilimanjaro region at same hospitals and to similar study population, where over 65% of patients who were septic had no Tuberculosis (TB), also despite of TB diagnosis and antituberculosis therapy, mortality rate was significant higher as to those whose no etiological pathogens was determined and treated with empirical antibiotic, 15% against 24% (Byashalira *et al*., 2019).

Kibong'oto Infectious Diseases Hospital (KIDH) located in northern Tanzania, was the national center for managing tuberculosis patients far back to colonialism and through independence of Tanganyika in 1961. In 2010, the scope of managing tuberculosis was expanded by the government of Tanzania and the name was changed from Kibong'oto National TB Hospital to KIDH, a move which further equipped the hospital with capacity to manage Tuberculosis (TB), TB/HIV, TB/Diabetic, Multi drug resistant TB, Extensive drug resistant TB, HIV and other infectious diseases. The KIDH has 340 beds ready for admitting and managing both infectious and non-infectious disease patients. All Laboratory procedures were done at KIDH laboratory, an accredited laboratory by the Southern African Development Community Accreditation Services (SADCAS).

Kilimanjaro Christian Medical Centre is one among the four Zonal Consultant hospitals in Tanzania. It was formally established in 1971 as a Zonal Referral Consultant hospital owned by the Evangelical Lutheran Church of Tanzania (ELCT) under the Good Samaritan Foundation (GSF). It is located in the foothills of the snowcapped, Mount Kilimanjaro, Tanzania. Kilimanjaro Christian Medical Centre is a referral hospital with a catchment area of over 15 million people in Northern Tanzania. The hospital is a huge complex facility with 500-800 inpatients in 630 official beds, 90 canvas, 40 baby Incubators, 1852 medical students and 1300 staff. This hospital was established in order to serve the northern, eastern and central zone of Tanzania. Its record in Medical Services, Research, and Education has significant influence in East Africa and beyond.

Mawenzi Regional Referral hospital is the government facility. It serves as referral center for all district hospitals and other peripheral health-care facilities in the region. It is one of the old regional referral hospitals in Tanzania as it was established as a dispensary to serve Germany military casualties before 1920 during German colonial days and later Africans. There after the hospital was expanded to accommodate several wards required in those days. In 1956 the hospital was upgraded to be the regional hospital and now regional referral hospital. The Hospital has bed capacity of 310 beds with OPD Capacity of 600-1000 per day.

3.2 Study Design and Recruitment

This was a prospective cohort study where patients were recruited at KIDH, KCMC and Mawenzi hospitals.

3.3 Study Population

Study population for this study were people living with HIV/AIDS (PLHIV) who were presenting with features of sepsis at study facilities.

3.3.1 Sample Size Determination and Sampling

The desired sample size was derived by the formula:

$$n_1 = \frac{\left[Z\alpha\sqrt{(1+1/r)P^*(1-P^*)} + Z_\beta\sqrt{\frac{p_1(1-p_1)}{r} + p_2(1-p_2)}\right]^2}{(p_2 - p_1)^2}$$

$$P^* = \frac{P2+rP1}{r+1}$$

$$n_1 = \frac{\left[1.96\sqrt{(1+1/2)0.2(1-0.2)} + 0.84\sqrt{\frac{0.15(1-0.15)}{1} + 0.24(1-0.24)}\right]^2}{(0.24 - 0.15)^2}$$

$n_1 = 56.078 \sim 56$ patients

$N = n_1 \times 2 = 112$ patients

Adjusting for 14.8% drop out
Sample size (N) = 129 patients

Whereby, n is the sample size, assumption of significance level of (α) 0.05%, power of (β) 80%, ratio of (r) 1:1 comparison subject per study subject, 15% mortality rate (P1) in group without TB and (P2) 24% in comparison group with TB, adjustment for drop-out/loss to follow

up of 14.8% (Byashalira *et al*., 2019; Charan & Biswas, 2013). Purposive sampling was used, where all patients who met inclusion criteria after screening were recruited into the study.

Study patients were followed for 28 days at an interval of 3, 5, 10 and 28 days of anti-TB treatment and broad-spectrum antibiotics. Patients with positive urine TB-LAM and or Xpert MTB/RIF test result received a standard fixed-dose anti-TB containing rifampicin (150 mg), isoniazid (75 mg), pyrazinamide (400 mg) and ethambutol (275 mg) [RHZE]. Those with negative urine TB-LAM and Xpert MTB/RIF test results received a broad-spectrum antibiotic composed of a third-generation cephalosporins (ceftriaxone) or oral ampicillin-cloxacillin as per Tanzania standard guideline. Antifungal and antimalarial drugs were prescribed to patients with positive Cryptococcal antigen test for cryptococcal meningitis and malaria, respectively. Antibiotics were modified after drugs susceptibility testing results showed resistance to the initially prescribed broad-spectrum antibiotics.

All patients were evaluated for either worsening or improvement of danger signs at day 3, 5, 10 and 28 days. Assessment for the outcome (survived or died) was performed at 28 days of treatment. Patients who recovered and were discharged from the hospital were contacted by telephone either directly or through their next of kin.

3.3.2 Inclusion Criteria

Inclusion criteria for this study were:

(i) Patients with age of 18 years or above

(ii) Willingness to participates by signing written informed consent

(iii) Having at least one of the following danger signs according to WHO guide:

(a) Body temperature ($<36.0°C \geq 39°C$)

(b) Tachycardia (>120 beats/min)

(c) Tachypnoea (>30 breaths/min)

(d) Unable to walk unaided

(e) CD4 cell count ≤ 100 cells/mm^3

(f) White blood cell count > 12000 /mm^3 or < 4000 /mm^3

3.3.3 Exclusion Criteria

Exclusion criteria for the study were:

(i) Severe Anemia (HB less than 5 gm/dl)

(ii) Bleeding tendency disorders e.g., Hemophilia

(iii) Obvious localized lesions/wounds

3.4 Clinical Data Collection

Clinical data including body temperature, respiratory rate, systolic blood pressure, heart rate and neurological examination were score according to the modified early warning signs score (MEWS) (Appendix 3). Ability of patient to carryout physical activities were assessed and scored by using karnofsky score (Appendix 2). Patients scoring MEWS above 6 and/or Karnofsky score < 50% were classified as severe sepsis.

3.5 Specimen Collection and Handling

3.5.1 Urine and Sputum Sample Collection

Study patients were instructed to collect 10 mLs of urine in the Polypropylene Random Copolymer urine container and labeled with unique patient identification number for TB LAM analyses. A Urine catheter was used to collect urine for patients who were unable to void spontaneously. These samples were collected on the day of enrollment and tested at the point of screening. Patients were instructed to collect at least 2 mLs of sputa into falcon tube and labeled with unique patient identification number. For patients with dry cough or not coughing special techniques such as early morning deep breathing and chest massage/percussion were done to stimulate sputum. Sputum samples for Xpert analyses were processed in the same day as in accordance with previous publication instructions (Byashalira *et al*., 2019; Mbelele *et al*., 2017; WHO, 2019).

