COMMUNITY ACQUIRED BACTEREMIA IN HAART NAÏVE HIV -1 INFECTED ADUALT PATIENTS WITH FEVER AT KENYATTA NATIONAL HOSPITAL NAIROBI

THIS DISSERTATION HAS BEEN WRITTEN IN PART FULFILMENT OF THE DEGREE OF MASTERS OF MEDICINE (INTERNAL MEDICINE), UNIVERSITY OF NAIROBI

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DECLARATION

I certify that this dissertation is my original work and has not been presented for a degree at any other University.

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DEDICATION

To Dad and Mum who have always been my greatest support.

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CD4	CD4+ lymphocytes
ELISA	Enyme linked immuno-absorbent assay
HIV	Human immunodeficiency syndrome
IL-1	Interleukin 1
IL-4	Interleukin 4
IL-6	Interleukin 6
KNH	Kenyatta National Hospital
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
NTS	Non Typhi Salmonella
TNF	Tumor Necrosis factor
WHO	World Health Organization

ABSTRACT

Objective: To determine the prevalence of bacteremia, the bacterial isolates, the drug sensitivity pattern of the bacteria isolated and the association between the CD4+ counts and bacteremia in HIV-1 patients with fever who present as acute medical admissions in Kenyatta National Hospital.

Design: Hospital based Cross-sectional study.

Setting: The accident and emergency department was the point of recruitment before admission to any of the seven medical wards at the Kenyatta National Hospital.

Patients: A total of 271 HIV-1adult patients with fever who had not been on antibiotics in the past 14 days including anti-tuberculous drugs and Trimethoprim/ Sulfamethoxazole or HAART and had not been admitted to a hospital in the previous14 days were recruited by systematic sampling at the point of admission.

Main measures: Clinical diagnosis, WHO staging, CD4+ count (CDC 1993-revised classification), bacteremia, bacteria isolates, drug sensitivity patterns.

Results: The male to female ratio of 1:1.5. 69.9% of the participants in the study were married and most of them belonged to a low social economic status. 48.8% of the patients had Community acquired pneumonia. 81.5% were in WHO stages III and IV. 74.5% had AIDS with CD4+ counts <200 cells/ul. The prevalence rate of bacteremia was 15.9% (95% CI, 11.5-20.3). The commonest bacteria isolates were *Staphylococcus aureus* at a prevalence rate of 32.6%, *Streptococcus pneumoniae* and *Salmonella typhi* at 16.3% each, *Echerrichia coli* and *Staphylococcus epidermidis* at 11.6% each. 71.4 % of *Streptococcus pneumoniae* were resistant to penicillin but were sensitive to amoxycillin/clavulanic acid. The prevalence of MRSA and MRSE were 21.4% and 40% respectively. 42.8 % of the *Salmonella typhi* were resistant to ciprofloxacin but were fully sensitive to ceftriaxone. There was no association between bacteremia and CD4+ count categories (p=0.566) However it was noted that most of the isolates were found in patients with AIDS.There was a significant association between Meningitis and gastroenteritis with WHO stage III with a p-value of <0.0002 and <0.007 respectively.

Conclusions: Bacteremia is a major problem in HIV-1 patients. *Streptococcus pneumoniae* still remains a major pathogen in this population with emergence of *Staphylococcus aureus* and *Salmonella typhi* bacteremia. Most of the isolated bacteria were resistant to the first line antibiotics used in Kenyatta National Hospital.

LITERATURE REVIEW

Bacteremia is a common problem in patients infected with HIV-AIDS and contributes to the morbidity and mortality in this population. Sub-Saharan Africa bears the burden of HIV and AIDS than any other region of the world. An estimated 22.5 million people were living with HIV at the end of 2007 and approximately 1.7 million additional people were infected with HIV during that year in Sub-Saharan Africa. In just the past year, the AIDS epidemic in Africa has claimed the lives of an estimated 1.6 million people in this region. More than eleven million children have been orphaned by AIDS. {1}

The 2007 UNAIDSAIDS epidemic update indicates that 5 % of Kenyan adults are infected with HIV. {1}. In Kenya, studies done in the last decade showed a rise of HIV prevalence from 18.7 % to 40 % among hospital admissions. {24,25} Katherine et al in a retrospective study in Hlabisa hospital done in South Africa, between 1991 and 1998 showed an 81% increase in patient admissions with a 43 fold increase in number of patients admitted with AIDS. {22}

In sub-Saharan Africa, the number of people receiving antiretroviral therapy treatment increased more than eight-fold between 2003 and 2005, and more than doubled in 2005 alone. Most of that trend is due to increased treatment access in a few countries (notably Botswana, Kenya, South Africa, Uganda and Zambia). {1}

The human immunodeficiency virus (HIV)-1 extends microbial mechanisms for survival to a unique degree, activating normal host processes to assist in replication. The body mounts immune responses to HIV-1, including neutralizing antibody, virus-specific T helper cells and cytotoxic T lymphocytes (CTL). However, the virus is not fully contained, inevitably resulting in progressive disease and the development of the acquired immunodeficiency syndrome (AIDS) in the vast majority of infected individuals. The cellular immune system, consisting of the CD4 (helper T cell) and the CD8 (cytotoxic T cell) responses, are strongly affected by HIV-1 infection. {2}

The symptoms of AIDS are primarily the result of opportunistic infections. Most of these conditions are infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages. {3}

Fever is the principal and sometimes the only manifestation of serious infection in the immuno-compromised patient. Although a number of fever patterns have been associated with various infectious or non-infectious illnesses, no pathognomonic pattern or degree of fever has been clearly associated with a specific infection in immuno-compromised patients. There is also no pattern of fever that can be used to rule out a noninfectious cause. {16}

Patients who are profoundly immuno-compromised can (albeit rarely) have serious local or systemic infections in the absence of fever. Fever can also be suppressed or muted by immunosuppressive agents that may be part of the therapeutic regimen, especially steroids and nonsteroidal anti-inflammatory agents. However, patients with infection usually have fever despite the use of these agents. {16}

Normally, a potent, complex immunologic cascade ensures a prompt protective response to microbial invasion in humans. A deficient immunologic defense may allow infection to become established; however, an excessive or poorly regulated response may harm the host through a maladaptive release of endogenously generated inflammatory compounds. The most widely investigated cytokines are tumor necrosis factor, interleukin-1, and interleukin-8, which are generally pro-inflammatory, and interleukin- 6 and interleukin-10, which tend to be anti-inflammatory. {5}

A trigger, such as a microbial toxin, stimulates the production of tumor necrosis factor and interleukin-1, which in turn promote endothelial cell– leukocyte adhesion, release of proteases and arachidonate metabolites, and activation of the clotting process. IL-1 and TNF are synergistic and share many biologic effects, and their inhibition improves organ function and survival in animal models of sepsis. {5}

Fever is a manifestation of the release of pro-inflammatory cytokines (IL-1*a*, IL-1*b*, IL-4, IL-6, and TNF *a*) from macrophages, lymphocytes, fibroblasts, epithelial cells, and endothelial cells as a consequence of infection or inflammation. {16}

Bacterial infections are common in HIV-infected patients because of abnormalities in humoral, cellular, and mucosal immunity. HIV-infected patients have an increased risk of bacteremia during bacterial infections. The prevalence of bacteremia in HIV infected patients who have fever and are hospitalized ranges from 5 to 28%.{16}. In Kenya in consecutive prevalence of Hospital based studies on patients admitted with an acute illness to the medical wards in the Kenyatta National Hospital, 26.3% of HIV–1 patients were found to have bacteremia in 1988-1989, 24.3% in 1992 and 13.7% in 1997. Approximately 30 to 40 % of these patients were febrile. Tests to determine CD4+ counts were not accessible during these study periods. {6, 17}

Grant et al in Abidjan, Côte d'Ivoire in a Hopital based study found that 20% of HIV patients admitted to the Infectious Disease Unit had a bacteremia. The median CD4+ counts in the patients with bacteremia was 58 x 10^6/l {20} Tumbarello et al in a retrospective Hospital based case control study in Italy, including all HIV-infected patients admitted from 1 January 1985 to 31 December 1993 established that the rate of bacteraemia in the total yearly HIV-related admissions in a University Hospital in Italy increased from 4% in 1985 to 13% in 1993. {29} Piroon in a retrospective study in Khon Kaen University, Thailand found prevalence of bacteremia in HIV-infected patients to range between 20—30% each year. However in this study CD4+ counts were not done and only 56% of the patients were febrile. {15}

The spectrum of organisms responsible for infectious complications in immuno-compromised hosts is daunting, since virtually any organism can become invasive if host defenses are severely impaired. Bacteria represent the immediate threat to most immuno-compromised hosts. {16}

