THE ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN PATIENTS WITH BLOOD CULTURE POSITIVE SEPSIS AT THE ACCIDENT AND EMERGENCY DEPARTMENT KENYATTA NATIONAL HOSPITAL.

DR. OTIENO GEORGE OCHIENG’
H58/79718/2012
DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTERS OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI

2016
DECLARATION

I Dr. Otieno George Ochieng' declare that this thesis is my original work and that to the best of my knowledge it has not been presented for the award of a degree in any other university.

Signed ………………………..

Date ………………………..
SUPERVISORS’ DECLARATION

This thesis is submitted with our approval

1. Dr. Loice Achieng
   Consultant Physician, Infectious Disease Specialist,
   Lecturer,
   Department of Clinical Medicine and Therapeutics,
   University of Nairobi

   Signed ………………………….   Date ……………………

2. Prof. Elijah Ogola,
   Consultant Physician and Cardiologist,
   Associate Professor, Internal medicine,
   Department of Clinical Medicine and Therapeutics,
   University of Nairobi

   Signed ………………………….   Date ……………………

3. Prof. Walter Jaoko,
   Consultant, Infectious Disease Specialist,
   Professor of Medicine (Microbiology),
   Department of Microbiology,
   University of Nairobi

   Signed ………………………….   Date ……………………
# TABLE OF CONTENTS

- DECLARATION .................................................................................................................. ii
- SUPERVISORS’ DECLARATION .................................................................................. iii
- TABLE OF CONTENTS ................................................................................................. iv
- LIST OF FIGURES AND TABLES ............................................................................... viii
- ACKNOWLEDGEMENTS .......................................................................................... ix
- ABSTRACT .................................................................................................................... x
  - Background ............................................................................................................... x
  - Study objective .......................................................................................................... x
  - Methodology .............................................................................................................. x
  - Results .........................................................................................................................
  - Conclusions .............................................................................................................. xi
- 1.0 INTRODUCTION AND LITERATURE REVIEW ......................................................... 1
  - 1.1 Background ........................................................................................................... 1
  - 1.2 Definitions ............................................................................................................ 1
  - 1.3.0 Diagnosis of sepsis ............................................................................................ 2
    - 1.3.1 Clinical features of sepsis .............................................................................. 2
    - 1.3.2 Common foci of sepsis ..................................................................................... 3
    - 1.3.3 Laboratory methods useful in approaching a septic patient ............................ 3
    - 1.3.4 Blood Culture .................................................................................................. 3
  - 1.4 Organisms causing sepsis ....................................................................................... 4
  - 1.5 Susceptibility patterns among patients with blood culture positive septicemia ..... 5
  - 1.6 Recognition and response to sepsis in Africa and Kenya-situation analysis ........................ 5
  - 1.6 Problem statement ............................................................................................... 6
- 2.0 STUDY JUSTIFICATION .......................................................................................... 7
- 3.0 RESEARCH QUESTION AND HYPOTHESIS ......................................................... 8
- 4.0 OBJECTIVES .......................................................................................................... 8
  - 4.1 Broad objective .................................................................................................... 8
  - 4.2 Specific objectives ............................................................................................... 8
  - 4.3 Secondary objective ........................................................................................... 8
- 5.0 METHODOLOGY ...................................................................................................... 9
  - 5.1 Study location ....................................................................................................... 9
  - 5.2 Study design ......................................................................................................... 9
  - 5.3 Study population .................................................................................................. 9
    - 5.3.1 Case definition ............................................................................................... 9
LIST OF ABBREVIATIONS

ACCP- American College of Chest physicians
AIDS- Acquired Immune Deficiency Syndrome
ARDS- Adult Respiratory Distress Syndrome
BA- Blood Agar
BAP- Blood Agar Plate
BHI - Brain Heart Infusion
BP- Blood Pressure
BSI- Blood stream infection
CAP- Chocolate Agar Plate
CBA- Chocolate Blood Agar
CLSI- Clinical and Laboratory Standards Institute (CLSI)
CONS- Coagulase Negative *Staphylococcus aureus*
COPD- Chronic Obstructive Pulmonary Disease
CNS - Central Nervous System
DM- Diabetes mellitus
ESBL- Extended spectrum beta lactamase
GNB- Gram negative bacteria
GPB- Gram positive bacteria
HIV- Human Immunodeficiency Virus
ICU- Intensive Care Unit
KNH- Kenyatta National Hospital
MH - Mueller Hinton
MIC Minimum Inhibitory Concentration
MODS - Multiple Organ Dysfunction Syndrome
MRSA- Methicillin Resistance *Staphylococcus aureus*
NaCl- Sodium Chloride
PI- Primary Investigator
RR- Respiratory Rate
SIRS - Systemic Inflammatory Response Syndrome
SCCM- Society of Critical Care Medicine
TSI- Triple Sugar Iron
UTI- Urinary tract Infection
VRSA- Vancomycin Resistant Staphylococcus aureus
LIST OF FIGURES AND TABLES

FIGURES
Figure 1: Flow chart demonstrating the recruitment process........................................17
Figure 2: A Pie Chart showing frequency of infection by site....................................20

TABLES
Table 1a: Baseline socio-demographic characteristics.................................................18
Table 1b: Baseline clinical characteristics .....................................................................18
Table 2: Presenting complaints (in percentage frequency) ...........................................19
Table 3: Frequency of isolation per organism...............................................................21
Table 4: Original raw data on individual susceptibility patterns per organism ..........22
Table 5: Susceptibility patterns (in percentage) per organism groups.........................23
ACKNOWLEDGEMENTS

I would sincerely want to thank the Almighty God for strengthening me through and through.

Thanks to all my three supervisors for the commitment they ensured despite their busy schedules.

I would also like to express my heartfelt appreciations to my entire family and especially my wife Lilian who embodies my inspiration.

All my colleagues were very helpful with the thesis writing, I owe them gratitude.
ABSTRACT

Background

Sepsis and septic shock are a major cause of morbidity and mortality globally. The case fatality rate in the developing countries is twice that in the developed world.

Being a treatable cause of mortality, a lot of research has gone into determining the causes of death in patients who present with sepsis. Key among them is the fact that most patients especially in the developing countries present late having gone into septic shock and end organ damage. In addition, most centers in the developing world do not have antimicrobial sensitivity patterns to inform appropriate empiric therapy early in the disease.

Study objective

To determine the antimicrobial susceptibility patterns among patients with blood culture positive sepsis at the accident and emergency department Kenyatta National Hospital, Nairobi-Kenya.

Methodology

This was a descriptive cross sectional study. The study participants were adults attending the accident and emergency department of Kenyatta National Hospital. A total of 288 patients with SIRS were screened and an informed consent obtained on 232 patients who met the study criteria.

A targeted history and examination to identify the foci of infection was performed then two blood culture samples were obtained per patient using a standard, aseptic process.

The samples were sent to the University of Nairobi microbiology laboratory for incubation and susceptibility testing (using disc diffusion method) within three hours of collection.

Results

We recruited 232 patients out of which 120 (51.7%) were female and 15% were HIV positive. Twenty four percent of our patients were referred from another facility. A third (28%) had used antibiotics prior to recruitment.