3.5.2 Blood Sample Collection

Venipuncture skin site was disinfected with swab containing BD isopropyl 70% Alcohol (Ellis, 2018). Using 20 mLs syringes, 20 mLs of blood were drawn and distributed in equal volume into both anaerobic and aerobic BACT/ALERT® Culture Media bottles. In addition, 10 mLs of blood were drawn and distributed as follows: 2 mLs were dispensed into plain BD vacutainer tubes for CRP and CrAg analysis, 4 mLs into BD vacutainer tubes containing EDTA for CBC

and 4 mLs into BD vacutainer tubes containing citrate for coagulation profile. For patients with CD4 counts done more than 6 months, an additional 4 mLs of blood was drawn into BD vacutainer tubes containing EDTA for CD4 counts. Samples were labeled with unique patient identification number, and they were transported using safety laboratory cooler box to KIDH laboratory for analysis on the same day.

3.6 Laboratory Samples Analyses

Laboratory procedures for each test were done according to manufacturer recommendation and KIDH laboratory accredited standard operating procedure. For antibiotic susceptibility testing guidelines from the Clinical laboratory and standard institute (CLSI) were used.

3.6.1 Determination of Mycobacterium Tuberculosis by TB LAM Test

The Determine™ TB LAM assay (Alere Inc., Waltham, MA), was performed at point of screening as per manufacturer's instructions and the existing standard procedures at KIDH laboratory. In brief, 60 µL of the urine sample was applied to specimen site of Determine™ TB LAM Ag test strip and was incubated at room temperature for 25 minutes. The test strip was visually assessed for occurrence of visible patient and control bars. If two bar lines for both the patient and control were formed the results were interpreted as positive. A test was negative if a line appeared at control bar only and results were documented in Case report form (CRF).

3.6.2 Determination of Mycobacterium Tuberculosis by Xpert MTB/RIF Assay

Xpert MTB/RIF assay (Cepheid, USA) was performed as per manufacturer instructions and existing standard of procedure at KIDH laboratory (Byashalira *et al*., 2019; Mbelele *et al*., 2017). Briefly, 1 mL of sputum was mixed with 2 mLs of sample reagent (Sodium Hydroxide and Isopropanol) (1:2), and shaken to homogenize, followed by 15 minutes incubation at room temperature. Thereafter, 2 mLs of homogenized sputum was transferred into the Xpert MTB/RIF cartridge (Cepheid, USA) before it was loaded into the GeneXpert machine for automatic DNA extraction, amplification and detection of *M. tuberculosis* complex (MTBC).

3.6.3 Determination of C-Reactive Protein (CRP)

The C-reactive protein tests were performed using automated clinical chemistry analyzer XL 180 as per manufacturers recommendation (ERBA Mannheim). In brief, blood sample in redtop tube were centrifuged at 3000 rpm for 5 minutes and 500 µL of serum was pipetted into

specimen tube and loaded into machine, displayed results were recorded on case reporting forms (Fig. 5).

Figure 5: CRP analysis using ERBA (Mannheim) automated clinical chemistry analyser XL 180

3.6.4 Determination of Complete Blood Count

Complete blood count was done by automated hematology analyzer (DYMIND DH76) as per manufactures recommendation (DYMIND BIOTECH, China). Briefly, the blood sample in EDTA tube was well mixed using roller blood mixer, then loaded to the automated analyzer where results were displayed and recorded.

3.6.5 Determination of Coagulation Profile (PT/APTT/ INR)

Coagulation profile assessment was done using Semi-automatic coagulation analyzer ECL 412 as per manufacturers recommendation (ERBA Mannheim). Briefly, blood sample in sodium citrate tube was centrifuged at 1000 rpm for 5 minutes, then 100 µL of plasma was pipetted to testing cuvettes which were inserted in the testing channels. Specific reagents were subsequently added as per protocol. Results were displayed and documented.

3.6.6 Blood Culture and Identification

Blood culture and antibiotic susceptibility testing were done according to the standard microbiological procedures prescribed by the Clinical and Laboratory Standards Institute (CLSI). In Brief, both anaerobic and aerobic BACT/ALERT® Culture Media bottles were loaded and incubated into BACT/ALERT® 3D blood culture system (bioMérieux, Durham,

NC, USA) at 37°C for five days (Fig. 6 a). Positive blood cultures were detected by a positivity signal in the machine and it was considered negative if no signal was displayed after 5 days. A gram stain was done. Positive blood cultures were sub-cultured into sheep blood agar and chocolate agar and incubation at 35°C in 5% CO_2 for 24 to 48 hours (Fig. 6). A positive culture from these solid media underwent gram staining and biochemical tests identification for bacterial isolates as previously described by Altheide (2020) and Boyanova (2018). In brief, for gram staining identification suspension of loopful colony from solid media was smeared on clean glass slide, air dried and heat fixed.

Primary (crystal violate and Gram's Iodine) stains were poured and kept for one minute each with water rinsing in between. Then washed with ethanol for 20 seconds and water rinsing before adding secondary stain (safranin) for one minute and washed with water. The Air-dried slides were then observed under microscope, gram positive bacteria appeared purple coloured while gram negative appeared red coloured. For biochemical identification, coagulase and catalase test were performed, where colonies from culture were added in test tubes containing plasma (for coagulase test) and hydrogen peroxide (for catalase test). The tubes were monitored for formation of clot/clump (for coagulase test) and air bubbles (for catalase test).