The predominant organisms isolated from seropositive patients in Kenya were *Salmonella typhimurium* and *Strepotococcus pneumoniae*. Gilks et al in a study done on acute admissions to medical wards showed that *Strepotococcus pneumoniae* prevalence was 7.4% and Salmonella was 11%.{6}. Arthur et al three years later on a similar population showed that *Strepotococcus pneumoniae* prevalence was 10.5 % whereas Salmonella was 10.5 %.{17}

Tumbarello et al in a study done in Italy, found that the more common aetiological agents of bacteraemia were: *Staphylococcus aureus* (29.7%), non-typhoidal species of *Salmonella* (14.1%), *Staphylococcus epidermidis* (10.9%), *Streptococcus pneumoniae* (8.4%), *Pseudomonas aeruginosa* (7.6%), *Klebsiella pneumoniae* (6%), *Acinetobarter* species (4.4%) and *fi haemolytic streptococci* (2.8%).{29}

Piroon in Thailand demonstrated that *Salmonella spp* were the most common pathogen making up to 63.6% of the isolated bacteria in of HIV-infected patients. {15} In North America and European countries, Staphylococcus species are the most common causes of bacterial infections and bacteremia due to the high rate of intravenous drug use. {14}

The common sources of infections are pneumonia and gastroenteritis but most of them are unknown. Piroon in Thailand could not establish the primary source of bacteremia in most patients with bacteremia, especially in Salmonella bacteremia. {15} Pedro-Botet et al in a retrospective study done in a University hospital in Spain showed a definite or probable source of infection in 80.7% of the episodes. Respiratory and gastrointestinal foci were the most frequent sites of infection in 22.9% and 19.3% patients, respectively. Other sites of infection included the skin in 11%, the endocardium in 8.3%, and the urinary tract in 7.3% and other intra-abdominal foci in 4.6 %. {14}

Pedro-Botet et al also demonstrated a decrease in the prevalence of bloodstream infections in HIV-positive patients on HAART which was attributed to the improvement of the immunologic state. {14}

Non-typhi salmonellae (NTS) has had a high incidence in HIV infected persons with *Salmonella typhimurium* and S*almonella enteriditis and* are implicated in most invasive disease in AIDS. {7, 8, 18} Non typhoid salmonellae are responsible for recurring bacteremia, which may represent up to 20% of episodes of bacterial septicemia in both adults and children with AIDS. {15}In Kenya NTS bacteremia was detected in 11 % of HIV-seropositive individuals on admission to hospital (Gilks C *et al.,* 1990). {6}

In a study carried out by Kariuki et al NTS were isolated from 11.3 % of patients admitted with diarrhea and or bacteremia to the Kenyatta National Hospital. 68.8% of the patients had HIV-1. {19}

Grant et al in a study done in Abidjan, Côte d'Ivoire in 1997 found that among HIV-positive patients, non-typhoid salmonellae were the most frequent organisms, isolated from 12% of HIV patients: *Salmonella enteritidis* making up 57% of non-typhoid Salmonella isolates and *Salmonella typhimurium* 26%. {20} Piroon M in the Thai study, established that salmonella species made up 62.2% of the community-acquired bacteremia. {15}

Kariuki et al demonstrated that over 47% of isolates were resistant to three or more of the readily available drugs including ampicillin, cefuroxime, chloramphenicol, co-trimoxazole, streptomycin and tetracycline. Only 16% of NTS were sensitive to all ten antibiotics tested, 20% were resistant to one agent (usually streptomycin or tetracycline) and 17% were resistant to two agents (usually streptomycin and tetracycline or tetracycline and ampicillin). {19} NTS has also been shown to have resistance to cotrimoxazole but sensitive to ceftriaxone, and fluoroquinolones {15}

Streptococcus pneumoniae and *Mycobacterium tuberculosis* are the leading causes of respiratory illnesses. *S. pneumoniae* is the most frequent cause of otitis media, sinusitis, and bronchitis. However it is the invasive syndromes of necrotizing pneumonia and

bacteremia, and meningitis to which HIV patients are particularly susceptible. {18} Pneumonia is the commonest presentation of pneumococcal bacteremia. {21}

Bacterial pneumonia is more frequent in HIV-positive persons than in seronegative controls, and the risk is highest among those with CD4 lymphocyte counts below 200 per cubic millimeter and among injection-drug users. The organisms identified most commonly were *Streptococcus pneumoniae, Staphylococcus aureus,* and *Haemophilus influenzae.* {10}

United States -based studies in the past decade have shown *S. pneumoniae* is still the single most common defined pathogen in nearly all studies of hospitalized adults with community-acquired pneumonia. {9} Nicola Jones et al in a South African study reported an 8.2-fold increase of *S. pneumoniae* bacteremia in HIV-seropositive adults, compared with HIV seronegative individuals. {21}In the several studies done in Kenya, all HIV-positive patients with *S. pneumoniae* bacteremia presented with acute community-acquired pneumonia. In 1988-1999 7%, 1992, 10.5%, and 1997, 7% of patients had *S. pneumoniae* bacteremia. {17}

Heather et al in a study looking at the susceptibility of *S. pneumoniae* to penicillin, showed that among HIV infected adults there has been a significant trend towards a greater proportion of infections due to serogroups 6, 19, 23, and 14 commonly found in children. {26} Scott et al in a study in the Coastal region of Kenya after analysis 284 *S. pneumoniae* isolates showed that there was no particular serotype association with HIV. Almost all serotypes were found to cause invasive disease in the absence of adequate immunity. {27}

The most striking result in studies done is the association between the occurrence of bacterial pneumonia and reduced CD4 lymphocyte counts. Although bacterial pneumonia occurred with virtually all CD4 lymphocyte counts, approximately one third of initial episodes occurred in persons with fewer than 50 CD4 lymphocytes per cubic millimeter,

and approximately two thirds occurred in persons with fewer than 200 CD4 lymphocytes per cubic millimeter. {10}

A significant increase in the occurrence of penicillin-resistant *S. pneumoniae* in HIV-infected adults was reported in South Africa in a study by Nicolas et al. Serotypes/serogroups 6, 14, 19, 23 accounted for 88.9% of penicillin-resistant isolates. {21} Heather et al demonstrated that HIV-positive patients had significantly more penicillin-resistant isolates than did HIV-negative patients, 29.7% HIV-positive patients vs. 8.6% HIV-negative patients. {26}

JUSTIFICATION OF STUDY

Although fever is a common symptom in HIV patients, it is nevertheless non-indicative of the actual underlying infection. It is therefore important that a full microbiological analysis be performed on such patients in order to arrive at an appropriate diagnosis and subsequent treatment.

The prevalence of bacteremia in HIV patients in KNH was 26.3% in 1988–1989 24.3% in 1992 and 13.7% in 1997. The commonest organisms found were *Strepotococcus pneumoniae* and Non Typhi Salmonella. {6, 17} In the three studies only 30-40 % of patients were febrile at presentation. Ten years later there are no current studies to show any prevalence or reflect any change in the prevalence and pattern of bacteremia.

Multiple and blind antibiotic therapy in HIV patients with fever, cotrimoxazole prophylaxis, HAART and the natural evolution of microbes to antibiotic pressure necessitate microbial studies.

With the increased numbers of patients and maturing of HIV/AIDS epidemic in Kenya these figure are expected to change with different clinical significance hence the study.

MAIN OBJECTIVE

To determine the prevalence of bacteremia in HIV-1 patients, with fever and the level of immunosuppresion at which it occurs.

SPECIFIC OBJECTIVES

1. To determine the prevalence of bacteremia in HIV-1 patients with fever at the KNH.
2. To determine the bacterial isolates in patients with fever in HIV-1 at KNH.
3. To determine the drug sensitivity pattern of the bacteria isolated in patients with fever in HIV-1 at KNH
4. To describe the association between the CD4+ counts and bacteremia in HIV-1 patients with fever in KNH.

METHODOLOGY

STUDY DESIGN AND AREA

This was a hospital-based cross-sectional survey that was conducted at Kenyatta National Hospital accident and emergency department between June and August 2007. KNH is a tertiary referral hospital located in Nairobi, the capital city of Kenya. Though most patients in the institution live in Nairobi, a good proportion are also referred from the district and provincial hospital countrywide. Up to 40% of the patients in the medical wards are HIV positive. Most patients seen at KNH are of low social economic status. The population is representative of almost all ethnic groups in Kenya.