Infections of the respiratory system were the most frequent (26%) followed by soft tissue infections (20%).
Out of the 232 blood samples drawn for culture, a total of 15 (6.5%) grew pathologically significant bacteria. The most common organisms were gram positive bacteria with Coagulase negative *Staphylococcus aureus* being the most common at 40%.

As far as the susceptibility to antibiotics was concerned, the majority of the bacteria were susceptible to the most commonly used antibiotics, with the least resistance against carbapenems and third generation cephalosporins. The highest resistance was against the penicillins and the macrolides.

**Conclusions**

Majority of our patients were from the community and the most common causative organisms were gram positive bacteria with high susceptibility to the commonly used antibiotics. Coagulase negative *Staphylococcus aureus* was the most commonly isolated organism. Most organisms were sensitive to levofloxacin, third generation cephalosporins and carbapenems.
1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Sepsis is recognized worldwide as a major cause of mortality(1), with an increasing incidence of eight to thirteen percent annually(2-4). Adhikari et al. in 2010 estimated an incidence of 19 million sepsis cases worldwide per year(5). Great gains have been made in the understanding of the pathophysiology of sepsis and the therapeutic approaches in its management but this has only created a small positive dent in outcomes due in part to the lack of compliance by clinicians(6). The number of hospitalizations for sepsis are still reported as doubling over the last 10 years with more people being hospitalized for sepsis compared to heart attack in the western countries(7).

Early identification of SIRS and the focus of infection(8) coupled with timely initiation of appropriate therapy with intravenous fluids and antimicrobial agents is key in averting mortality due to sepsis(6, 9). The choice of antimicrobial therapy must be directed by the local antimicrobial susceptibility patterns if we are to reduce mortality and the development of resistance.

These concepts are outlined in the six hour bundles as given by the Surviving Sepsis Campaign Guidelines. Compliance to this guideline has been shown to improve survival from sepsis and septic shock(10, 11).

1.2 Definitions

Hippocrates first described sepsis as the process by which flesh rots, swamps generate foul airs, and wounds fester(12). Currently the term refers to systemic inflammatory response (SIRS) to an infection.

Given the complexity of sepsis, guidelines that define at what point in the continuum of sepsis a patient resides have been developed by the American College of Chest physicians and the Society of Critical Care Medicine(13-15). This continuum includes SIRS, sepsis, severe sepsis, septic shock and multi-organ dysfunction syndrome (MODS).

a) Systemic inflammatory response syndrome (SIRS) is the systemic inflammatory response to a variety of severe clinical insults. The response is manifested by two or more of the following conditions:
(1) Temperature >38 degrees Celsius or <36 degrees Celsius;
(2) Heart rate >90 beats per minute;
(3) Respiratory rate >20 breaths per minute or Pa CO2<32 mm Hg; and
(4) White blood cell count >12,000/cu mm, <4,000/cu mm, or >10% immature (band) forms

b) Sepsis is the SIRS attributable to an infection
c) Severe sepsis is sepsis associated with organ dysfunction, hypoperfusion, or hypotension. Hypoperfusion and perfusion abnormalities may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status.
d) Septic shock is sepsis plus a systolic blood pressure <90 mm Hg or a reduction of SBP by more than 40 mm Hg from baseline in the absence of other causes for hypotension.
e) Multiple organ dysfunction syndrome (MODS) is sepsis with a dysfunction of more than one organ.

1.3.0 Diagnosis of sepsis

The diagnosis of sepsis is mainly dependent upon clinical findings(16). Over the past 10 to 15 years great advancement has been made in defining markers useful both for diagnosis and prognostication(6). Despite these achievements, it has become increasingly evident that the clinician’s role in picking the clinical spectrum that defines SIRS is an important entry point (8). One also needs to recognize patients’ risks of sepsis including chronic diseases such as AIDS, malignancies, COPD, diabetes and the use of immunosuppressive agents. Old age, male gender, the offending infectious agent and timelines for therapy are thought to be the main determinants of the severity of sepsis and organ dysfunction(4, 17).

1.3.1 Clinical features of sepsis

The clinical features of sepsis vary based on infection site, the infectious agent, type of acute organ dysfunction, co-morbidities and duration from onset of illness(15). The most commonly affected organs are the respiratory and cardiovascular systems. Respiratory compromise is classically manifested as acute respiratory distress syndrome (ARDS)(18) while cardiovascular compromise manifests primarily as hypotension or an elevated serum lactate level. The brain when affected manifests as delirium or altered mental status while acute kidney injury presents as anuria or oliguria. Critical illness polyneuropathy and myopathy are also common, especially in patients with a pro-longed ICU stay. Paralytic ileus, elevated
aminotransferase levels, altered glycemic control, thrombocytopenia and disseminated intravascular coagulation, adrenal dysfunction, and the euthyroid sick syndrome are all common in patients with severe sepsis(14).

1.3.2 Common foci of sepsis

It is the identification of a probable or confirmed focus of infection that changes a diagnosis from SIRS to sepsis. Identifying a definite site of infection is possible in upto 70 to 80 percent of patients (19-21) and among these 20 to 30 percent have sterile cultures or questionable microbiologic isolates (22, 23). According to a study by Cohen et al in 1999, the most common site of infection is the respiratory system in almost half of the cases followed by the abdominal and genitourinary systems with the skin and soft tissue foci forming a minority (24).

1.3.3 Laboratory methods useful in approaching a septic patient

Sepsis is majorly a clinical diagnosis with most of the laboratory work up done for purposes of monitoring the patient management e.g. serum lactate, blood gases and other measures of end organ damage(1). There are however useful diagnostic tests such as blood cultures, cultures of fluids or samples from the foci of infection and procalcitonin levels (5). The latter test is an accurate indicator of bacterial blood infection but does not help determine the exact aetiology of sepsis. Blood cultures therefore, remains the main diagnostic test especially in so far as defining the aetiology of sepsis and in determining antibiotic susceptibility.

1.3.4 Blood Culture

Blood culture is a common laboratory investigation where blood is inoculated into culture medium and incubated. Positive blood cultures are the accepted proof of serious infection, but blood cultures are positive in only approximately 30 percent of patients (20, 25, 26).

The media used in most blood culture bottles support the growth of medically important bacteria and fungi. Anaerobic bacteria grow adequately in the aerobic blood culture bottle, hence separate anaerobic bottles are infrequently used(27, 28).

The yield of blood culture is increased by a number of factors e.g. increasing the volume of blood sampled to greater than ten milliliters per culture bottle(29), the number of samples obtained i.e. two or more cultures drawn either simultaneously from different sites or over a period of not less than an hour apart within the first 24hours(30). In an article by Federico
G.N in 2007, the cumulative sensitivity of blood cultures collected during a 24 hour period of sepsis diagnosis was as follows; 73.1 percent of mono-microbial episodes were detected with the first blood culture, 89.7 percent with the first two blood cultures, 98.2 percent with the first three and 99.8 percent with the first four(30). This was consistent with the finding from the Mayo clinic study in 2004 that suggested a detection rate of about 90 percent when two blood cultures were drawn and an increasing yield with more than two cultures especially in patients with low level bacteremia or patients with prior antibiotic exposure(31).