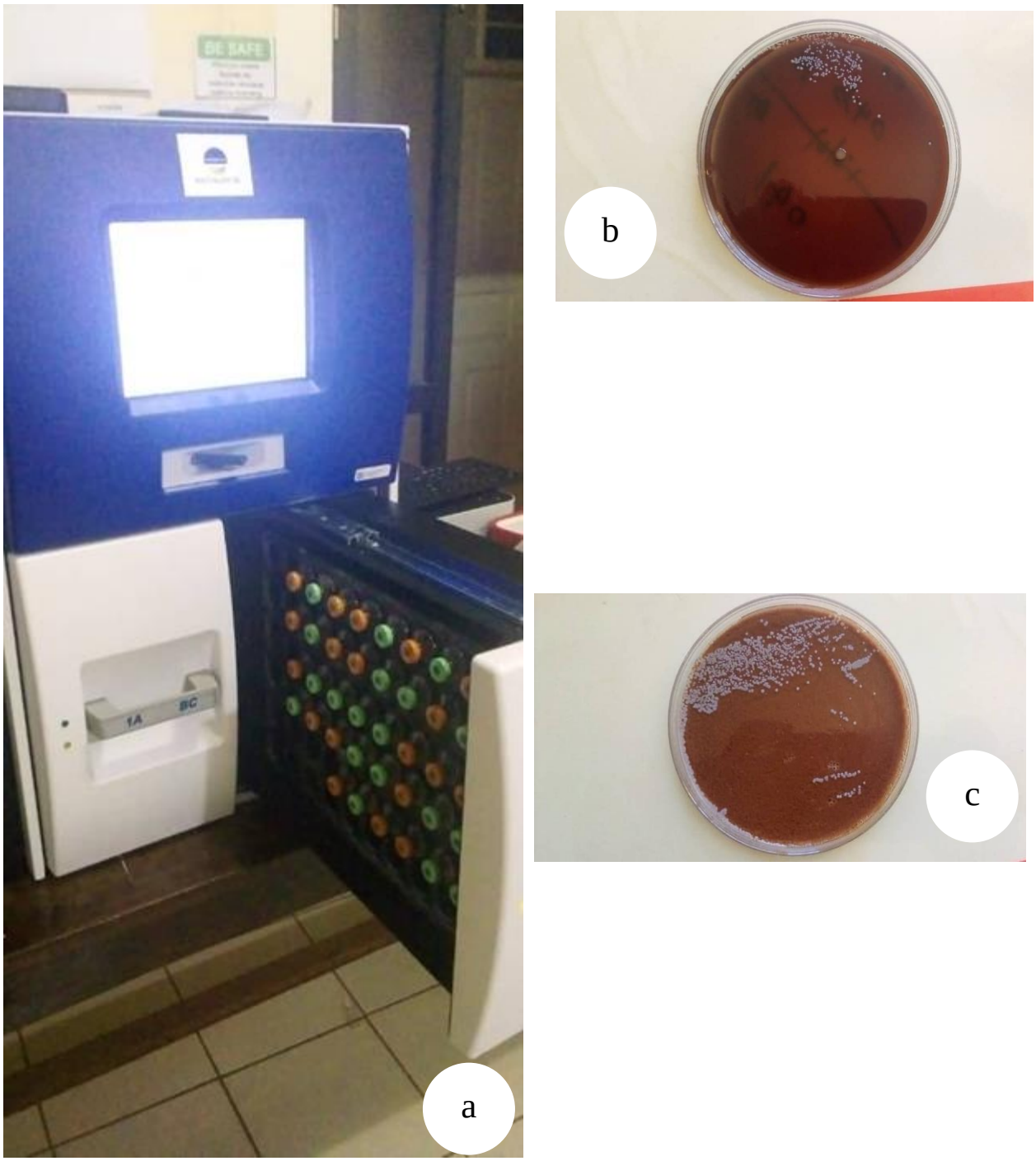

Figure 6: Specimen for blood culture incubated in automated BACT/ALERT system (a), then positive culture bottle sub cultured in solid media for identification process (b, c)

3.6.7 Antibiotic Susceptibility Test (AST)

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) with commonly used antibiotics as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (Weinaten *et al*., 2021). In brief, colonies from solid culture were inoculated on Mueller-Hinton Agar. The plates were incubated at 37°C for 24 hours, then inhibition zones were interpreted according to CLSI guidelines (Weinaten et *al*., 2021). The following antibiotic disks (HiMedia, India) were tested: ciprofloxacin (5 mcg),

Ampicillin (10 mcg) Cloxacillin (30 mcg) gentamicin (10 mcg), Sulphamethoxazole-Trimethoprim (1.25/23.75 mcg), Vancomycin (30 mcg), Amoxicillin (30 mcg), Ceftriaxone (30 mcg), Azithromycin (30 mcg), Cephalexin (30 mcg), Erythromycin (15 mcg), Amoxiclav (30 mcg), Clindamycin (2 mcg), Chloramphenicol (25 mcg) and Meropenem (10 mcg). Respective zones of inhibition were evaluated as Susceptible (clear zone of inhibition) or Resistant (no inhibition zone) (Fig. 7).

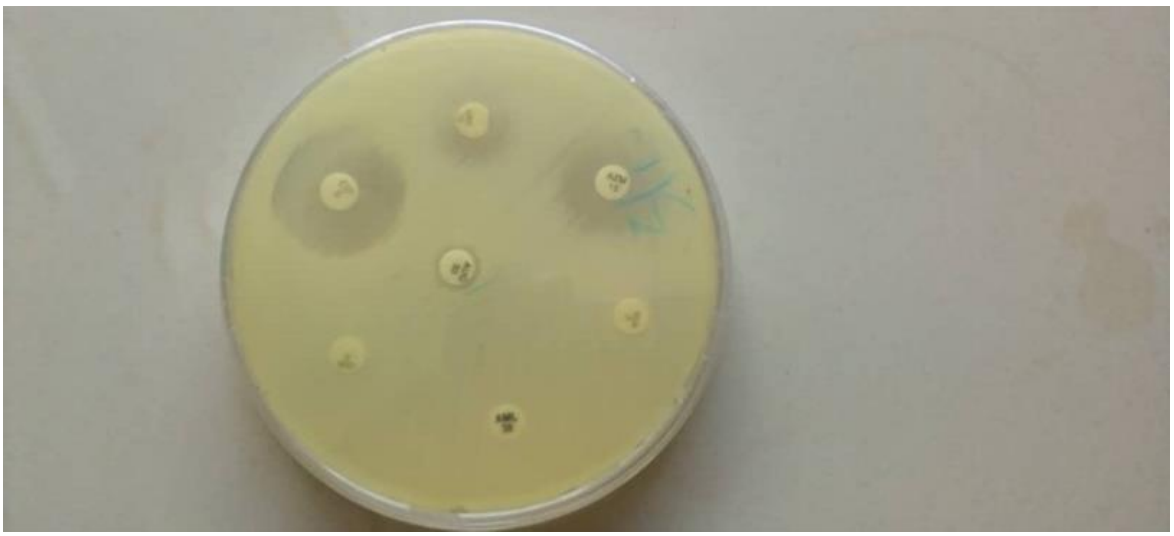

Figure 7: Antibiotic susceptibility pattern of *S aureus* isolates against seven (7) antibiotic drugs using agar well diffusion method on Petri Plate

3.7 Data Management and Statistical Analysis

Data were recorded in a clinical case report form, entered before statistical analysis. The patients were categorized into those with and without TB detected by either Xpert® MTB/RIF or Urine LF-LAM. Continuous variables such as age was described as mean (SD) and were compared using an independent student *t*-test. Accordingly, proportions were used for categorical variables like, gender, malnutrition measured by BMI <18.5 kg/m^2, INR (higher if $\geq$ 1.8), CRP (higher if $\geq$ 10 mg/L). A conventional sepsis score for predicting outcome was also measured by MEWS with scores above 6 considered high. Severity of illness was further characterized and categorized as severe at a Karnofsky score <50%. Chi-Square or fisher's exact test compared proportions among patients with and without TB. A Venn diagram was used to display co-infections among PLHIV. The median time to mortality was estimated using the Kaplan-Meier method, and was compared across different regimens using a log-rank test as described by Gillespie *et al*. (2014). A $p < 0.05$ was considered significant. All analyses were performed using Statistical Package for Social Sciences version 24.0 (IBM SPSS, Armonk, NY, USA).

3.8 Ethical Clearance

The Ethical approval to conduct the study was obtained from the KIDH-NM AIST-CEDHA Health Research Ethical Committee-KNCHREC (KNCHREC00043/03/2021) (Appendix 4) and permission to perform the study at Kibong'oto infectious diseases hospital, KCMC and Mawenzi regional referral hospital was pursued from hospital authorities. Participants were requested to affirm in participating in the study through the informed consent form and participants who did not contribute in the study received normal routine care according to their condition and presentations. All data collected were assigned identification code for confidentiality purposes and later securely archived for future reference.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Etiological Pathogens among PLHIV with Features of Sepsis

(i) Baseline Characteristics of Patients with and without TB Sepsis

Among 137 PLHIV screened, 98 (71.5%) were enrolled (Fig. 8). Of these 98 patients, 39 (39.8%) were male. Their mean age (SD) was 44 (12.9) years. In total, 84 (85.7%) were on ART, 56 (57.1%) had CD4+T cells < 100 cells/µL and 63 (64.3%) were malnourished. Patients' socio-demographic and clinical characteristics are shown in Table 1. The TB sepsis was significantly higher in young patients compared to older patients [mean (SD) age 39 (12.9) vs 46 (12.4), p = 0.009]. Using MEWS score at 7-8 for sepsis, 16 (25.6%) of patients without TB scored in that higher range compared to 17 (47.2%) with TB (p = 0.025). 27 (32.1%) patients who were on ART had TB compared to 9 (64.3%) patients who were not on ART (p = 0.016). C-reactive protein levels were significantly high in Tb patients (>10 mg/L) compared to patients without TB (p < 0.001, Table 1).