SAMPLE SIZE ESTIMATION

The sample size was calculated using formula below: {11}

$$N = \frac{(Z1-\alpha)^2 \cdot P \cdot (1-P)}{\frac{2}{d^2}}$$

N	Minimum sample size
α	Level of significance = 5%
P	Prevalence of bacteremia in HIV patients with fever
d	Degree of precision = 4 %
$(Z1-\alpha)^2$	1.96 (from tables of standard normal distribution) corresponds to 95% confidence interval

The prevalence rate of bacteremia in HIV patients in Kenya in 1997 was 13.7 %. {17}
Thus the sample size is:

$$\frac{1.96^2 \times 0.13 \times 0.87}{0.04^2} = 271 \text{ patients}$$

The minimum sample size required was 271 patients.

Study population

All patients admitted to the medical wards at KNH aged 18 years and above presenting with fever in HIV-1 infection between June and September 2007.

CASE DEFINITION

Bacteremia was defined as two or more cultures positive for the same micro-organism of blood samples obtained on the same occasion from different sites.

Fever was defined as a single reading of axillary temperature above $\geq 37.5^0$C

HIV infection was defined as a positive HIV serologic test using the rapid test kits: Uni-Gold™ HIV and Determine™ HIV-1/2

PATIENT SELECTION

INCLUSION CRITERIA

1. Adults (>18 years) who are HIV positive
2. Body temperature (single axillary reading) of $\geq 37.5^0$C
3. Informed written consent

EXCLUSION CRITERIA

1. Patients who have taken an antibiotics in the past 14 days and less including anti-tuberculous drugs and Trimethoprim / Sulfamethoxazole
2. HIV-1 patients on HAART
3. Patients who were admitted to a hospital in the last 14 days

CLINICAL PROCEDURE

All eligible acute medical admissions were consecutively recruited into the study until a sample size of 271 was achieved. Patients or their next of kin were interviewed and a questionnaire was filled. (Appendix I). Those whose HIV status was not known were subjected to the Diagnostic Counseling and Testing. The 271 patients underwent a through history taking and physical examination and a clinical impression was made. The patients were thereafter staged according to the WHO guidelines. (Appendix II). There was no laboratory or radiological data obtained to support the clinical impression made.
A blood sample of about 22 ml was taken: 20mls for a set of two blood cultures and 2ml for CD4+ counts. All subjects were categorized according to the CDC 1993-revised classification system for HIV infection by CD4+ T cell categories. (Appendix VI, VII)

LABORATORY METHODS

GUIDELINES FOR BLOOD CULTURE COLLECTION

I. TIMING

Blood cultures were drawn prior to the institution of antibiotics and this did not jeopardize the management of the patient.

II. VOLUME OF BLOOD PER SET

Twenty milliliters of blood were obtained for a set of two blood cultures.

III. SITE OF BLOOD CULTURE

Blood was obtained from two different sites of either peripheral venous or arterial sites.

IV. PREPARATION OF THE SITE FOR CULTURE

1. After the vessel site was selected, a 5 cm area of skin was disinfected by swabbing concentrically with 70% alcohol, from the venepuncture site outward.
2. The site was cleansed once again, this time with 10% povidone-iodine or 2% tincture of iodine again in a circular motion.
3. The iodine was allowed to dry completely before performing venepuncture. This took 1 - 2 minutes.
4. While waiting for the site to dry, the plastic cap covering each blood culture bottle was removed, and the rubber stopper was decontaminated with 70% alcohol. 22 ml of blood was withdrawn in total.
6. Needles were not changed between venepuncture and inoculation of the bottles, or between bottles.
7. The iodine solution from the skin was removed with alcohol. This minimized the possibility of hypersensitivity

V. BLOOD CULTURE

1. 10 milliliters of blood was collected into 50 milliliters of supplemented Brain Heart infusion (BHI) broth to provide a blood broth dilution factor of 1:5.
2. The inoculated media was taken to the Center for Microbiology Research-KEMRI within two hours for processing.
3. Blood culture bottles were incubated at 35°C in a CO_2 incubator.
4. Subcultures were made every morning in a quarter of 5% Sheep Blood Agar (SBA), Chocolate Agar (CA) and MacConkey's Agar.
5. All the isolates were processed according to standard bacteriological procedure such as :

    a. Colony morphology
    b. Gram staining characteristics
    c. Spot tests like
        i. Catalase: Adding hydrogen peroxide to a culture sample or agar slant. If the bacteria in question produce catalase, they will convert

the hydrogen peroxide and oxygen gas will be evolved. The evolution of gas causes bubbles to form and is indicative of a positive test

ii. Oxidase: Presence of cytochrome oxidase can be detected through the use of an Oxidase Disk which acts as an electron donator to cytochrome oxidase. If the bacteria oxidize the disk (remove electrons) the disk will turn purple, indicating a positive test. No color change indicates a negative test

d. Biochemical characteristics

e. Enzymatic detections like

i. Urease: Members of genus *Proteus* are known to produce urease. Urease was detected by plating bacteria onto an amide containing medium, specifically urea. When urea is broken down, ammonia is released and the pH of the medium increases (becomes more basic). This pH change is detected by a pH indicator that turns pink in a basic environment. A pink medium indicates a positive test for urease.

ii. Coagulase: In differienting between pathogenic and non-pathogenic strains of *Staphylococcus.* The sample in question is usually inoculated onto 0.5 ml of rabbit plasma and incubated at 37 degrees celsius for one to four hours. A positive test is denoted by a clot formation in the test tube after the allotted time

f. Anti-microbial susceptibility tests by disc diffusion technique as per Clinical Laboratory Standards Institute (CLSI-USA)

g. Minimal inhibitory concentrations were undertaken on salmonella typhi isolates.

CD4+ Count

Two milliliters of blood was taken and transported to the laboratory in EDTA bottles within one hour of collection. CD4+ was will be determined by the automated flow cytometry analyzer, Fascaliber (benedict dick, USA).

FEASIBILITY OF STUDY

The study was carried out in the Accident and Emergency Department of Kenyatta National Hospital. This department is accessible to patients 24 hours a day, seven days a week. An average of seven patients presented with fever and were found to have HIV-1 every day.

The Accident and Emergency Department provided Diagnostic Counseling and Testing (DCT) services and thus new diagnoses of patients with HIV 1 were made.

The Center for Microbiology Research-KEMRI is located approximately 200 meters away from Kenyatta National Hospital. Samples were delivered to this premise within two hours of collection.

DATA HANDLING AND ANALYSIS

Data was cleaned, coded and entered into the microcomputer using SPSS/PC + Version 15. The study variables analysed were bacteremia, bacteria isolated, drug sensitivity patterns, CD4+ counts, WHO staging and the clinical diagnosis. Data analysis involved descriptive statistics such as mean, medians, and standard deviation for continuous variables and proportions and frequency distributions categorical variables.

To show association in the various variables such as bacteremia and bacteria isolated, WHO staging and clinical diagnosis the Pearson Chi square test was applied. The fischer's exact test was used where the data analysed was in small numbers. Associations

were measured and considered statistically significant at a p-value of 0.05 or less. Drug sensitivity patterns were presented in form of proportions. Summarized data was presented in form of tables, pie charts and graphs.

BENEFICIARY OF RESULTS

The patients were the direct beneficiaries of the study as all blood culture results and the sensitivity patterns where applicable were communicated to their primary doctor for appropriate management.

All eligible patients were referred to The Comprehensive Care Clinic in KNH for further follow up.

The protocol for antibiotic use in febrile HIV 1 patients use was derived from the sensitivity patterns of the bacteria grown.