Systematic studies conducted on the timing of blood cultures and the optimal interval between successive tests have constantly revealed that it is ideal to collect two or more blood cultures one to several hours apart(32, 33) however, because of technical challenges it has been proven as sufficiently appropriate to collect blood from two separate sites within minutes of each other (34).

The type of organism also affects the yield of culture with *Staphylococcus aureus* being the easiest to detect with the first culture sample at a detection rate of 90 percent while *Pseudomonas aeruginosa* and *Candida albicans* the least detectable (at 60 percent) with the first sample.

Recommendations by the infectious Disease society of America and the American Microbiology Association encourages the following: Drawing of upto 20mls of blood per patient, disinfecting the venupuncture site with chlorhexidine or 2%iodine tincture, avoiding drawing of blood from catheters, concurrent submission of blood drawn from a venupuncture when catheter tips are being cultured, use of two to three blood culture bottles per adult with at least one aerobic and one anaerobic and lastly, never refrigerating blood before incubation(35).

**1.4 Organisms causing sepsis**

Most studies indicate that of all the causes of bacteremia, 44–48% are Gram-negative and 44–49% gram-positive organisms(4, 24). An increasing role of gram-negative bacteria has been documented among blood isolates, especially in healthcare-associated infections with *Escherichia coli* being the most common(19). The proportion of fungemia is clearly dependent on whether the sepsis is healthcare- or community-acquired (24). There is also a clear variation between prevalence of causative organisms in studies conducted in different countries.
An increase in Gram-negative organisms as a cause of healthcare-acquired infections is increasingly being reported, and the proportion of healthcare-acquired infections from the *Candida* species has doubled from 5.8% in 1999 to 11.3% in 2003(19). The risk of candidemia is related to the degree of immunosuppression.

1.5 Susceptibility patterns among patients with blood culture positive septicemia.

Different geographical regions have varying susceptibility and resistance patterns necessitating the need to profile organisms in order to come up with the appropriate empiric therapies. In a cross sectional observational study by Fayyaz M et al in Rawalpindi, Pakistan, *Staphylococcus spp* were 100% susceptible to vancomycin and linezolid. The susceptibility of *Enterobacteriaceae spp* was 85.7% to amikacin. Tigecycline had only 61.2% sensitivity and imipenem 59.2% sensitivity against *Enterobacteriaceae spp*(36).

Gohel et al also did a retrospective study in India (2007) in which he demonstrated that gram positive isolates were most sensitive to vancomycin, linezolid, teicoplanin and clindamycin. Gram negative isolates were most sensitive to carbapenems, colistin, aminoglycocides and tigecycline. Methicillin resistant *Staphylococcus aureus* (MRSA) were found in 70.6% of cases with 21.6% of the isolates being vancomycin resistant *Staphylococcus aureus* (VRSA). He also demonstrated that *Enterobacteriaceae spp* had very poor susceptibility (0-12%) to quinolones, penicillins and cephalosporins. Extended spectrum beta lactamase producers (ESBL) were detected in 39.6% of the isolates(37).

Usha et al in 2007 demonstrated that ampicillin had a resistance of 86.1% contrasted with that of piperacillin at 57.7% among gram negative isolates. The gram positive organisms had a resistance of 70% to erythromycin(38).

These patterns together with similar ones across the world are a worrying trend and pose a threat to the fight against blood stream infection.

1.6 Recognition and response to sepsis in Africa and Kenya-situation analysis

Africa hosts 90% of the infectious disease burden in the world with a mortality rate higher compared to that in the developed nations (50-80% compared to 30-40%)(39, 40). The main contributions to mortality are; high burden of disease, late presentation compounded by limited and ill equipped facilities, high rate of antimicrobial resistance, minimal data
available on local susceptibility patterns and thus irrational use of antibiotics and minimal emphasis on preventive medicine (26).

In a retrospective study by Mulat et al in 2013 in Gondar University Hospital, Ethiopia, antibiotic resistance was found to be a big problem with the level of gram negative antimicrobial resistance ranging between 20-100% while that of gram positive being 23-58% (41), this is consistent with other studies elsewhere in Africa (42, 43).

It is clear that timely and appropriate use of antibiotics is currently the only effective way to control bacteremia (44). Therefore, the rising trends of resistance to antimicrobial agents are a public health concern especially in resource limited settings. This is because of the limited availability of effective new generation antimicrobial agents in the background of paucity of data as regards antimicrobial susceptibility.

The yields of blood cultures among septic patients locally is quite dismal, and a survey of the most commonly used laboratories gave us a culture positivity rate ranging between 4 percent to 20 percent. We believe that this study will form part of the greater data base from which useful information on antimicrobial sensitivity patterns will be derived.

1.6 Problem statement

The trends discussed above especially rising antibiotic resistance in developing nations is concerning. The need to tailor empiric antibiotic therapy based on local patterns and de-escalate upon receiving blood culture results is key. We, therefore, should equip the available microbiology laboratories so that we may continuously profile the aetiologies of sepsis and their antimicrobial susceptibility in our setting. An informed choice of antibiotics in our setting will reduce mortality from sepsis while also bringing down the cost of healthcare through reduced days of hospitalization.
2.0 STUDY JUSTIFICATION

Sepsis is a major cause of morbidity and mortality in African and across the world. We have clearly defined ways of improving these outcomes.

Key among them is the creation of antibiogram that helps in the early initiation of appropriate empiric antibiotic therapy. De-escalation of antibiotics is also dependent upon the isolation of a particular causative agent and the culture and susceptibility patterns thereof.

There is currently no data in Kenya to describe the antibiotic susceptibility patterns in the vast majority of our hospitals especially within the public sector.

This study will help develop an antibiogram for Kenyatta National Hospital(KNH).
3.0 RESEARCH QUESTION AND HYPOTHESIS

What are the appropriate antimicrobial choices for septic patients presenting in KNH accident and emergency department?

Null hypothesis

The current antimicrobial therapy for sepsis in the accident and emergency department KNH is adequate and appropriate.

4.0 OBJECTIVES

4.1 Broad objective

To determine the antimicrobial susceptibility patterns among patients with blood culture positive sepsis at the Accident and Emergency department Kenyatta National Hospital, Nairobi-Kenya.

4.2 Specific objectives

1. To identify causes of blood culture positive sepsis among patients attending the accident and emergency department Kenyatta National Hospital

2. To determine the antimicrobial susceptibility patterns among patients with blood culture positive sepsis at the Kenyatta National Hospital accident and emergency department.

4.3 Secondary objective

To describe the foci of infection in patients with blood culture positive sepsis at KNH.
5.0 METHODOLOGY

5.1 Study location

The study was conducted in the accident and emergency department of Kenyatta National Hospital, Nairobi. Kenyatta National Hospital is the national referral facility and the teaching hospital for the University of Nairobi Medical School.

5.2 Study design

This was a descriptive cross-sectional study.

5.3 Study population

Patients aged above 18 years attending the accident and emergency department with a diagnosis of sepsis.

5.3.1 Case definition

Any patient with suspected sepsis whose blood culture was positive for any pathologic bacteria.

Inclusion.

All patients had:

1. Features of SIRS and a suspected focus of infection.
2. Age of 18 years and above
3. Given an informed written consent or a assent.

Exclusion

Patients undergoing routine dialysis at Kenyatta National Hospital renal unit.