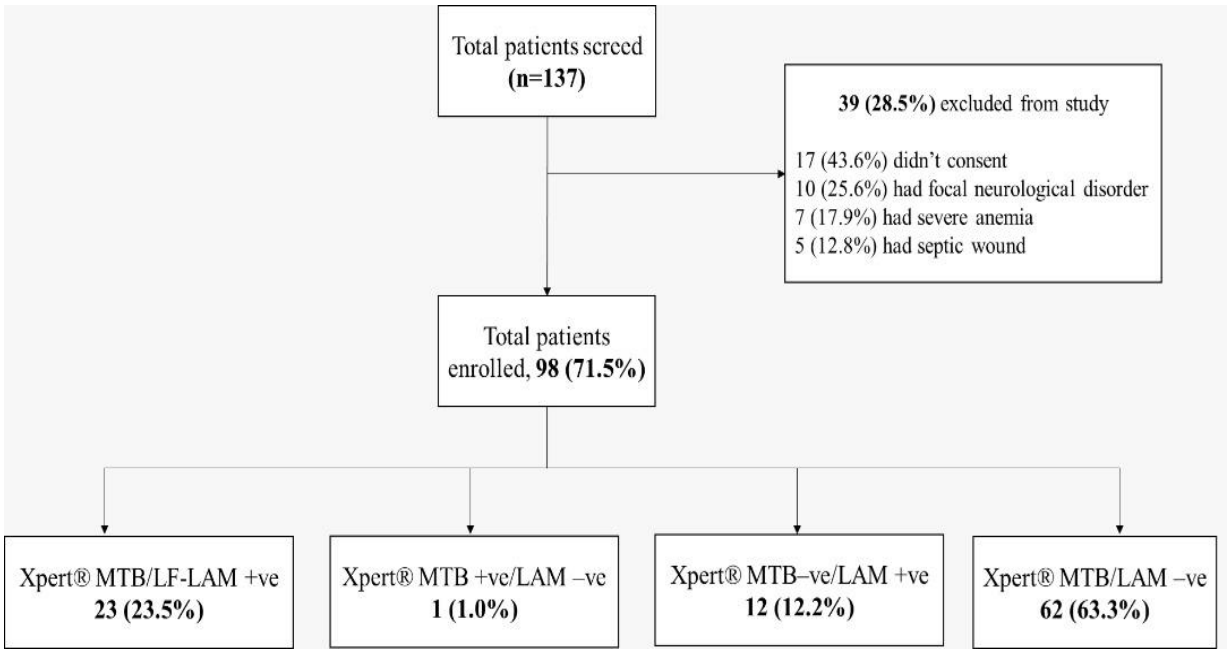

Figure 8: Study procedure including TB Lateral Flow Urine Lipoarabinomannan Assay and Xpert MTB/RIF Assay results

Table 1: Socio-demographic and clinical characteristics of PLHIV with and without TB (N=98)

Variables	Overall n (%)	Positive TB n (%)	Negative TB n (%)	*p* Value
Age (yrs.), mean (SD)	44 (12.9)	39 (12.9)	46 (12.4)	0.009*
Gender				
Male	39 (39.8)	14 (38.9)	25 (40.3)	0.975 †
Female	59 (60.2)	22 (61.1)	37 (59.7)	
Nutritional status, BMI (Kg/M^2)				
<16.0	21 (21.4)	7 (19.4)	7 (11.3)	0.436 †
16.0-18.4	42 (42.9)	15 (41.7)	27 (43.5)	
18.5 -24.9	42 (42.9)	14 (38.9)	28 (45.2)	
Karnofsky score (%)				
<50	57 (58.2)	21 (58.3)	36 (58.1)	0.742 †
≥50	41 (41.8)	15 (41.7)	27 (41.9)	
MEWS				
<3	1 (1.0%)	1 (2.8)	0 (0.0)	0.025 †
4-6	50 (51.0%)	12 (33.3)	37 (59.7)	
7-8	34 (34.7%)	17 (47.2)	16 (25.8)	
>8	13 (13.3%)	6 (16.7)	9 (14.5)	
Duration of illness (weeks)				
<2	30 (30.6)	6 (16.7)	24 (38.7)	0.032 †
2-4	46 (46.9)	20 (55.6)	26 (41.9)	
>4	22 (22.4)	10 (27.8)	12 (19.4)	
On ART				
Yes	84 (85.7)	27 (32.1)	57 (67.9)	0.016 †
No	14 (14.3)	9 (64.3)	5 (35.7)	
Absolute CD4 counts (cells/µL)				
<100	56 (57.1)	21 (58.3)	35 (56.5)	0.520 †
100-199	29 (29.6)	12 (33.3)	17 (27.4)	
≥200	13 (13.3)	3 (8.3)	10 (16.1)	
Hemoglobin level (g/dl)				
<11.0	31 (31.6)	11 (30.6)	20 (32.3)	0.974 †
≥11.0	67 (68.4)	25 (69.4)	42 (67.7)	

Variables	Overall n (%)	Positive TB n (%)	Negative TB n (%)	*p* Value
INR				
< 0.8	18 (18.4)	5 (13.9)	13 (21.0)	0.082 †
0.8-1.1	24 (24.5)	10 (27.8)	14 (22.6)	
> 1.1	40 (40.8)	19 (52.8)	22 (35.5)	
C-reactive protein (mg/L)				
<10	49 (50.0)	2 (5.6)	47 (75.8)	0.000 †
10-50	33 (33.7)	19 (52.8)	14 (22.6)	
>50	16 (16.3)	15 (41.7)	1 (1.6)	

ART: Antiretroviral therapy, MEWS: Modified early warning signs, SD: standard deviation, INR: international normalized ratio, BMI: Body mass index, TB: Tuberculosis
** p-value by t-test*
† p-value by Chi-square or fisher's exact test

(ii) Pathogens Causes of Sepsis in TB and non-TB patients with Advanced HIV Disease

All 98 PLHIV provided urine for TB-LAM and sputum for Xpert® MTB/RIF testing. In 98 patients, urine LF LAM increased TB sepsis detection from 24 (24.5%) by Xpert MTB/RIF in sputum sample to 36 (36.7%) by both tests (Fig. 8). Table 2 and Fig. 9 show that sepsis in 23/98 (23.5%) of 98 PLHIV was due to only one bacterial species (n = 15), two bacterial species (n=1), bacteria/fungi coinfection (n=1), Bacteria/malaria coinfection (n=1), fungi only (n = 4), and malaria only (n = 1). In total, 11 (30.6%) out of 36 patients with TB were co-infected by other microbial agents compared to 12 (19.4%) of 62 participants without TB (Table 2). The *S. aureus* was the commonest cause of sepsis in both patients with (n = 4) and without tuberculosis (n = 6). Etiological agents for TB sepsis were not identified in 50 (51.0%) of 98 patients (Fig. 9).