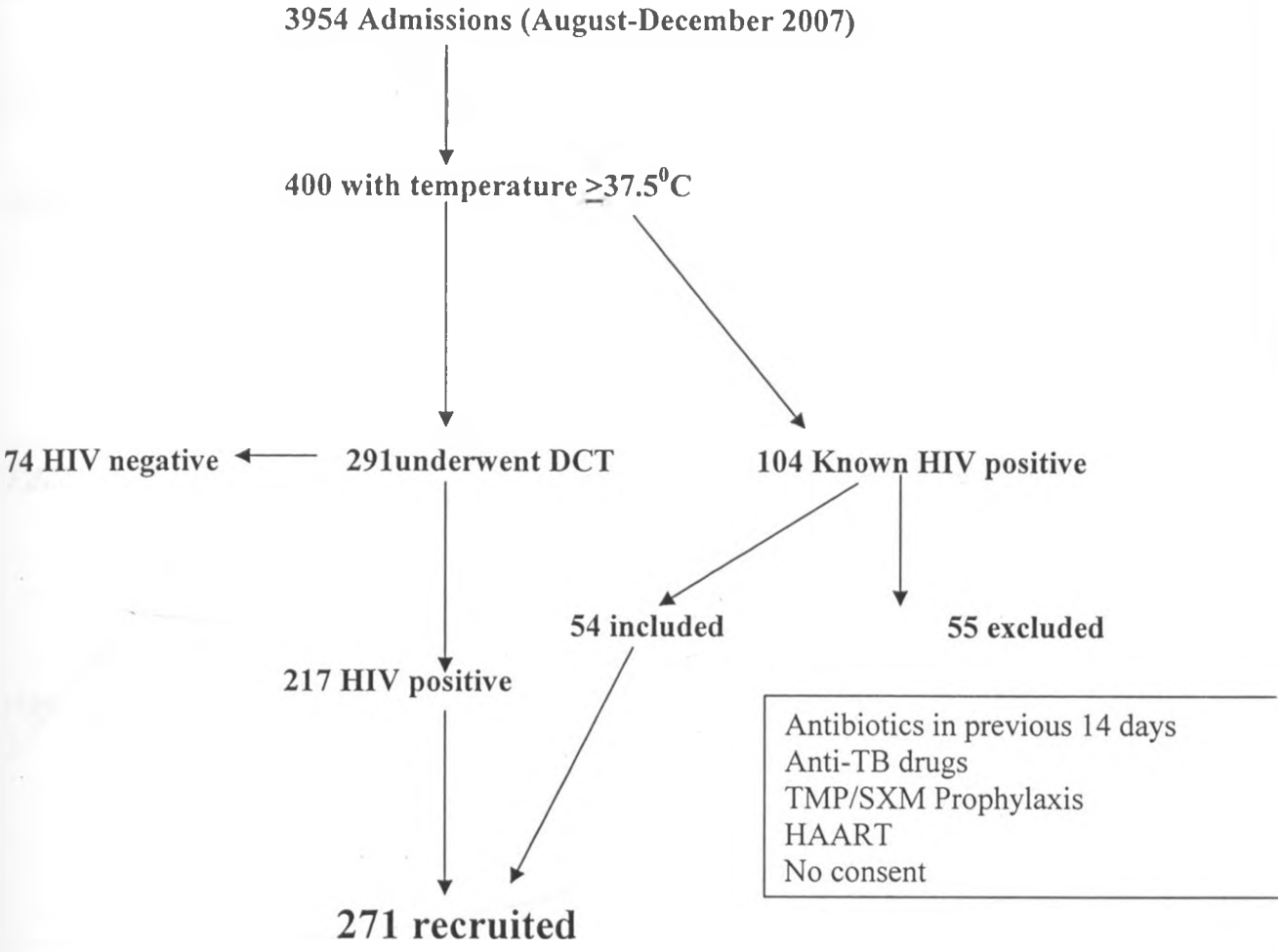
ETHICAL APPROVAL:

This study was carried out after approval of the University of Nairobi and the Kenyatta National Hospital Ethics Review Committees.

The intentions, benefits and risks were fully explained to eligible subjects (Appendix VIII). Only those who gave a written consent were recruited.

RESULTS:

Figure 1: RECRUITMENT OF PATIENTS



DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION

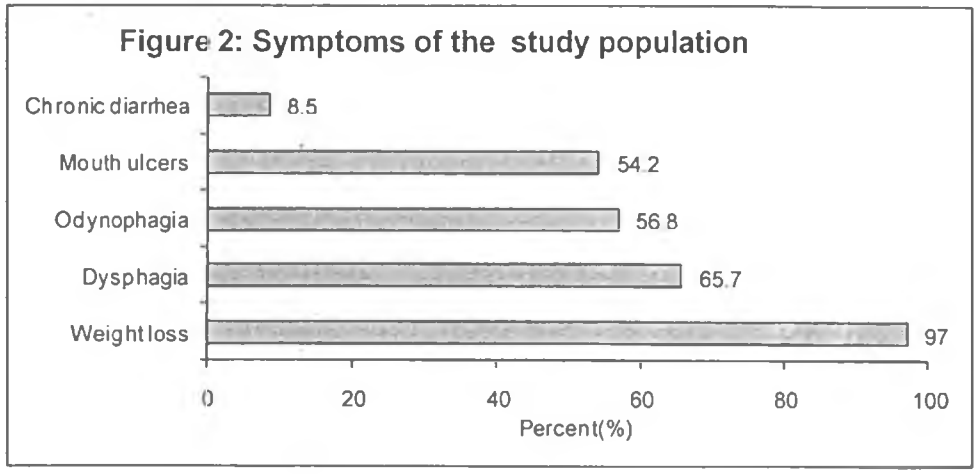
Table 1: Demographic Characteristics of the study population

DEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION		
		n (%)
Sex	Female	161 (60)
Marital Status	Single	67 (24.7)
	Married	165 (60.9)
	Widowed	20 (7.4)
	Separated/divorced	17 (6.3)
Education level	Primary	176 (64.9)
	Secondary	79 (29.2)
	Tertiary	14 (5.2)
Age	<20	4.8 (1.1)
	20-29	31 (31.9)
	30-39	45.2 (45.2)
	40-49	19 (15.9)
	>49	5.9
Residence	Urban	262 (97.4)

Females made up 60% of the study population. The male to female ratio was 1:1.5. 45% of the patients were in the age range of 30-39 years (median=32 years), 60.9% of the patients were married, 64.7% of the patients had attained primary school education and up to 97.4 % were living in the urban areas.

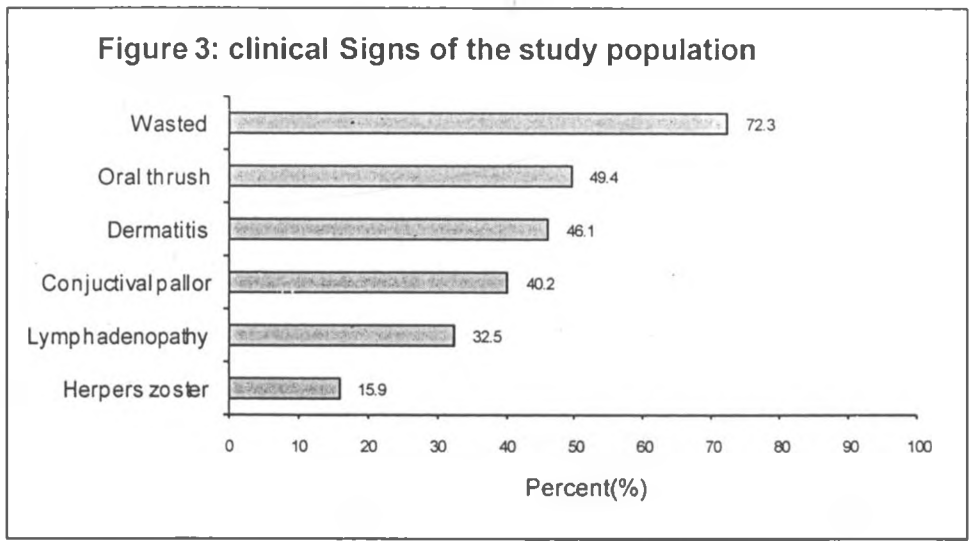
CLINICAL DATA OF STUDY POPULATION

Presenting symptoms of study population



Two hundred and sixty three patients (97 %) reported remarkable weight loss, 178 (65.7 %) had dysphagia and 154 (56.8%) had odynophagia. Only 8.5 % reported having had chronic diarrhea. (Figure 2)

Clinical signs of study populations



72.3% of the study population had wasting and 49.4% had oral thrush. One patient had varicella infection. (Figure 3)

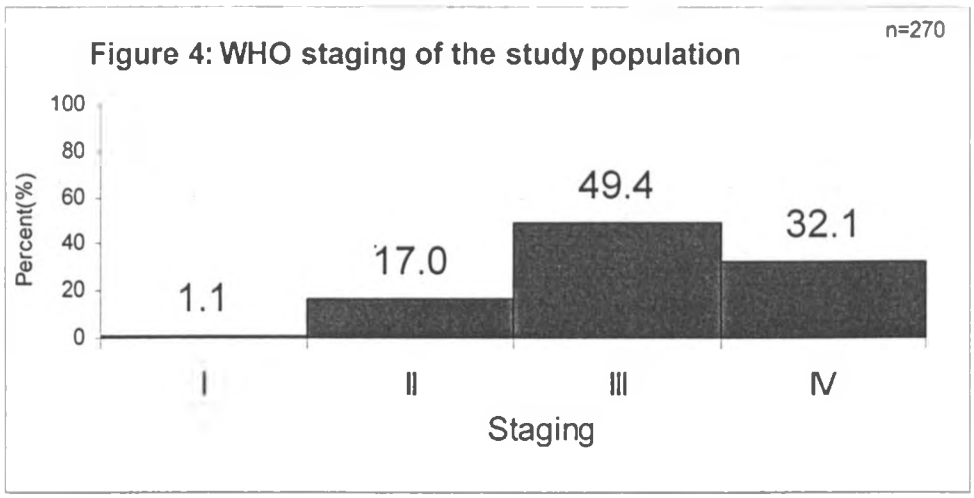
Possible Foci of Infection in the Study population