5.4 Sample size calculation.

The sample size was made using the formula for estimating prevalence (Daniel et al 1999):

\[ n = Z^2 \frac{p(1-p)}{e^2}, \]

\[ =228. \]
Where $n$ is the sample size

$Z$ is the constant for a desired confidence interval of 95% and equals 1.96.

$P$ is the rate of culture positivity=18.2% (41) and $e$ is the margin of error= 0.05

5.5 Clinical methods

Files of patients presenting at the accident and emergency department of KNH were screened consecutively to identify those who had a diagnosis of sepsis. Patients whose cards had a diagnosis of sepsis or whose vital signs had any two of PR >90, RR >20, Temp >38 or < 36 degrees Celsius and systolic BP < 90, were identified. These patients were then reviewed by the study assistant (a registered clinical officer by training) or the primary investigator to confirm that they met the case definition. The patients who met this criterion were then educated on the details of the study by the study assistant or the primary investigator. A written informed consent was then administered.

Those who consented underwent a targeted history and examination to capture their biodata (age, marital status, level of education), chief presenting complaints to aid in identification of site/foci of infection, history and duration of antibiotic use in the preceding one week, any history of chronic kidney disease and HIV status.

Two blood culture samples were then taken plus a sample from a suspected focus of infection if any, as detailed below.

5.6.0 Laboratory Methods

5.6.1 Sample Collection

The procedure was explained to the patient using a standard written procedure consent form.

Once the patient or the next of kin gave a written or verbal consent, the patient was guided to lie in a supine position and the cubital fossa located on both arms (the two sites for drawing blood).

The skin at the venupuncture site was then prepared meticulously using a 0.55 chlorhexidine in 70% alcohol solution.
The disinfectant was allowed to evaporate on the skin surface for about 1 minute before blood was withdrawn.

A 21 gauge needle affixed on a 20 cc syringe was introduced carefully into the anterior cubital veins of either side of the arms and 10 cc of venous blood collected on either side.

The blood was inoculated into each of the culture bottles (two aerobic bottle each containing 100 cc of culture medium-blood culture broth and Tryptic soy broth) through a disinfected diaphragm.

Whenever the procedures were completed, the patient would be appreciated and the procedure area cleared for use by the next client.

### 5.6.2 Sample transportation and processing

The samples were kept at room temperature and transported to the University of Nairobi microbiology laboratory within three hours. On receipt, the two sample bottles were incubated overnight at 35 - 37°C in the incubator.

The following day, subcultures were made from each bottle and were plated on blood agar (BA), chocolate blood agar (CBA), and MacConkey (MAC) agar plates for discrete colonies. These again were incubated overnight and for up to 48h. if no growth was obtained.

If no microbial growth was obtained after first subculture, further subcultures were done on the third and sixth day of incubation of the original sample bottle after which a final report (No Microbial Growth Obtained) was submitted.

In case of (an isolate) growth, investigations were done to try and identify the isolate as much as possible as it’s indicated below.

1. Colonial morphology study on each medium.
2. Gram stain.
3. Biochemical tests.

The isolated bacteria were:

1. *Staphylococcus aureus*.
2. *Staphylococcus epidermidis*.
3. *Streptococcus pyogenes*. 
4. *Escherichia coli.*
5. *Proteus mirabilis.*

**S. aureus isolates.**

Yellowish and white pigmented species on BA and CBA, also beta hemolytic on BA, lactose fermenting on MAC agar, gram positive (clustering) cocci, catalase positive and were coagulase test positive as well as novobiocin sensitive.

**S. epidermidis.**

White pigmented species on BA and CBA. Non hemolytic on BA and lactose fermenting on MAC agar. Gram positive (clustering) cocci, catalase positive and were coagulase negative but were novobiocin sensitive.

**Str. Pyogenes.**

Whitish pin point colonies on BA and CBA. Beta- hemolytic colonies on BA. Did not grow on MAC agar. Gram positive cocci mainly in chains, catalase negative and were bacitracin sensitive.

**E. coli.**

Whitish colonies on BA and CBA. The isolates were the non hemolytic type on BA. Were lactose fermenting on MAC agar and were gram negative bacilli. Indole test was positive and citrate test negative.

**P. mirabilis.**

Grayish swam on both BA and CBA. Were non lactose fermenting on MAC agar and were gram negative bacilli. Urease test was positive, citrate positive, indole negative and did not ferment sucrose (K/A) in TSI agar.
**Klebsiella spp.**

White mucoid colonies on BA and CBA, non hemolytic on BA and were lactose fermenters on MAC agar. Isolates were Simmons citrate agar positive but indole test negative.

**Interpretation of culture**

All bacteria isolated from blood were considered as clinically significant organisms whenever growth occurred in both bottles.

All gram negative organisms were deemed pathogenic even if growth occurred in one bottle.

**Susceptibility Testing**

After identification, antimicrobial susceptibility testing was done. Kirby-Bauer(disk-diffusion) method on Mueller-Hinton agar medium was used. Inoculation was done in a standard manner utilizing 0.5 McFarland standard. The choice of the Antimicrobial Disks applied was dictated upon as per the Research Project Protocol and per Clinical and Laboratory Standards Institute (CLSI) guideline (45).

The results were read after 24 hours incubation at 37°C, and the diameters of growth inhibition around the discs measured and interpreted as susceptible or resistant as per CLSI guidelines.
Interpretation of susceptibility results

The diameter of inhibition were measured using calipers and then the value obtained compared to the Clinical and Laboratory standards Institute (CLSI) (45).
6.0 QUALITY CONTROL AND ASSUARANCE

The laboratory of reference had an in-built internal control and a regular external control.

7.0 DATA MANAGEMENT AND ANALYSIS

7.1 Study variables:

The study data variables were-:

i. Presence/absence of a microbe in blood
ii. The type/profile of microbe isolated
iii. Susceptibility to antimicrobial agents
iv. Focus/site of infection
v. Patient biodata e.g. age and gender.
vi. Whether or not a patient has known chronic kidney disease or self reported HIV positive
vii. History of use of antibiotics in the preceding seven days.

7.2 Data Collection and Management

Data was collected using two different tools with a corresponding unique identifier for each patient: Study proforma and laboratory result form.

Double data entry technique was used to enter data into MS Access where the study proforma and the laboratory result form were linked.

Data checking and validation was done in MS Excel and a copy archived. All analyses were performed using R (ver. 3.1.2) [R Core Team, 2013] and packages rms (ver. 4.3-1) [F. Harrell, 2014] for analysis, Gmisc for plot and table output (ver. 1.1), and knitr (ver ) [Xie, 2013] for reproducible research.

7.4 Data protection and study dissemination plan

The raw data collected during this study was properly secured under lock and key and was only accessible to the primary investigator, the supervisors and the ethical committee. The documents did not bear the names of the study participants but unique identifiers only known to the primary investigator.
All the results of each study participant were relayed to the attending resident (registrar) in the respective wards within 12 hours of the release of such results for purposes of managing the study participant.