Table 2: Other aetiologies of sepsis than *M. tuberculosis* in PLHIV (N=98)

Pathogen isolated	Positive TB patients (n = 36)	Negative TB patients (n = 62)
No other pathogen	25 (69.4%)	50 (80.6%)
Staphylococcus aureus	4 (11.1%)	6 (9.7%)
Streptococcus pneumoniae	3 (8.3%)	2 (3.2%)
Klebsiella pneumoniae	0 (0.0%)	1 (1.6%)
Shigella spp.	0 (0.0%)	1 (1.6%) *
Coagulase negative Staphylococcus	1 (2.8%)	1 (1.6%)
Cryptococcal spp.	3 (8.3%)	2 (3.2%) †
Plasmodia spp.	0 (0.0%)	2 (3.2%) †

*Was isolated in same patient with *Streptococcal pneumonia* infection

†One patient had bacteria coinfection (*Staphylococcus aureus*)

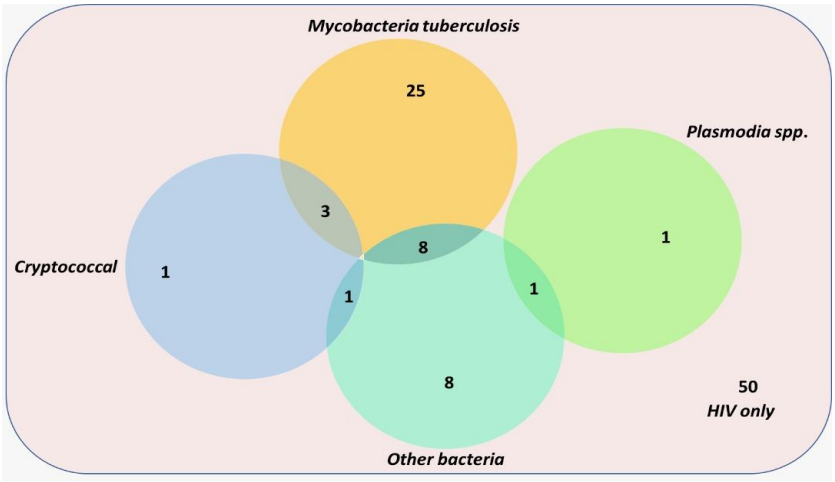

Figure 9: Venn diagram showing the different patterns of coinfections observed in the study

4.1.2 Antibiotic-resistance patterns of isolated bacteria from people living with HIV/AIDS

The antibiotic resistance profiles of the isolates to antibiotics are given in Table 3. Generally, isolates bacteria showed high resistance to most of the antibiotics tested. For example, S. *aureus resistance* ranged from 10% for Gentamycin to 90% for Sulphamethoxazole-Trimethoprim (Co-trimoxazole). Also, *S. pneumoniae* was 20% resistant to Ceftriaxone, cephalexin, Sulphamethoxazole-Trimethoprim and Azithromycin (Table 3). All isolated bacteria exhibited multi drug resistance (MDR) patterns for tested antibiotics. There is no significant difference in sepsis severity and clinical outcome among patients with susceptible vs resistance bacterial pathogens (Table 4).

Table 3: Antibiotic-resistance patterns of bacteria isolated from PLHIV with sepsis

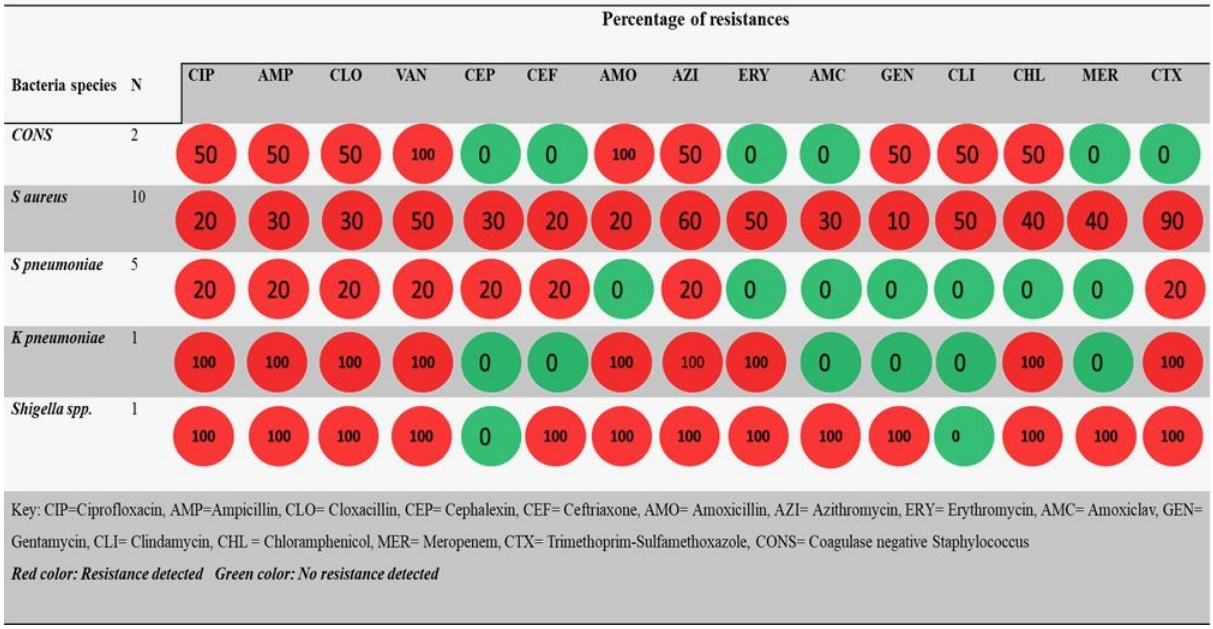

Table 4: Variable characteristics among patients with Susceptible vs Resistant bacteria

Variables	Overall n (%)	With Susceptible pathogens n (%)	With Resistant pathogens n (%)	*p* Value
Age (yrs) Mean (SD)		33 (SD 10)	48 (SD 12)	
Sex				
male	6 (33.33)	2 (33.3)	4 (66.7)	0.710
female	12 (66.7)	3 (13.6)	9 (86.4)	
Nutritional status (Kg/M^2)				
<16	4 (22.2)	2 (50.0)	2 (50.0)	0.272
16.0 – 18.5	5 (27.8)	2 (40.0)	3 (60.0)	
18.5-24	9 (50.0)	1 (11.1)	8 (88.9)	
Karnofsky score (%)				
<50	11 (61.1)	3 (27.3)	8 (72.7)	0.952
>50	7 (38,9)	2 (28.6)	5 (71.4)	
TB status				
Positive	11(61.1)	3 (27.3)	8 (72.7)	0.554
Negative	7 (38.9)	2 (28.6)	5 (71.4)	
INR				
<0.8	1 (5.6)	0 (0)	1 (100)	0.758
0.8-1.8	8 (44.4)	2 (25.0)	6 (75.0)	
>1.8	9 (50.0)	3 (33.3)	6 (66.7	
MEWS				
<3	1 (5.6)	0 (0)	1 (100.0)	0.671
4-6	8 (44.4)	2 (25.0)	6 (75.0)	
7-8	5 (27.8)	1 (20.0)	4 (80.0)	
>8	4 (22.2)	2 (50.0)	2 (50.0)	
CRP				
<10	5 (27.8)	1 (20.0)	4 (80.0)	0.758
10-50	8 (44.4)	2 (25.0)	6 (75.0)	
>50	5 (27.8)	2 (40.0)	3 (60.0)	
Clinical outcome				
Died	2 (11.1)	1 (50.0)	1 (50.0)	0.457
Survived	16 (88.9)	4 (25.0)	12 (75.0)	

4.1.3 Associated Factors with Clinical Outcomes among PLHIV with Sepsis

Among 98 patients, 9 (9.2%) died with mean (SD) age of 30 (7.7), whereas 28-day survivors had mean age of 45 (12.7), p =0.001]. Mortality was also significantly higher among malnourished compared to those with normal nutrition status (p =0.001), severe forms of diseases measured by Karnofsky scored below 50% compared to those with a score above 50% (p =0.028), high modified early warning signs score [MEWS, 8 (88.9%) vs. 1(11.1%), p =0.001] and higher INR 8 (88.9%) vs. 1 (11.1%), p =0.025)]. There was no significant difference in mortality rate among TB patients with/without other pathogens and those without any pathogen detected (HIV alone) [5 (55.6%) vs 1(11.1%) vs 3(33.3%), p = 0.238], (Table 5 and Fig. 10).