Table 2: Possible foci of infection

Clinical diagnosis	= n	Percentage
Pneumonia	118	43.5%
Meningitis	50	18.5%
Gastroenteritis	37	13.7%
A febrile illness	31	11.4%
Skin	8	3%
Others	43	15.9%

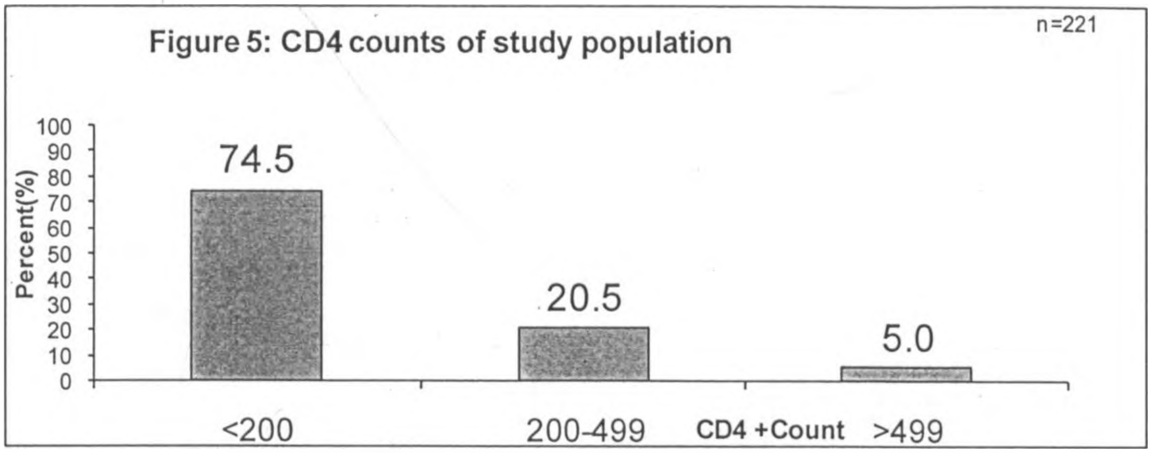
One hundred and eighteen patients (43.5%) had acute pneumonia as the clinical diagnosis, whereas fifty (18.5 %) had meningitis, thirty seven (13.7%) had gastroenteritis, thirty one (11.4%) had a febrile illness, eight patients (3%) had a skin pathology where as forty three (15.9%) were grouped as others (pyelo-nephritis, hepatitis, pulmonary tuberculosis, peritonitis and cholecystitis). One patient had a psoas abscess and another cervical abscess.

WHO staging of study population



The majority of the patients presented with WHO stage III and IV making up 81.5 %.

CD4 counts of the study population:

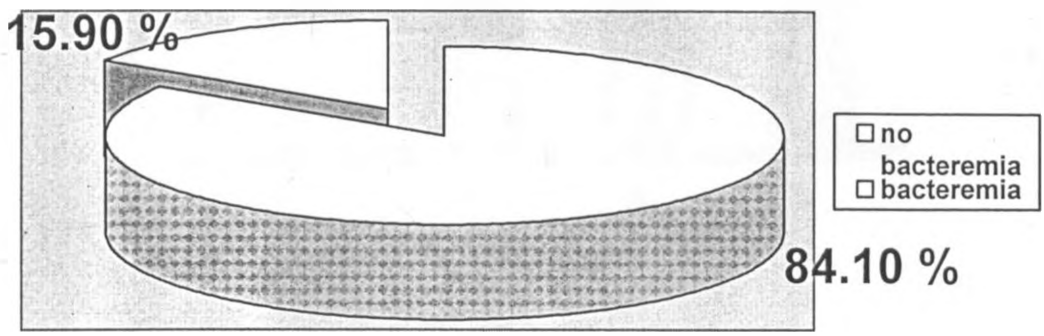


74.5 % of the patients had a CD4+ counts were less than <200cell/ul, mean of 147 .52 (±203) and a median of 69

MICROBIOLOGY

Prevalence of bacteremia:

Figure 6: Prevalence of Bacteremia



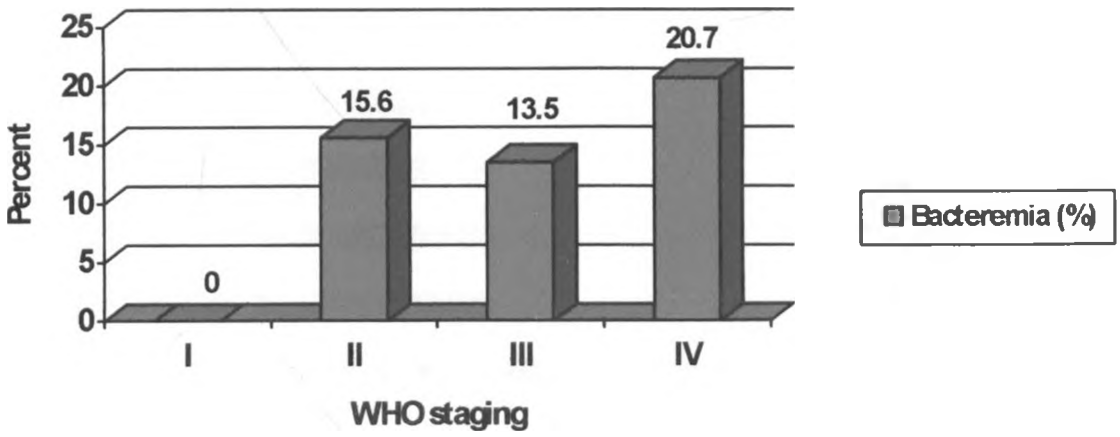
Forty three patients (15.9%) were found to have bacteremia. (95% CI, 11.5-20.3)

Bacteremia in relation to CD4+ counts

Bacteremia was evident in 14.6% of patients with CD4 counts below 200, 20.0% in CD4 counts ranging from 200-499 and 9.1 % with CD4 counts above 500. Nine patients (20.9%) with bacteremia did not have a CD4 count result. There was no significant difference of bacteremia in various CD4 count categories (pvalue=0.566).

Prevalence of Bacteremia in various WHO stages

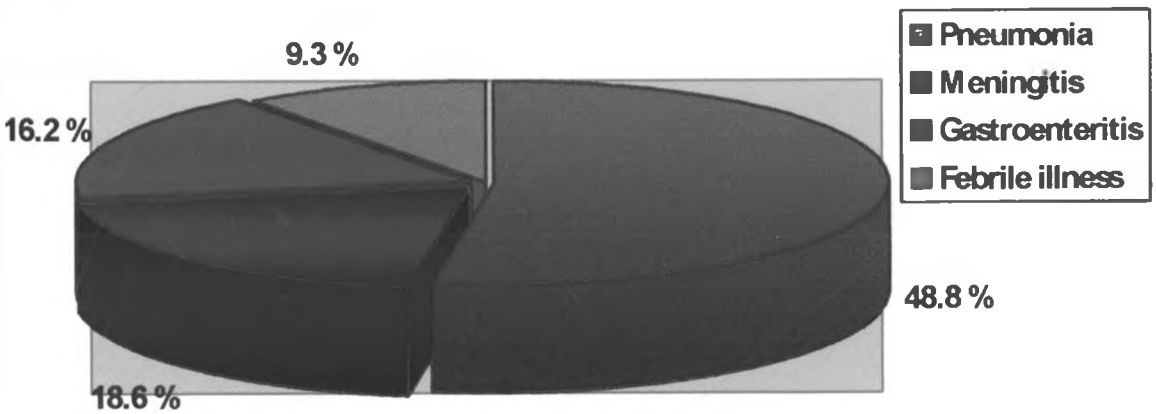
Figure 7: Prevalence of Bacteremia in various WHO stages



20.7 % of bacteremia was found in patients in WHO stage IV. There was no significant difference in bacteremia in patients in various WHO stages, with a p-value of 0.46. (Figure 7)