8.0 ETHICAL CONSIDERATIONS

This study was undertaken after approval by the Department of Clinical Medicine and Therapeutics and the Kenyatta National Hospital / University of Nairobi Ethics and Research Committee. Enrolment into the study was purely voluntary after obtaining written informed consent (Appendix 1 and 2). Invasive procedures (blood sample collection) were done as part of the usual care of patients with sepsis and no additional risks to the study participants were experienced. All the predetermined ethical conditions were adhered to as per the approved protocol.
9.0 RESULTS

This study was carried out between February and April 2015. Two hundred and eighty eight patients were screened for the presence of sepsis. We excluded 56 patients who did not meet the study criteria. This process is represented in the flow chart below (figure 1).

Figure 1: Flow chart demonstrating the recruitment process

9.1 Baseline characteristics

A total of 232 patients were recruited and analyzed. Females were 120 (52%). The median age of the patients was 37.8 years (IQR 1.825) years. Most of the patients 176 (76%) were from the community with only 56(24%) coming as referrals from another facility. The prevalence of HIV among the study subjects was 15% and a total of 64(28%) had used antibiotics one week prior to the date of recruitment.

The average of all the recorded vital signs was temperature 37.6 degrees Celsius, systolic BP 118, respiratory rate 26 and pulse rate 110. In addition the mean white cell count was 15.5 X 10^3 cells/mm3. A total of 23(9.91%) patients had severe sepsis as defined by a SBP below 90mmHg. These baseline findings are summarized in table 1 below.
Table 1a: Baseline socio-demographic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female n(%)</td>
<td>120 (52%)</td>
</tr>
<tr>
<td>Male n(%)</td>
<td>112(48%)</td>
</tr>
<tr>
<td>Age (median+IQR)</td>
<td>37.8 (±1.825)</td>
</tr>
<tr>
<td>Marital status n(%)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Married</td>
<td>177 (76%)</td>
</tr>
<tr>
<td>Separated</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Single</td>
<td>45 (19%)</td>
</tr>
</tbody>
</table>

Table 1b: Baseline clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(n) Frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referral state</td>
<td></td>
</tr>
<tr>
<td>Community</td>
<td>176 (76%)</td>
</tr>
<tr>
<td>Hospital</td>
<td>56 (24%)</td>
</tr>
<tr>
<td>HIV Positive (self reported)</td>
<td>34 (15%)</td>
</tr>
<tr>
<td>Prior antibiotic use</td>
<td></td>
</tr>
<tr>
<td>Among Community referrals</td>
<td>31(17.6%)</td>
</tr>
<tr>
<td>Among Hospital referrals</td>
<td>33(58.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>64(28%)</td>
</tr>
<tr>
<td>Vital signs</td>
<td></td>
</tr>
<tr>
<td>Temperature (mean+/SD) (degrees Celsius)</td>
<td>37.6 (±1.3)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Normal n (%)</td>
<td>196(84.5%)</td>
</tr>
<tr>
<td>Hypotensive n (%)</td>
<td>36(15.5%)</td>
</tr>
<tr>
<td>RR (mean+/SD) (breaths per minute)</td>
<td>26 (± 14)</td>
</tr>
<tr>
<td>PR (mean+/SD) (beats per minute)</td>
<td>110 (± 16)</td>
</tr>
<tr>
<td>White blood cells (mean+/SD) (X109/L)</td>
<td>15.5(±9.0)</td>
</tr>
<tr>
<td>Range (0.6-56.2)</td>
<td></td>
</tr>
</tbody>
</table>
9.2 Focus of infection

The most common presenting complaint was headache 81 (35.3%), followed by cough 50 (21.6%) confusion 40 (17.7%), and abdominal pain 31 (13.4%). The remaining symptoms were only present less than 10 percent of the times as demonstrated in the table 2 below.

Table 2: Presenting complains (in percentage frequency)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>n(% Frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>82 (35.3%)</td>
</tr>
<tr>
<td>Confusion</td>
<td>41 (17.7%)</td>
</tr>
<tr>
<td>Convulsion</td>
<td>15 (6.5%)</td>
</tr>
<tr>
<td>Cough</td>
<td>50 (21.6%)</td>
</tr>
<tr>
<td>Chest pain</td>
<td>41 (17.7%)</td>
</tr>
<tr>
<td>Dysuria</td>
<td>11 (4.7%)</td>
</tr>
<tr>
<td>Frequency</td>
<td>11 (4.7%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>31 (13.4%)</td>
</tr>
<tr>
<td>Wound</td>
<td>22 (9.5%)</td>
</tr>
</tbody>
</table>

The foci of infection was identifiable in a majority of patients 176 (75.8%) with 56 (24.1%) not having any obvious focus of infection.

Respiratory infections were the most common at 26%. The second most common infection was the skin and soft tissue 20% followed by the abdomen 13%, CNS 12% and UTI at 6% as shown in the pie chart below (figure 2).
Figure 2: A Pie chart showing focus of infection by percentage.

9.3 Laboratory findings

A total of 232 blood samples were collected and analyzed by culture for possible growth of bacterial organisms. Only 20 (8.6%) samples cultured any bacteria. Out of these, 15 (6.5%) met the criteria for pathologically significant growth.

Of all the culture cases 11(73.3%) were gram positive whereas a minority 3(26.7) were gram negative.

The most common gram positive organism was CONS 6 (40.0%) followed by Str. pyogenes 3 (20.0%), S.aureus 2 (13.3). All the gram negative bacteria namely K.pneumonias, E.coli were isolated at an equal frequency of 6.7% each except P.mirabilis at 13.3%.

This information is summarized in table 3 below.
Table 3: Frequency of isolation per organism

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative <em>Staphylococcus aureus</em></td>
<td>6</td>
<td>40.0</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>3</td>
<td>20.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>2</td>
<td>13.3</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1</td>
<td>6.7</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1</td>
<td>6.7</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>15</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

9.5 Sensitivity patterns

A total of seventeen antibiotics were tested for the susceptibility of both the gram negative and gram positive organisms using the disc diffusion method. In general, gram positive organisms were greatly (more than 90%) susceptible to meropenem, ceftriaxone, imipenem, amoxicillin/clavulenic acid, levofloxacin and Novobiocin. The gram negative organisms were mostly susceptible to Amikacin, levofloxacin, imipenem and meropenem. All the organisms demonstrated pan-resistance to erythromycin. The sensitivity patterns are shown in tables 4 and 5 below:
Table 4: Original raw data on individual susceptibility patterns per organism

<table>
<thead>
<tr>
<th>Drug</th>
<th>S. aureus</th>
<th>S. aureus</th>
<th>Klebsiella</th>
<th>E. coli</th>
<th>CONS</th>
<th>CONS</th>
<th>CONS</th>
<th>CONS</th>
<th>S. pyogenes</th>
<th>S. pyogenes</th>
<th>P. mirabilis</th>
<th>P. mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin/Clavulan</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Penicillin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>
Table 5: Susceptibility patterns (in percentage) per organism groups