Table 5: Factors associated with mortality among PLHIV with sepsis (N=98)

Variable	Died (n = 9)	Survived (N = 89)	*p* Value
Age (yrs) Mean (SD)	30 (7.7)	45 (12.7)	0.001
Gender			
Male	6 (66.7%)	33 (37.1%)	0.084
Female	3 (33.3%)	56 (62.9%)	
Nutritional status			
<16	6 (66.7%)	8 (9.0%)	< 0.001
16-18.5	3(33.3%)	39 (43.8%)	
>18.5	0 (0.0%)	42 (47.2%)	
Karnofsky score			
<50	9 (100%)	48 (53.9%)	0.028
≥50	0 (0.0%)	41 (46.1%)	
MEWS			
<3	0 (0.0%)	1 (1.1%)	< 0.001
4-6	1 (2.0%)	49 (55.1%)	
7-8	2 (5.9%)	38 (42.7%)	
>8	6 (85.7%)	1 (1.1%)	
Duration of illness (weeks)			
<2	0 (0.0%)	30 (33.7%)	0.08
2-4	7 (77.8)	39 (43.8%)	
>4	2 (22.2%)	20 (22.5%)	
ART use			
No	2 (22.2%)	12 (13.5%)	0.386
Yes	7 (77.8%)	77 (86.5%)	
Absolute CD4 count			
<100	5 (55.6%)	51 (57.3%)	0.357
100-199	4 (44.4%)	25 (28.1%)	
>200	0 (0.0%)	13 (14.6%)	
Hemoglobin			
<11	4 (44.4%)	27 (30.3%)	0.386
>11	5 (55.6%)	62 (69.7%)	
INR			

Variable	Died (n = 9)	Survived (N = 89)	*p* Value
<0.8	0 (0.0%)	18 (24.3%)	0.025
0.8-1.8	1 (4.2%)	23 (31.1%)	
>1.8	8 (19.5%)	33 (44.6%)	
Isolated pathogens			
TB with/without other pathogens	5 (55.6%)	31 (34.8%)	0.513
Other microbes	1(11.1%)	11(12.4%)	
HIV only	3 (33.3%)	47 (52.8%)	

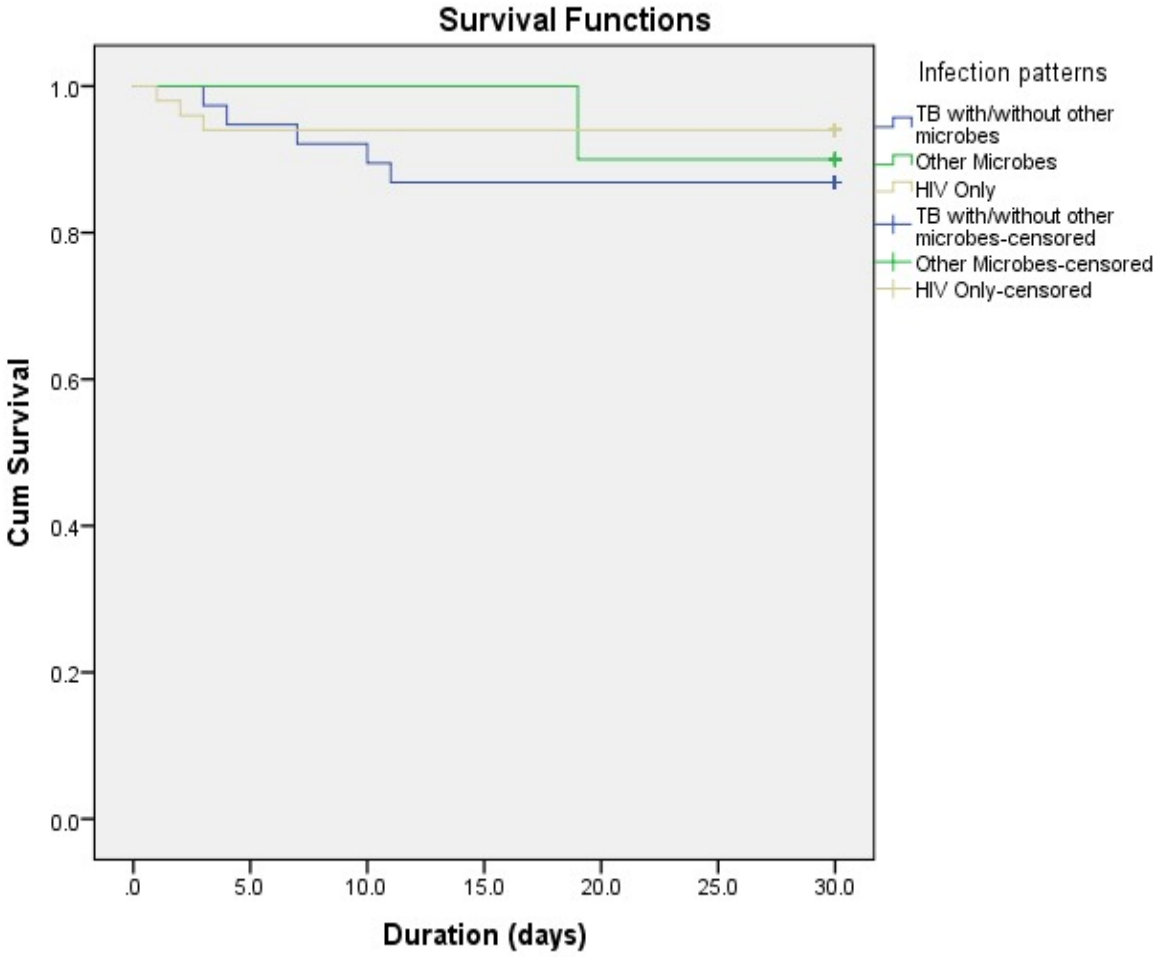

Figure 10: Kaplan-Meier curves for estimated mortality in PLHIV with sepsis stratified by isolated pathogens

4.2 Discussion

This study reports on the spectrum of aetiological agents of sepsis, phenotypic antibiotic susceptibility of isolated bacteria and factors linked with clinical mortality among PLHIV presenting with features of sepsis, from Kilimanjaro region in Tanzania.