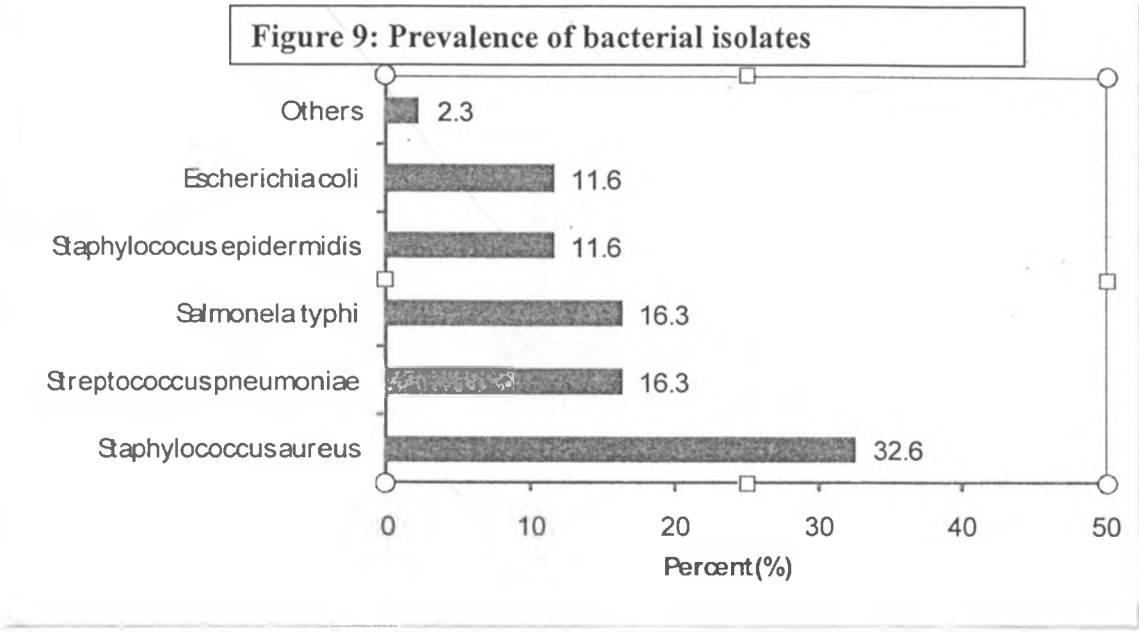
Bacteremia and the clinical diagnosis of the study population.

Figure 8: Distribution of bacteremia in various clinical diagnosis.



Majority of patients with bacteremia had acute pneumonia. There was however no correlation between bacteremia and clinical diagnosis made.

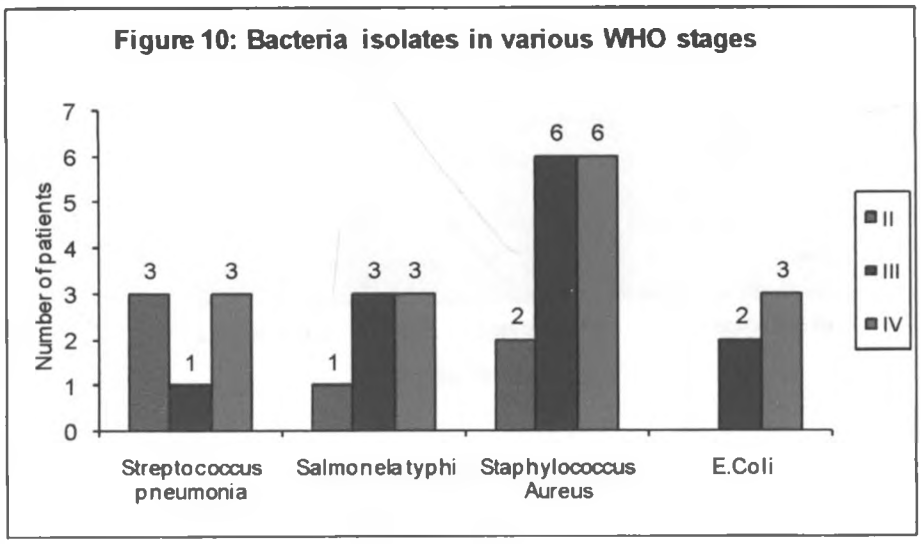
Bacterial isolates



Staphylococcus aureus was the commonest organism with a prevalence of 32.6 %, *Streptococcus pneumonia* and *Salmonella typhi* had a prevalence of 16.3 % each.

57.1% of the patients with *Streptococcus pneumoniae* had a clinical diagnosis of pneumonia. *57.1 % of patients with Salmonella typhi* had gastroenteritis. The majority of patients with staph aureus had pneumonia for diagnosis. There was no focus of skin infection noted in the patients with staphylococcus bacteremia. *Klebsiela pneumoniae* was isolated in a patient with pneumonia.

Bacteria isolates and WHO stages

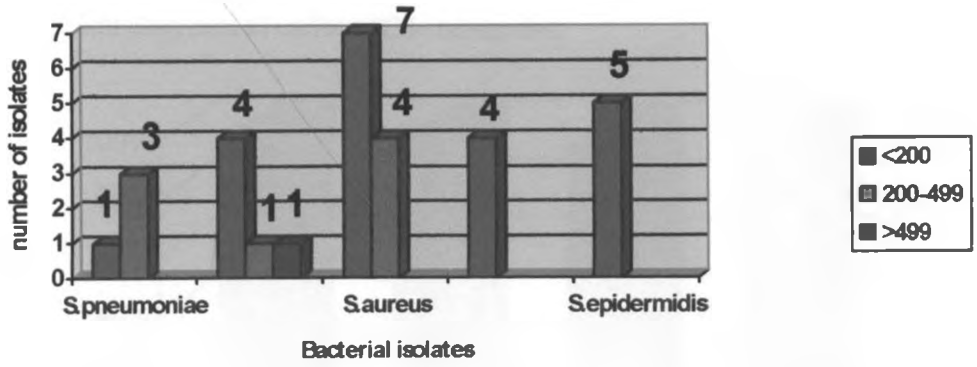


Streptococcus pneumoniae was isolated equally in patients in both WHO stages II and IV. *Salmonella typhi*, *Staphylococcus aureus*, *Echerichia coli* and *Staphylococcus epidermidis* were predominantly isolated in WHO stage III and IV.

Micrococcus species was isolated in a patient in WHO stage IV, whereas *Proteus mirabilis*, *Proteus vulgaris* and *Klebsiella pneumoniae* were found in patients in WHO stage III. Gram positive bacilli were isolated in a patient in WHO stage II.
There was however no significant difference in WHO staging and bacterial isolates occurrence.

Bacteria isolates at various CD4+ counts

Figure 11: Bacterial isolates at various categories of CD4+ counts.



Streptococcus pneumonia and *Klebsiella pneumoniae* was isolated in predominatly in patients CD4+ counts of 200 and 499 cell/ul. However *Salmonella typhi, Staphyloccccus aureus Echerichia coli, Staphylococcus epidermidis, Proteus mirabilis, Proteus vulgaris* and gram positive cocci were isolated in patients with CD4 counts below 200 cell/ul. There was no correlation between CD4+ counts and bacterial isolates.

Clinical diagnosis in comparison with WHO stages

Table 3: Clinical diagnosis at various WHO stages

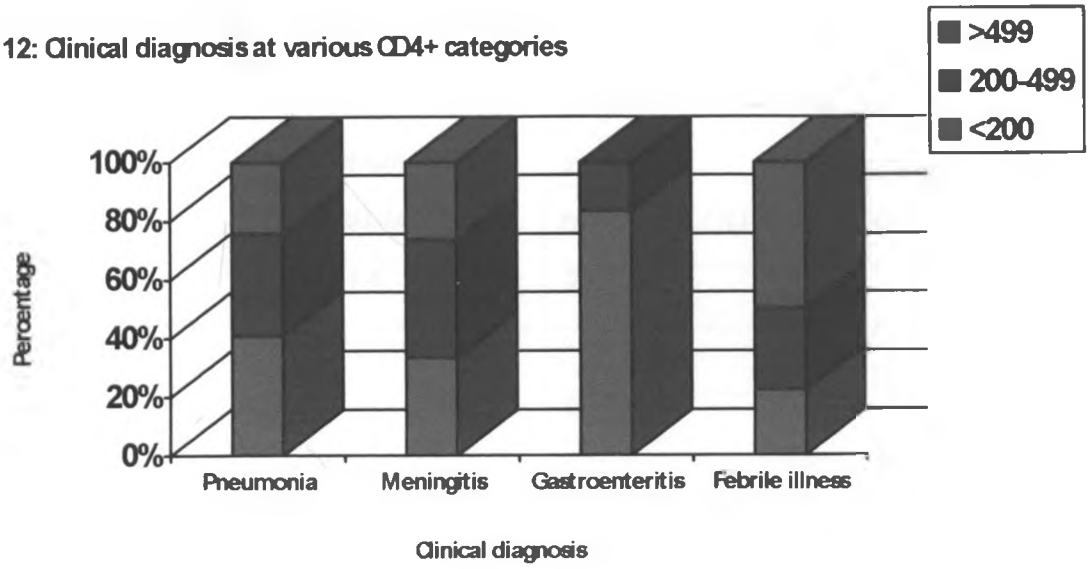
	WHO STAGES (%)				*p-value*
	I	**II**	**III**	**IV**	
Pneumonia	**33.3**	**50**	**42.5**	**44.8**	**.82**
Meningitis	**0**	**2.2**	**27.6**	**19.9**	**.0002***
Gastroenteritis	**0**	**10.9**	**8.2**	**24.1**	**.007#**
Febrile illness	**33.3**	**15.2**	**10.4**	**10.3**	**.51**

*** OR 3.32 (1.63-6.88) # OR 3.24 (1.51-7.0)**

There was a statistically significant correlation in patients in WHO stage III and Meningitis with a p-value of 0.0002 and in Gastroenteritis and WHO stage IV with a p value of 0.007.