<table>
<thead>
<tr>
<th>No</th>
<th>Drugs</th>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ceftriaxone</td>
<td>11(100%)</td>
<td>3(75%)</td>
</tr>
<tr>
<td>2</td>
<td>Cefotaxime</td>
<td>9(81.8%)</td>
<td>3(75%)</td>
</tr>
<tr>
<td>3</td>
<td>Cefuroxime</td>
<td>9(81.8%)</td>
<td>2(50%)</td>
</tr>
<tr>
<td>4</td>
<td>Cefepime</td>
<td>8(72.7%)</td>
<td>3(75%)</td>
</tr>
<tr>
<td>5</td>
<td>Cefoxitin</td>
<td>7(63.6%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>6</td>
<td>Meropenem</td>
<td>10(90.9%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>7</td>
<td>Imipenem</td>
<td>10(90.9%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>8</td>
<td>Amoxicillin/clavulinic acid</td>
<td>10(90.9%)</td>
<td>2(50%)</td>
</tr>
<tr>
<td>9</td>
<td>Amoxicillin</td>
<td>4(36.4%)</td>
<td>1(25%)</td>
</tr>
<tr>
<td>10</td>
<td>Penicillin</td>
<td>4(36.4%)</td>
<td>1(25%)</td>
</tr>
<tr>
<td>11</td>
<td>Levofloxacin</td>
<td>11(100%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>12</td>
<td>Ciprofloxacin</td>
<td>9(81.8%)</td>
<td>2(50%)</td>
</tr>
<tr>
<td>13</td>
<td>Vancomycin</td>
<td>8(72.7%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>14</td>
<td>Teicoplanin</td>
<td>3(27.3%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>15</td>
<td>Novobiocin</td>
<td>10(90.9%)</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>16</td>
<td>Amikacin</td>
<td>8(72.7%)</td>
<td>4(100%)</td>
</tr>
<tr>
<td>17</td>
<td>Erythromycin</td>
<td>0(0.0%)</td>
<td>0(0.0%)</td>
</tr>
</tbody>
</table>
10.0 DISCUSSION

Most of our patients (89.2%) were sixty years or younger with a mean age of 38 years. This finding is unique in that most studies have documented blood stream infection (BSI) among a predominantly elderly group. For example in a retrospective study by Cisterna et al in Spain(46), most of the patients were above the age of sixty. Other studies across Africa show a similar finding as that of Cisterna et al. This may have an impact on the type of organisms isolated given the difference in co-morbidities expected in this study population compared to that in other studies.

We also had a predominantly (52%) female population presenting with BSI. This is consistent with data across most African studies. A study by Dagnew et al in Gondar Ethiopia consisted of 212(54.35%) females(41) while that by Shevin in Uganda had 59.2% females(47). However most western data suggest that males have a 1.28 times higher risk of getting sepsis compared to females(48).

A total of 34 (15%) of our patients were HIV positive. This was a large number and may have a bearing on the younger mean age of our patients with BSI and definitely on the focus of infection. In a study by Huson et al in 2014 younger people with HIV had a higher prevalence of BSI than older people (30% versus 20%). In Uganda’s Mulago hospital a study by Shevin et al revealed an even higher proportion of patients with sepsis as HIV positive 84.9% and in this population the average age of septic patients was 34.8 years(47).

Despite a larger number of our patients having come from the community (76%), a sizable proportion (28%) of the total population had used antibiotic within a week before recruitment. Out of the 64 patients with prior antibiotic use a total of 30 were from the community (17.0% of all patients from the community), suggesting a high rate of over the counter antibiotic use. Other studies elsewhere demonstrate an even higher percentage of prior antibiotic use e.g. 73.9% in a study by Awad et al in Khartoum, Sudan(49).

A total of 34(60.7%) of the patients referred from other facilities had been given antibiotics prior to referral. This may have altered the presentation of our patients e.g. the severity of illness, rate of blood culture positivity and resistance patterns. Unfortunately we could not capture the types of antibiotics used because not all patients had a detailed referral letter.
10.1 Focus of infection

We were able to deduce the focus of infection using symptomatology in up to 176(75.9%) of the cases. The most common symptom with significance as to the focus of infection was cough at 21.6% followed by chest pain and confusion at 17.7% each. This is congruent with the findings in a study by Marc and Cohen et al(24, 50). Although they both found that respiratory infections were the most common, they described skin and soft tissue infections as the least common unlike in our study where it was the second most common. However, in both this study and that by Cohen et al the abdominal infection was the third most frequent. The focus of infection seems to be dependent on whether the infection was community or hospital acquired with most studies on hospital-acquired infections like that by Cisterna(46) and Franco Moreno being predominantly of the genitourinary system while the community based ones being mainly of the respiratory system(51). We however recognize the limitation in using general symptoms to determine the focus of infection since most symptoms overlap systems.

10.2 Culture positivity

Out of a total 232 blood samples collected and analyzed, we were able to isolate organisms in 20(8.6%). Out of these 75 percent were truly pathogenic giving a low culture conversion rate of only 6.5%. The culture positivity rate in most studies across the world is between 12-20%(41, 51). A survey done across most hospitals in Nairobi, Kenya, demonstrated a culture positivity rate of between four to fifteen percent. Our culture conversion rate of 6.5% is lower than most of the studies elsewhere in the world. A plausible explanation is the difference in the patient populations—we had quite a good number of HIV patients (15% self reported). In addition, the severity of sepsis and the high percentage of patients with prior antibiotic exposure may explain these findings. Given that the most common focus of infection was the respiratory system, one would have expected organisms such as Streptococcus pneumoniae. However, the high rates of antibiotic exposure prior to culture may have interfered with the growths of such fastidious organisms. Although the laboratory quality assurance was ensured through working in an accredited facility, a further improvement in our clinical and laboratory methods ranging from specimen collection to transportation and exploration of other diagnostic modalities like the molecular methods may increase our percentage yields. The other unexplored area that would explain these low culture rates would be the non-infectious aetiologies of SIRS as well as the non-bacterial causes such as fungi and viruses.
These were not looked into as much as they might have been implicated in the causation of sepsis in our patients.

Twenty five percent of our culture positive results were contaminants; all of which were Coagulase negative *Staphylococcus aureus*. The contamination rate varies with each region and is majorly determined by the collection techniques and laboratory precautions used. In a study by Franco Moreno in new Delhi, India, the contamination rate was as high as 75.9% of the 243 samples analyzed while in another study by Aznar et al it was only 7.5% (52). The universally acceptable contaminant rate is less than five percent (53). The single most common isolated contaminant in most BSI studies is CONS (Chand Wattal, New Delhi).

Most of the organisms isolated in this study were gram positive (78.6%). Most studies indicate that 44-48% of the causes of bacteremia are gram negative with 44-49% being gram positive (4, 48). This could be explained by the fact that most studies on BSI are inpatient hospital based studies with nosocomial infections as compared to our study in which 76% of the patients were from the community.

10.3 Sensitivity patterns

The overall sensitivity rate was high for both the gram positive and gram negative bacteria with most antibiotic having susceptibility rates of above 60%. The drugs that showed pan resistance across the board were erythromycin, amoxicillin, penicillin and teicoplanin, ciprofloxacin, cefuroxime for gram negative organisms and erythromycin, teicoplanin, ciprofloxacin, amoxicillin, penicillin and ciprofloxacin for gram positive organisms.

The most sensitive antibiotics across the two groups of organisms were ceftriaxone, the carbapenems and levofloxacin with susceptibilities above 90%.