4.2.1 Etiological Pathogens among PLHIV with Features of Sepsis

Mycobacteria tuberculosis (MTB) was the primary coinfection and major etiological agent of sepsis among PLHIV in the Kilimanjaro region of Tanzania. *Mycobacteria tuberculosis* is known to have a high capacity to evade the immune system and remain dormant within the body awaiting reactivation during conditions of immune suppression, which may account for high TB prevalence (Zhai *et al*., 2019) among PLHIV. This study observed a prevalence of active TB in more than one-third of patients with advanced HIV/AIDS disease. These findings support the high TB prevalence of 38% was previously reported in other studies in the HIV/AIDS population (Alebachew *et al*., 2016; Lewis *et al*., 2019; Moore *et al*., 2019). Apart from TB, co-infection with solitary or multiple pathogens including bacteria like *S. aureus,* fungi like *Cryptococcus spp.,* a causative agent for meningitis, and parasites like *Plasmodia spp.,* a causative agent for malaria, were commonly detected. This finding is in agreement with a recently reported increase in gram-positive bacteria recovered among patients presenting with septicaemia (Sun *et al*., 2012). The wide spectrum of aetiological agents and possible polymicrobial infection in one patient found in my study can partly be attributed to altered cell-mediated and humoral host immunity (Huang *et al*., 2019; Van der Poll & Opal, 2008) as well as malnutrition. This diverse cause of sepsis in patients with advanced HIV disease calls for optimal diagnostic algorithm not only to confirm diagnosis but also to guide clinical management decisions.

A combination of Xpert® MTB/RIF assay and urine LF-LAM in this study increased TB detection from 24% to 37%. This incremental yield of TB case detection in people living with HIV by both tests was previously reported in Kilimanjaro region by Byasharila *et al*. (2019) and in one systematic review and meta-analysis reported by Bar *et al.* (2020). In resource-limited countries like Tanzania, Xpert® MTB/RIF is a frontline diagnostic test for detecting TB in PLHIV (WHO *et al.,* 2019). However, Xpert® MTB/RIF requires adequate and quality sputum testing for optimal diagnosis, which is not commonly achieved in immunocompromised, critically ill patients with paucibacillary disease like that reported in this study. This findings support the WHO recommendation of using urine LF-LAM for detecting TB among patients with advanced HIV diseases with CD4 +T cells <100 cell/µL (WHO, 2019). Additionally, in the study significantly higher CRP value were found in patients with advanced HIV with TB co-infections, which is in agreement with Ciccacci *et al*. (2021) who found similar trend in a cohort of Kenyan HIV patients who participated in the DREAM program. These findings and others, complement a systematic review and meta-analysis that recommends CRP as a potential biomarker for screening active TB in high-risk populations including PLHIV (Ciccacci *et al*., 2021; Yoon *et al*., 2017). The CRP is a biomarker

predominantly released in acute phase following a response to IL-6 mediated bacterial blood stream infections such as TB (Escadafal *et al*., 2020).

4.2.2 Antibiotic-Resistance Patterns of Isolated Bacteria from People living with HIV/AIDS

In this study high antibiotic resistance to both first-line and second line antibiotics were observed among isolated bacterial isolates. Similar findings have been observed in other studies where antibiotic resistance was equally detected among first and second line antibiotic in various populations including PLHIV (Jemal *et al*., 2020; Manyahi *et al*., 2020; Marwa *et al.*, 2015; Ngalani *et al*., 2019). The observed high antibiotic resistance in PLHIV may be due to their frequent and prolonged use for the treatment of recurrent infections owing to compromised immunity as also described by Ragavan Rameshkumar and Arunagirinathan, (2018). The high rate of cotrimoxazole resistance observed in this study may be due to the fact that this drug is widely and prolonged used as prophylaxis against HIV/AIDS-associated opportunistic infections. Similar findings were observed by Morpeth *et al.* (2008) where, Trimethoprim-sulfamethoxazole (cotrimoxazole) prophylaxis led to cotrimoxazole resistance and other antibiotics such as ampicillin, chloramphenicol, ciprofloxacin, and nalidixic acid . Generally undetermined antibiotic-resistance to PLHIV are frequently associated with unfavorable treatment outcomes (Friedman *et al.*, 2016). In the current study, early drug susceptibility testing detected antibiotic-resistance in over 70% of patients hence informing on their regimen changes which improved their treatment outcomes. These findings are in agreement with that of Harbarth *et al.* (2007) whereas the assortment of effective antibiotics as guided by drug susceptibility testing improved patients' survival and cure rate. Therefore, routine antibiotic susceptibility testing may be part of the diagnosis algorithm of sepsis in PLHIV.

4.2.3 Associated Factors with Clinical Outcomes among PLHIV with Sepsis

The study found relatively low overall mortality (9.2%) compared to 16.5% previously reported from a similar cohort in the same setting in Tanzania by Byasharila *et al*. (2019) and in other cohorts such as 21.5% from South Africa and Ghana (Ogyiri *et al*., 2019; Schutz *et al*., 2019) and 20% reported from Brazil in 2017 (Da Silva Escada *et al.*, 2017). As expected, mortality was high in patients with the severe form of sepsis as clinically measured by high MEWS score, a Karnofsky score $\leq$ 50%, malnutrition and coagulopathy biomarker like INR tested (Birhanu *et al*., 2021; Moore *et al.*, 2017; Workie *et al.*, 2021). Previous studies have argued that early diagnosis and treatment of sepsis limit progression from mild to uncontrolled

pro-inflammatory cascades leading to end organ damage, coagulopathy, clinical deterioration and ultimately death (Birhanu *et al.*, 2021; Byashalira *et al.*, 2019). Certainly, early treatment of active TB confirmed by either Xpert® MTB/RIF or urine LF-LAM tests as well as initiation of effective empirical broad-spectrum antibiotic for bacterial infection, anti-fungal therapy for cryptococcal meningitis could partly explain the low mortality in the present study. In addition, low mortality in the current study can also be due to pathogen-guided evidence-based early clinical decision made in support of implementing WHO recommendations to evaluate for worsening or improvement of danger signs at day 3, 5, 10 and 28 days (Organization, 2016). This argument support integration of additional optimal microbiological investigations to recover other causes of sepsis from different sources including but not limited to blood, sputum and cerebrospinal fluids (Attia *et al.*, 2019).

The main strength of this study was the holistic application of not only multiple diagnostic tools to confirm the diverse causes of sepsis (TB, bacterial, fungal and parasitic) in patients with advanced HIV diseases from their sputum, urine and blood, but also early initiation of effective therapy and vigorous monitoring to enforce early clinical decisions. The use of tests other than the TB diagnostic tools to identify bacterial, fungal and parasitic causes of sepsis addresses prior limitations reported by Byasharila *et al.* (2019).

Nonetheless, the study has limitations. First, in this study etiological agents of sepsis were unable to identify in over half of patients with advanced HIV diseases this may be due to the limited performance profile of blood culture in recovering fastidious bacteria and viruses. Deploying mycobacterial blood culture methods as well as a more sensitive multiple pathogen molecular assays such as the RT-qPCR TaqMan® card (Moore *et al*., 2019) and metagenomics sequencing technologies would have increased the chance for detecting more putative pathogens (Gu *et al*., 2021). Lastly, this study was done during COVID-19 pandemic but SARS-CoV-2 was neither presumed to cause sepsis nor tested.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From this study it has been shown that, *Mycobacteria tuberculosis* in combination with other bacteria, fungi, and parasitic infections were common etiological agents of sepsis in PLHIV/AIDS either in solitary or parallel infection pattern. It was also shown that most of isolated bacteria showed multi drug resistance against antibiotics, including Trimethoprim-sulfamethoxazole (cotrimoxazole) which is used for prophylaxis against opportunistic pathogens. On assessment of factors associated with mortality, it was manifested that mortality was predicted by higher scores of conventional sepsis/severity grading tools, severe malnutrition and elevated International normalized ratio (INR).