Clinical diagnosis in comparison to CD4+ counts

Figure 12: Clinical diagnosis at various CD4+ categories



There was no significant occurrence of a clinical diagnosis in a particular CD4+ category. However gastroenteritis was mainly in patients with CD4+ counts below 200 cell/ul.

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS

Table 4: Antimicrobial susceptibility patterns for Gram positive bacteria

Antibiotics	*Streptococcus pneumoniae resistance (%)*	*Staphylococcus aureus resistance (%)*	*Staphylococcus epidermidis resistance (%)*
Oxacillin	71.4	21.4	40
Amoxy/clavulanic acid	0	-	-
Vancomycin	0	0	0
Linezolid	0	0	0
Ciprofloxacin	-	7.1	-
Erythromycin	14.3	21.4	-
Septrin	28.5	21.4	40

Streptococcus pneumoniae resistance to penicillin was high at 71.4 % but all isolates were sensitive to amoxycillin/clavulanic acid, linezolid and vancomycin. The prevalence of MRSA and MRSE were 21.4% and 40% respectively.

Table 5: Antimicrobial susceptibility pattern of Gram negative bacterial isolates

Antibiotics	*Salmonella typhi resistance (%)*	*Echerichia coli resistance (%)*
Ampicillin	0	20
Chloramphenical	100	60
Septrin	100	60
Ciprofloxacin	42.8	20
Ceftriaxone	0	0
Nalidixic acid	100	60

Salmonella typhi isolates were fully sensitive to both ceftriaxone. 42.8 % of *Salmonella typhi* had resistance to ciprofloxacin. There was complete resistance to septrin, chloramphenicol and ampicillin.

Discussion:

The females in the study population were 60% with a male to female ratio of 1:1.5, corresponding to the current epidemiological profile of HIV infected patients in Kenya. {23} Studies done by Busulwa and Obilo during the same study period showed a male to female ratio of 1:1.4 (unpublished data). The mean age was 33.5±8 years and was comparable to 34.6± 9.1 found in previous studies of similar population. {6, 17} 69.9% of the participants in the study were married reflecting the heterosexual mode of transmission in our environment, had a low level of education, were casual laborers and resided in the Nairobi province. They belong to a low social economic status which the Kenyatta National Hospital serves.

Acute pneumonia was diagnosed in 48.8 % of the study population, while the other common clinical diagnoses included meningitis, gastroenteritis and a febrile illness. The majority of the patients were in WHO stage III and IV. AIDS was found in 74.5% of the study population. This was the first contact with the health care systems for most patients and reflects on the impact of late diagnosis of HIV in our community. Previous studies conducted in Kenyatta National Hospital found 26-34% of patients as having clinical AIDS. {6, 17}

In our study the prevalence of bacteremia was 15.9%. This was in keeping with previous studies done at least a decade ago which showed a prevalence ranging from 5 to 28 %.{16} In Kenya consecutive prevalence studies showed that 26.3% of HIV–1 patients were found to have bacteremia in 1988-1989, 24.3% in 1992 and 13.7% in 1997. {6, 17}The prevalence also matches other studies done in west Africa where Grant et al in Abidjan, Côte d'Ivoire looking at patients admitted to the Infectious Disease Unit found a similar prevalence of 20%. {20} Piroon in Thailand found prevalence of bacteremia in HIV-infected patients to range between 20-30% each year. {15} Tumbarello et al stablished that the rate of bacteraemia in the total yearly HIV-related admissions in a University Hospital in Italy increased from 4% in 1985 to 13% in 1993. {29}

Staphylococcus aureus was the commonest organism isolated with a prevalence rate of 32.6 %. These patients however did not have focus of skin infection and none were intravenous drug abusers. This high prevalence may be accounted for by either a high prevalence of nasopharyngeal colonization in these patients or neutropenia which have been previously implicated. *Staphylococcus aureus* had been found to be the most prevalence organism in patients with fever in the major referral hospital in Nairobi. (Revathi G –unpublished data). In North America and European countries, Staphylococcus species is the most common cause of bacterial infections though these population groups have a high prevalence of intravenous drug use. {14, 29} Staphylococcus was isolated in patients with severe immonusuppression, with a mean CD4 + count of 89.2 cell/ul. This results were similar to those of Senthilkumar et al, in a study done in Italy where they reported a mean CD4+ cell count of 52 cells/ul in patients *Staphylococcus aureus* bacteremia. {28}

The prevalence of *Streptococcus pneumonia* was 16.3 % .This was surprising as almost 50% of the study population presented with pneumonia, and streptococcus pneumonia remains the commonest cause of pneumonia in this population. *Mycobacterium tuberculosis* and *Pneumocystiis jiroveci* infections may have accounted for some of the pneumonias. The prevalence rate however was similar to studies done in Africa and Europe. {6, 17, 29}*Streptococcus pneumoniae* was isolated in patients with a mean CD4 count of 289 cells /ul. *Streptococcus pneumoniae* has been shown to occur in all stages of HIV disease though there seem to be a higher incidence in patients with AIDS. {10}

There was a new pattern of bacteremia with *Salmonella typhi* having a prevalence of 16.3 %. An epidemic of *Salmonella typhi* was not reported during the study period. In all previous studies the non typhi salmonella were showed to cause bacteremia in HIV patients with salmonella typhi accounting for a small number. {6, 17, 29} Recurrent Salmonella bacteremia is an AIDS defining disease. The mean CD4+ counts in patients with salmonellosis was 203 cells/ul, however 66.7 % of the patients had a CD4+ below 200 cells/ul.

Staphylococcus epidermidis had a prevalence of 11.6%, although it is usually a contaminant, in this study if it was cultured from both specimen bottles it was classified as a possible cause of bacteremia. This was supported by the fact that these patients were found to have a CD4 count of less than a 100 cell/ul with a mean of 93 cell/ul. Non of the patients had an intravenous catheter or was an intravenous drug user as this accounted for Staphylococcus epidermidis bacteremia found by Tumbarello et al. He found the prevalence rate to be 10.9% and the patients had neutropenia and a low CD4 + T-cell count <200 cells/mm^3. {29}

The prevalence of *Escherichia coli* was 11.6%. All the patients had a CD4+ count below 200 cells/mm^3 and a mean CD4 count of 42.6 cells/ul. Previous Kenyan studies had shown a similar prevalence although CD4+ counts were not done. {17}

Streptococcus pneumoniae showed high resistance to penicillin at 71.4 % unfortunately the MIC were not done to categorise the level of resistance. All isolates were sensitive to amoxycillin/clavulanic acid which is available at Kenyatta. The first line antibiotic in Kenyatta is crystalline penicillin and most patients tend to improve on it thus high the resistance may be of a mild degree which may be overcome by a high dose. The prevalence of MRSA and MRSE were 21.4% and 40% respectively. This necessitates the use of vancomycin in patients who do not response to first line drugs. *Salmonella typhi* isolates were fully sensitive to ceftriaxone but 42.8% had resistance to ciprofloxacin which is the first line drug for treatment of typhoid in Kenyatta National Hospital.

There was no association between bacteremia and CD4+ count categories (p=0.566). In HIV disease bacteremia may not reflect the severity of immnunosuppression. In our study the mean CD4+ counts for patients with bacteremia is 128 cell/ul. Grant et al in Abidjan, Côte d'Ivoire found the mean CD4+ count for patients with bacteremia was 58 cell/ul.{20} In his study he did not have any patients with pneumonia unlike our study where they made up half of our study population and bacteremia in these patient was found at a higher CD4+ count.