This data implies that resistance has developed among the commonly used b-lactam antibiotics especially penicillin G, and amoxicillin and that these should not be used in treating sepsis except with strong evidence from susceptibility results supporting their efficacy in a given individual. The extended spectrum penicillin, amoxicillin clavulanic acid, however has very good efficacy against most gram positive organisms (90.9%) but only effective against 50% of gram negative organisms. The other b-lactam antibiotics, namely the cephalosporin displayed varied effects. The second generation cephalosporin cefuroxime and Cefoxitin had opposite effects with cefuroxime being effective against most (81.8%) gram positive bacteria as is expected of this group of cephalosporins. Cefoxitin had an unexpected
efficacy against all gram negative bacteria and a modest effect against the gram positive ones. All the gram positive and a majority of gram negative organisms were susceptible to ceftriaxone (a third generation cephalosporin) This susceptibility mirrored that of cefotaxime. Majority of the gram negative organisms were also susceptible to cefepime and meropenem.

The glycopeptides vancomycin as expected was only effective against the GPB organisms (72.7% of gram positive and none of the gram negatives). Teicoplanin on the other hand had efficacy against only 27.3% of the gram positive and no effect against any gram negative bugs. This is surprising as the efficacy of teicoplanin against GNB is expected to be as good as if not better than that of vancomycin.

The organisms including the GPB were resistant to the only macrolide we tested but this may not be generalized to all macrolides.

Amikacin was effective against all the gram negative organisms with a relatively good efficacy (74%) against most GPB.

The discrepancy in the efficacy of fluoroquinolones, ciprofloxacin and levofloxacin can be explained by the generally high frequency among clinicians of prescribing the former two compared to the latter hence the high resistance patterns. This trend is seen across the world. Oscar et al demonstrated in his study among patients using fluoroquinolones in the community that most of this increase in ciprofloxacin resistance is driven by the wide spread use of fluoroquinolones and amoxicillin clavulenic acid leading to the emergence of extended spectrum beta lactamase (ESBL)(54).
11.0 CONCLUSION

Majority of our patients were from the community and the most common causative organisms were gram positive bacteria with high susceptibility to the commonly used antibiotics. Coagulase negative *Staphylococcus aureus* was the most commonly isolated organism. Most organisms were sensitive to levofloxacin, carbapenems and third generation cephalosporins.

12.0 LIMITATIONS OF THE STUDY

The study did not exclude those patients with prior exposure to antibiotics and this may affect the percentage of positive cultures. The clinical symptoms by themselves were not specific enough to ascertain the focus of infection.

We also did not give the quantitative response of each organism to every antibiotic since we used the qualitative disc diffusion method. This may make deductions such as minimum inhibitory concentrations (MIC) difficult.

It would be noted that it was not within the limits of this study to identify the viral, fungal and non-infectious aetiologies of SIRS thus the low rates of septicemia may not reflect the true infection rates among patients presenting with sepsis.

13.0 RECOMMENDATIONS

There is need to study a larger number of patients to enable us determine the factors that contribute to culture positivity.

Further studies to check specific strains of community acquired genetic mutations associated with the observed resistance patterns.

A continuation arm of this study is warranted to answer some of the above questions with the aim of guiding the development of an antibiogram for our setting.
14.0 REFERENCES


15.0 APPENDICES

APPENDIX 1- CONSENT EXPLANATION

1. STUDY PARTICIPANT CONSENT FORM

<table>
<thead>
<tr>
<th>Name</th>
<th>Qualification</th>
<th>Institution</th>
<th>Department</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. George Otieno</td>
<td>MBchB, Masters student</td>
<td>UoN/KNH</td>
<td>Clinical Medicine and Therapeutics</td>
<td>Resident</td>
</tr>
</tbody>
</table>

Emergency telephone number:

Dr. George Otieno, University of Nairobi, Tel: 0715512835

Investigator’s statement

I am asking you to be in a research study. The purpose of this consent form is to give you the information you will need to help you decide whether to be in the study. Please read this form carefully. You may ask questions about what you will be asked to do, the risks, the benefits and your rights as study participant, or anything about the research that is not clear in this form. When all your questions have been answered, you can decide if you want to be in this study or not.
Purpose and benefits

Those participating in this study have a suspected case of blood infection and will undergo treatment for the same. We are doing a study to find out what caused your blood infection and to determine what antibiotic it will best respond to. Usually one is treated based on the most likely cause and so you may be on some form of treatment already but this study will help to further clarify the specific organism for purposes of modifying your treatment and also to help generate data on the organisms causing blood infections within our setting and the antibiotics they respond to. We will keep your doctor informed about our findings within 12 hours of getting the results.

Procedures

You will be called into a private room where questions relating to your main complain, HIV status, use of antibiotics and whether or not you have a kidney disease will be asked. Your vital signs will be taken. Then, blood will be taken from your veins. This process involves locating a vein in your arm and then cleaning that area with a fluid that sterilizes the skin to prevent your blood from being contaminated by the skin bacteria. Then the vein will be punctured using a sterile needle attached to a syringe and 10mls of blood drawn. This process may be a little painful but just for a few seconds. The sample will be put in a culture bottle and sent to the University of Nairobi Microbiology laboratory an analysis which may take upto three days.

Risks, stresses or discomfort.

Some of the questions asked will be of personal nature. However, you are encouraged to answer them all to aid in strengthening the study. The questions will be asked in a private environment and confidentiality will be assured at all times to ensure your comfort.

The pain mentioned above may also be of concern but that only lasts for a short while.

Participation in the study will require you to commit your time. The whole process may take about 20 minutes.

Cost

You will be responsible for meeting the cost of standard care while at the hospital. However the cost of analyzing the culture will be incurred by the study investigator.
Confidentiality

Your confidentiality will be maintained at all times. The questionnaires will not have any names except unique identifiers known only to the study investigator. Only the investigator, the University of Nairobi ethics and research committee will have access to the information about you.

There shall be no mention of names or identifiers in the report or publications which may arise from the study. The information obtained will be used only for the purpose of the study and any raw data will be secured out of reach for all except the investigators and the ethics committee. Samples will be destroyed after use and shall not be used for any purposes outside what is outlined here.

You may withdraw from the study or refuse to answer any of the questions asked at any time without loss of benefit or penalty. Your participation in the study is voluntary and will be highly appreciated.

If you have any questions regarding the study, contact Dr Otieno through 0715512835.

In case of any ethical concerns please contact:

The Chairman, KNH/UON – Ethics and Research Committee
Hospita Road along Ngong Road
P.O BOX 20723, Nairobi (CODE 00202)
Telephone number (+254-020)2726300 ext 44355
Chairperson: Professor A.N. Guantai
Contact person: Esther Wanjiru Mbuba
Email: uonknhe@uonbi.ac.ke
FOMU YA MAELEZO

FOMU YA IDHINI YA MSHIRIKI WA UTAFITI

<table>
<thead>
<tr>
<th>Jina</th>
<th>Kiwango cha elimu</th>
<th>Chuo</th>
<th>Kitendo</th>
<th>Kiwango cha kikazi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dk. George Otieno</td>
<td>MBchB, UoN/KNH</td>
<td>Clinical Medicine and Therapeutics</td>
<td>Mwanafunzi</td>
<td></td>
</tr>
</tbody>
</table>

Namba ya simu ya dharura:

**Dk. George Otieno**, Chuo Kikuu cha Nairobi, Na: 0715512835

**Maelezo ya mtafiti:**

Ninaomba idhini yako ya kushiriki katika utafiti. Madhumuni ya fomu hii ya maelezo na ruhusa ni kukupa maelezo ya kutosha kukuwezesha wewe kufanya uamuzi wa kukubali kushiriki katika utafiti huu au la. Unatakiwa uisome fomu hii kwa makini. Unaweza kuulizamaswali kuhusu unachotakiwa kufanya, madhara za kushiriki katika utafiti huu, manufaa ya kushiriki, hakizakokama mshiriki na jambo lolote lisilwekalinalohusiana na utafiti huu. Maswali yako yote yakishajibiwa kwa urefu una hiari ya kukubali kushiriki au kukataa.