5.2 Recommendations

Following findings of this study, I recommend the following actions:

(i) Performing a wide-microbiological analysis in all PLHIV/AIDS patients presenting with sepsis in order to optimized timely diagnosis of all etiological pathogens since sepsis in PLHIV is caused by multiple pathogens either in solitary or parallel pattern.

(ii) The use of robust molecular assays (Polymerize chain reaction, metagenomics and sequencing) to optimize detection of microbes that can be missed by conventional diagnostic tests such as fastidious bacteria and viruses.

(iii) Performing diagnostic drug susceptibility test (DST) for all PLHIV/AIDS presenting with sepsis due to higher prevalence of antimicrobial resistance, this will help to identify and prescribing effective antibiotic and hence better management.

(iv) To look for alternative prophylaxis antibiotic against opportunistic pathogens since Trimethoprim-sulfamethoxazole (cotrimoxazole) has shown high level of resistance.

(v) Management of comorbidity such as malnutrition and coagulopathy since they are associated with higher mortality rate.

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APPENDICES

Appendix 1: Case Report Form

[For participants who are eligible for the study only]

Study site: _____

Interviewer's name: _____

Date of interview_____/_____/_____ (dd/mm/yyyy)

Participant ID ☐☐☐☐☐☐

Contact address: _____

Telephone number: _____

Alternative Phone number _____

Demographic information

1. Date of birth _____/_____/_____ (dd/mm/yyyy)
2. Age (years) _____
3. Sex ☐
    a. Male
    b. Female
4. Marital status ☐

a. Single b. Married c. Divorced/separated

d. Widow/widowed e. Cohabiting

f. Others (specify) _____

5. Education level ☐

a. None b. Primary education c. Secondary education

d. College/university e. Others (specify) _____

6. Occupation ☐

a. Peasant b. Business c. Employee

d. Student e. House wife f. Others (specify) _____

Anthropometrics

7. Weight _____ (Kg)
8. Height _____ (Cm)
9. MUAC _____

Clinical history

10. Karnofsky Score _____
11. Vital signs at the date of interview;
    a. Body temperature: _____ °C
    b. Pulse rate: _____ beats/min
    c. Respiratory rate: _____ breaths/min
    d. Blood pressure (Systolic: _____ mmHg Diastolic _____ mmHg)
    e. GCS _____
12. Duration of current illness _____
13. Other comorbidity/diagnosis _____
14. Date confirmed HIV positive; _____/_____/_____ (dd/mm/yyyy)
15. Date initiated ART _____/_____/_____ (dd/mm/yyyy)
16. Indicate the Highly Active Antiretroviral Therapy at the date of interview

_____, Date started _____/_____/_____ (dd/mm/yyyy)

17. Is the participant on Isoniazid Preventive Therapy (IPT)?
    a. Yes
    b. No

If yes, indicate the date started IPT _____/_____/_____ (dd/mm/yyyy)

18. Is the participant on Cotrimoxazole (CPT)?
    a. Yes

b. No

If yes, indicate the date started CPT _____/_____/_____ (dd/mm/yyyy)

19. CD4 count at the date of interview? _____cells/mm^3

20. HIV viral load at the date of interview? _____copies/ml

21. Antibiotic use within the last 7 days?

    a. Yes

    b. No

22. Which one (if possible, to recall?

_____

*† *Collect two urine specimens for LAM*

*† *Collect blood for culture, TAC qPCR, CRP, FBP, ESR, PT, PTT, INR, CrAG, CD4, Sputum for Xpert/MTB Rif, per protocol*

*† *Take the participant to Chest X-Ray*

End of Interview: Thank the study participant

RESULTS OF LABORATORY INVESTIGATIONS

1. White blood cell count at date of interview: _____ /mm^3
2. C-reactive protein at date of interview: _____ mg/L
3. Aerobic/ Anaerobic blood culture results: ☐
    a. *S. aureus* isolated
    b. *E. coli* isolated
    c. *Specify other (s)microorganism isolated:* _____
    d. Contaminated
4. TAC q PCR results: ☐
    a. *S. aureus* isolated
    b. *E. coli* isolated
    c. *Specify other (s)microorganism isolated:* _____

5. DST results from isolates of Aerobic/ Anaerobic blood culture results: ☐

    a. *Sensitives* _____

    b. *Resistant*_____

    c. Contaminated

Follow UP and Outcome assessment

Follow up day	**Temp**	**PR**	**RR**	**BP**
Day 3				
Day 5				
Day 10				
Day 28				

Final outcome at Day 28:

a) Survived (Improved)

b) Died (Date _____/_____/_____ (dd/mm/yyyy)

Appendix 2: Karnofsky performance status scale definitions rating (%) criteria

Performance status	Rating (%)
Normal no complaints; no evidence of disease.	100
Able to carry on normal activity; Minor signs or symptoms of disease	90
Normal activity with efforts; some signs or symptoms of disease.	80
Cares for self; unable to carry on normal activity or to do active work.	70
Requires occasional assistance, but is able to care for most of his personal needs.	60
Requires considerable assistance and frequent medical care.	50
Disabled; requires special care and assistance.	40
Severely disabled; hospital admission is indicated although death not imminent.	30
Very sick; hospital admission necessary; Active supportive treatment necessary.	20
Moribund; fatal processes progressing rapidly	10
Dead	0

Appendix 3: Modified early warning signs scores (MEWS)

Score	**3**	**2**	**1**	**0**	**1**	**2**	**3**
Systolic blood pressure (mmHg)	<70	70-80	81-100	101-199		≥200	
Heart rate (bpm)		<40	40-50	51-100	101-110	111-129	≥130
Respiratory rate (bpm)		<9		9-14	15-20	21-29	≥30
Temperature (˚C)		<35		35-38.4		≥38.5	
Neurological				Alert	Reacting to Voice	Reacting to Pain	Unresponsive

Appendix 4: Ethical clearance Certificate

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No: KNCHREC00043/03/2021 23rd APRIL 2021

Study Title: **Characterizing antibiotic-resistance patterns among people living with HIV with features of sepsis to improve clinical outcomes, in Northern Tanzania**

Study Area: **NORTHERN TANZANIA**

PI Name: DR. DONATUS BONIPHACE TSERE
Co-Invigilator:
Institutions: School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 23rd April 2021

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Research and Ministry of Health Community Development Gender Elderly and Children

Duration of Study Renewal: Subject to Renewal within ONE YEAR
Span From: 23rd APRIL 2021 to 22nd April 2022.

DocuSigned by: Simon Njeya 5BB798E863704AA...

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Mr. Simon Njeya
Secretary
KNCHREC

.....
Prof. Raymond Mosha
Chairperson
KNCHREC

RESEARCH OUTPUT

(i) Publication

Tsere, D. B., Shirima, G. M., Grundy, B. S., Heysell, S. K., Mpagama, S. G., Mziray, S. R., & Mbelele, P. M. (2022). Multiple pathogens contribute to human immunodeficiency virus-related sepsis in addition to Mycobacterium tuberculosis: A prospective cohort in Tanzania. *International Journal of Mycobacteriology*, 11(3), 241–248.

(ii) Poster Presentation

Tsere, D. B., Shirima, G. M., Grundy, B. S., Heysell, S. K., Mpagama, S. G., Mziray, S. R., & Mbelele, P. M. (2022). *Multiple pathogens contribute to human immunodeficiency virus-related sepsis in addition to Mycobacterium tuberculosis: A prospective cohort in Tanzania.*