Bacteremia was found in 20.7% of patients in WHO stage IV, 13.5% in WHO stage III and 15.6% WHO stage II. None of the patients in WHO Stage I had bacteremia. Bacteremia was evident in 14.6% of patients with CD4+ counts below 200, 20.0% in CD4+ counts ranging from 200-499 and 9.1 % with CD4+ counts above 500. The majority of patient with bacteremia had pneumonia at 48.8%, followed by meningitis at 18.6 %. The mean CD4+ cells in patients with pneumonia, meningitis and gastroenteritis were was 142, 145, and 137 cell/ul respectively. This was in keeping with the severe immnosuppression found in up to 70% of the study population. Grant et al in Abidjan had similar finding of mean CD4+ counts of 137 and 164 cell/ul in meningitis and gastroenteritis respectively.

Most of the meningitis patients were in WHO stage III with a p-value of <0.0002 whereas the majority of the patients with gastroenteritis were in WHO stage III with a p-value of <0.007. This is demonstrated by the WHO clinical staging whereby chronic diarrhea places the patient in stage III. The clinical diagnosis of pneumonia and nonspecific febrile illness were equally distributed in the four WHO stages with no significance difference.

Conclusion:

Bacteremia remains a major problem in HIV-1 patients with prevalence rates hardly showing a change in the past 10 years. *Streptococcus pneumoniae* still remains a major pathogen in this population. In this study we recorded the emergence of *Staphylococcus aureus* and *Salmonella typhi* bacteremia. The isolated bacteria were resistant to the drugs commonly used in Kenyatta National Hospital with 71.4% of *Streptococcus pneumoniae* being resistant to penicillin and 42.8% of *Salmonella typhi* showing intermediate resistance to ciprofloxacin. There were no evident association between CD4+ counts and bacteremia demonstrated in this study. However it was noted that most of the isolates were found in patients with AIDS.

Recommendations:

In this study the prevalence of bacteremia was 15.9 % therefore patients with fever in HIV should be investigated for bacteremia regardless of their CD4+ counts. Ceftriaxone is the drug of choice *Salmonella typhi* and amoxicillin/clavulanic acid for *Streptococcus pneumoniae*. More awareness and screening of HIV should be done in the population as most patients in this study presented with AIDS. Further studies are encouraged to confirm the emergence of new bacteremia such as Staphylococcus aureus and Salmonella typhi in HIV patients.

Limitations of the Study:

1. Undetected prior antibiotic use
2. Mycobacterial cultures were not done, and these are known to be causes of fever.
3. Some blood cultures took up to seven day to turn positive thus not available during the active management of patients.

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Appendix I

STUDY PROFORMA

DATE OF INTERVIEW ----------

PATIENT ☐☐☐☐☐☐☐ **NUMBER**

☐☐☐

STUDY NUMBER

1. **INITIALS** ----------
2. **SEX** 1) MALE 2) FEMALE ☐
3. **YEAR OF BIRTH**_____ ☐
4. **RESIDENCE** 1) RURAL 2) URBAN ☐
5. **MARITAL STATUS** 1) SINGLE 2) MARRIED ☐
   3) WIDOWED 4) DIVORCED / SEPARATED
6. **LEVEL OF EDUCATION**----------
7. **OCCUPATION**----------
8. **HISTORY**

	1) YES	2) NO
a. Weight loss	☐	☐
b. Body hotness / chills	☐	☐
c. Mouth sores	☐	☐
d. Dysphagia	☐	☐
e. Odynophagia	☐	☐
f. Chronic diarrhoea	☐	☐

9. PHYSICAL EXAMINATION

a. Wasting
b. Pallor
c. Oral thrash
d. Lymphadenopathy
e. Herpes zoster
f. Dermatitis
g. Systemic examination

10. CO-MORBID CONDITIONS **YES** **NO**

a. Pneumonia ☐ ☐
b. Meningitis ☐ ☐
c. Skin pathology ☐ ☐
d. Gastroenteritis ☐ ☐
e. Other ☐ ☐

11. WHO Clinical Staging I II III IV

12. Clinical stage of HIV ☐

13. CD4 counts ☐

14. Bacteria cultured ☐

15. Sensitivity patterns _____

Appendix II

WHO CLINICAL STAGING OF HIV DISEASE IN ADULTS AND ADOLESCENTS (2006 REVISION)

Clinical Stage I:

- Asymptomatic
- Persistent generalized lymphadenopathy

Clinical Stage II:

- Moderate unexplained weight loss (under 10% of presumed or measured body weight)
- Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)
- Herpes zoster
- Angular chelitis
- Recurrent oral ulceration
- Papular pruritic eruptions
- Seborrhoeic dermatitis
- Fungal nail infections

Clinical Stage III:

- Unexplained severe weight loss (over 10% of presumed or measured body weight)
- Unexplained chronic diarrhoea for longer than one month
- Unexplained persistent fever (intermittent or constant for longer than one month)
- Persistent oral candidiasis

- Oral hairy leukoplakia
- Pulmonary tuberculosis
- Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
- Unexplained anaemia (below 8 g/dl), neutropenia (below 0.5 billion/l) and/or chronic thrombocytopenia (below 50 billion/l)

Clinical Stage IV:

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)
- Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- Extrapulmonary tuberculosis
- Kaposi sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Chronic cryptosporidiosis
- Chronic isosporiasis
- Disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis)
- Recurrent septicaemia (including non-typhoidal Salmonella)
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy

Appendix III

Fascaliber Machine For CD4 Count (Benedict Dick USA)

Procedure

1. Take blood in an EDTA K_3 sterile bottle
2. Mix the blood to avoid clotting
3. Take 50 mcl of the blood into already made reagents (monoclonal antibodies) for CD4 and CD8 cells
4. Incubate for 1-2 hours to allow stating to take place
5. Fix the already stained samples with fixative
6. Read the machine within 30 minutes.

Appendix IV

CDC classification system for HIV infected adolescents and adults

CD4+ T-Lymphocyte Categories

The three CD4+ T-lymphocyte categories are defined as follows:

- Category 1: greater than or equal to 500 cells/mL
- Category 2: 200-499 cells/uL
- Category 3: less than 200 cells/uL

Clinical Categories

The clinical categories of HIV infection are defined as follows: Category A

Category A consists of one or more of the conditions listed below in an adolescent or adult (greater than or equal to 13 years) with documented HIV infection. Conditions listed in Categories B and C must not have occurred.

- Asymptomatic HIV infection

- Persistent generalized lymphadenopathy
- Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Category B consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria:

Examples of conditions in clinical Category B include, but are not limited to:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting greater than 1 month
- Hairy leukoplakia, oral
- Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess
- Peripheral neuropathy

Category C

Category C includes the clinical conditions listed in the AIDS surveillance case definition. For classification purposes, once a Category C condition has occurred, the person will remain in Category C.

Conditions included in the 1993 AIDS surveillance case definition

- Candidiasis of bronchi, trachea, or lungs

- Candidiasis, esophageal
- Cervical cancer, invasive
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)
- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain
- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent *
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV

Appendix V

CONSENT EXPLANATION

Introduction:

My name is Dr Kimeu Redemptar Mwelu. I am a postgraduate student pursuing a masters Degree in Internal Medicine in the University of Nairobi. Part of my training requires me to carry out a research project, which involves an evaluation of different aspects of persons who are ill.

I am doing a research on the various bacteria, which can be found in blood of patients with HIV and fever.

To carry out this research, I require to take the history of your illness and examine you. I will then draw 22 millilitres of blood from you for a blood culture and CD4+ counts. I will carry out these processes which are standard clinical evaluation methods. I will do them in a professional way.

Benefits:

This study is aimed at identifying bacteria affecting the patient at the time of the test and establishing the drug sensitivities. This will help in making a definitive choice of antibiotic in the patients.

The blood culture results either confirming a bacterimia and the sensitivity patterns, or denying the same will be discussed with the primary clinician who will take the necessary action.

Patients who will fit in the criteria for start of HAART will be referred to the Comprehensive Care Clinic (CCC).

Risks: History taking and physical examination have no risks. Taking a blood sample will cause a minor discomfort. The amount of blood for the test is not excessive to reduce your circulating volume unduly.

Participation: This is entirely voluntary. All information collected will be confidential. Participants have the right to withdraw from study without jeopardy to their current treatment.

I will now address other concerns that have not been covered in the explanation.

If you are ready and want to be part of this study I will kindly request you to sign here below.

The study has been approved by the Kenyatta National Hospital Ethics Research Committee of P.O Box 20723, Nairobi, Telephone, 2726300-9.

CONSENT

Consent by patient / next of kin for participation in the study.

I ..... Of .....

Hereby consent to participate in this study / research, the nature of which has been fully explained to me by DR.....of P.O BOX 46477-00100, Nairobi.

I am required to give a blood sample of 22 milliliters for a blood culture and CD4 + counts. I understand that DR Kimeu R M shall use the results of these tests for research work only.

Date..... Signed.....

I confirm that I have explained to the patient the nature of the study and tests to be done.

Date..... Signed.....