**Faida na manufaa**

Mtindo

Hatari na madhara
Baadhi ya maswali utakayoulizwa yatakuwa ya kibinafsi. Unashauriwa uyajibu yote kwa ajili ya uhakika wa utafiti huu. Maswali yataulizwa katika mazingira ya kibinafsina ya siri. Majibu hayo yatahifadhiwa na mtafiti. Maumivu yaliyotajwa awali yanaweza kukusababishia wasiwasi lakini ni ya muda mfupi sana
Kujiunga katika utafiti huu kunahitaji wakati wako takriban dakika ishirini.

Gharama
Gharama za matibabu ya hospitali yatakabiliwa na mshiriki. Gharama za kufanya uchunguzi unaohusika na utafiti huu zitakabiliwa na mtafiti.

Usiri
Mtafiti atahakikisha kuweka usiri wakati wote. Karatasi za maswali ya utafiti hayatakuwa na majina ya washiriki bali yatajulikana kwa nambari. Mtafiti, chuo kikuu cha Nairobi na kitengo cha utafiti pekee ndio watakaokuwa na uwezo wa kupata habari juu yako.
Katika kuandika ripoti ya utafiti huu hakutakuwa na majina wala maandishi yoyote ambayo yanaweza kumtambulisha mshiriki. Habari itakayopatikana itatumika kwa ajili ya utafiti peke yake.
Unaruhusiwa kujitooka utafiti wakati wowote au kukataa kujibu swali lolote ambalo litaulizwa, bila ya hofu yoyote wala madhara yoyote kwako. Ushirikiano wako katika utafiti huu ni wa hiari.

Ikiwa una maswali yoyote kuhusu utafiti huu, wasiliana na Dk Otieno kupitia simu nambari 0715512835.

Ikiwa una maswali yeyote kuhusu njia za utafiti huu, wasiliana na:

Mwenyekiti, KNH/UON – Kitengo cha utafiti,

Hospital Road , Ngong Road
S.L.P 20723, Nairobi (CODE 00202)
Simu nambari: (+254-020)2726300 ext 44355

Mwenyekiti : Profesa K.M. Bhatt
Mhusika wa mawasiliano: Esther Wanjiru Mbuba
Barua pepe: uonknh_erc@uonbi.ac.ke
APPENDIX 2- CONSENT /ASSENT FORM

CONSENT TO PARTICIPATE IN THE STUDY

Subject’s statement

This study has been explained to me. I volunteer to take part in this research. If I have questions later on about the research I can ask the investigator above. If I have questions about my rights as a research subject, I can call the University of Nairobi Ethics and Research Committee at 2726300. I will receive a copy of this consent form.

Signature of subject_______________________ Date______________________
Left thumbprint of subject___________________ Date____________________
Name of subject____________________________
Signature of witness (If thumbprint used)______________________________
Name of witness____________________________________________________
2. FOMU YA IDHINI

Mimi .............................................. kutoka ...........................................

Sahihi ........................................... Tarehe ..............................................
Shahidi..........................(mtafiti mkuu/msaidizi) Tarehe .........................

MAWASILIANO

Ukiwa namaswali yoyote ya ziada, unaweza kuwasiliana na wafuatao:

1. Dkt Otieno George (Mtafari Mkuu)
P.O Box 120 Kijabe.
Tel 0715512835

2. Kamati ya Maadili ya Utafiti katika Hospitali ya Kenyatta na Chuo kikuu cha Nairobi
SLP 20723 NAIROBI
Simu: 020-726300
APPENDIX 3- STUDY PROFORMA

BIODATA

Participant number ……………

Hospital- IP. No. …………………

SOCIAL-DEMOGRAPHICS

Age……………………

Gender: (tick one) Male …. Female …….

Marital status( Denote whether single, married ,divorced, separated).

Level of education: (tick one) None …. Primary …. Secondary …. Tertiary …. 

MEDICAL HISTORY

Type of referral (tick as appropriate)

- Community (from home)
- Hospital (from another facility)
- ICU/HDU
- Others (namely)………………

Primary complains. (Tick all the symptoms present to help identify possible foci of infection):

- Headache
- Confusion
- Convulsion
- Cough
- Chest pain
- Dysuria
- Urinary frequency
- Wound or ulcer
- Abdominal pain
- Diarrhea
- Chills/fever
- Others

Has the patient used any antibiotic in the last one week?

- Yes
- No

If the answer is yes, for how many days?--------

Vital signs: Blood pressure-------- Temperature ---------------

Pulse rate-------- Respiratory rate --------
What is the HIV status of the study participant?
- Unknown
- Positive
- Negative

Is the patient on dialysis for chronic kidney disease?
- Yes
- No

LABORATORY FORM

Type of sample taken -(tick appropriately)
- Blood for culture and sensitivity
- Sample taken from the source site.

What is the suspected site/focus of infection?(tick as applicable).
- Lung
- Abdomen
- Central nervous system
- Urinary tract
- Wound /soft tissue
- Cardiovascular
- Unknown
- Others ……………

What is the result of the culture after seven days?
- Positive
- No growth

If positive, which bacteria has been isolated ………………………

Has the susceptibility test been done? Yes_____ No _____.

(Please attach a copy of the antimicrobial susceptibility test result).
1. **Blood Specimen**

**Container:** Blood culture media set-aerobic and vacutainer with SPS (Sodium Polyanethol Sulfate).

**Patient preparation:** Disinfection of the venupuncture site with 70% alcohol followed by betadine disinfection

**Instructions:** Draw blood at time of febrile episode when possible and before any administration of antibiotic. Draw two sets from right and left arms, no more than three sets in 24 hours. Draw ten milliliters per set for adults.

**Transport to the laboratory:** Within 2hrs at room temperature.

**Storage before processing:** Incubated at 37°C on receipt in laboratory.

**Primary plating media:** Blood culture bottles
APPENDIX 5: KNH ETHICS APPROVAL LETTER

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19766 Code 00302
Telegram: varsity
(254-020) 2726300 Fax 44355

Ref: KNH-ERC/A/74

Dr. Otieno George Ochieng
Dept of Clinical Medicine & Therapeutics
School of Medicine
University of Nairobi

Dear Dr. Otieno

Research Proposal: The Antimicrobial susceptibility pattern in patients presenting with sepsis at the Accident and Emergency department, Kenyatta National Hospital (P624/10/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 20th February 2015 to 19th February 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period.

(Attach a comprehensive progress report to support the renewal).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.erc.uonbi.ac.ke

Protect to discover