BACTERIAL PATHOGENS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG NEONATES WITH SEPSIS AT KENYATTA NATIONAL HOSPITAL, NEWBORN UNIT.

BY
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DECLARATION

I declare that this dissertation is my own work and has not been published or presented for a degree in any other institution

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LIST OF ABBREVIATIONS

CBA Chocolate Blood Agar

CoNS Coagulase Negative Staphylococcus aureus

CME Continuous Medical Education

EONS Early Onset Neonatal Sepsis

E. coli Escherichia coli

GBS Group B Streptococcus

HIC High Income Countries

HDU High Dependency Unit

IMCI Integrated Management of Childhood Illnesses

KMC Kangaroo Mother Care

KNH Kenyatta National Hospital

Kshs Kenya Shillings

NICU Neonatal Intensive Care Unit

NBU New Born Unit

Staph aureus Staphylococcus aureus

Spp Species

STROBE-NI Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection

SPSS Statistical Package for Social Sciences

PSBI Possible Serious Bacterial Infection

WHO World Health Organization

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ABSTRACT

Background

Neonatal sepsis is a major cause of morbidity and mortality among neonates more so in low- and middle-income countries. There is variability of causative pathogens in neonatal sepsis between as well as within countries and this changes from time to time. Antimicrobial resistance is globally on the rise and Africa shares this trend. However, there are major gaps in routine antimicrobial surveillance reported in the region.

Objectives

The study aimed to describe the bacterial pathogens and antibiotic susceptibility of neonates with sepsis from the Newborn unit at Kenyatta National Hospital within a six months period. This study also determined some clinical characteristics of cases with culture positive sepsis to further put the matter into perspective.

Methods

A retrospective cross-sectional study design was used. Cases with blood culture positive results from the newborn unit were identified from January to June 2019. These details were used to trace patient files from the records department of KNH.

Data management and analysis

Case record forms were used for data collection then transferred to Microsoft EXCEL spread sheets on a password protected computer. Data verification, cleaning and coding was done before transfer to STATA version 13 for analysis.

Results

A total of 357 blood culture samples were analyzed from the NBU, of which 158 were positive (44.2%). More than half (54%) of the cases had late onset sepsis. Gram negative isolates were predominant at 58% and associated with poor outcome with mortality rate of 56.3% (p=0.007). *Klebsiella pneumoniae* was the leading isolate at 28.9%. High resistance rates above 95% were noted to cephalosporins. Vancomycin and amikacin had high sensitivities of 90% and 88% respectively, that of ciprofloxacin and meropenem was 87%. Majority of patients were discharged home alive, 53% although they had stayed longer in hospital with 25 days median duration of stay (p=0.0069).

Conclusion

Gram-negative sepsis predominated by *K. pneumoniae* accounts for majority of neonatal sepsis cases and is associated with high mortality and morbidity in our setting. Many isolates demonstrated high sensitivity to vancomycin, amikacin, ciprofloxacin and meropenem whereas very high resistance rates to commonly used antibiotics particularly, cephalosporins and benzylpenicillin were noted.

BACKGROUND

Globally, neonatal mortality rate in 2018 stood at 18 deaths per 1000 live births, meaning that approximately 7000 infants died daily within the 1st month of life (1).This accounted for 47% of all under five deaths, an increase from 40% in 1990 attributable to the relatively slower decline in neonatal mortality compared to mortality among children aged 1-59 months. Furthermore, 7% of neonatal deaths were due to sepsis, coming third after intra-partum related events and preterm birth complications which caused up to 11% and 16% of deaths respectively(1). The burden of neonatal sepsis continues to be high especially in low- and middle-income countries and is a priority sustainable development goal 3 which aims at ensuring healthy lives and promoting wellbeing for all at all ages. Target 3.2 focuses on ending preventable deaths among newborns and children under 5 years by 2030 while setting country targets of reduction in neonatal mortality to at least 12 per 1000 live births(2).

Sub-Saharan Africa had the highest neonatal mortality rate at 28 deaths per 1000 live births in 2018(1). Regionally, most East African countries except South Sudan (which had higher rates) had neonatal mortality rates between 12-25 deaths per 1000 live births(1). In Kenya, neonatal sepsis was the third leading cause of death in 2017 at 16% of neonatal deaths following preterm births and intra-partum related events which were 28% and 29% respectively(3). Worth noting is that the Kenya estimates of neonatal deaths attributable to sepsis was about two times the global rates which puts emphasis on the gravity of the local situation.

Neonatal sepsis is a clinical syndrome occurring in an infant 28 days or less(4). In 2005, the International Pediatric Sepsis Consensus Conference defined sepsis as; the presence of at least two of these four criteria, one of which being abnormal temperature or leukocyte count;

1. Core temperature of more than 38.5 or less than 36 degrees Celsius

2. Tachycardia, or bradycardia for children younger than 1 year old

3. Mean respiratory rate > 2SDs above normal or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the need for general anesthesia

4. Leukocyte count elevated or depressed for age or more than 10% immature neutrophils(5).

In low- and middle-income countries where laboratory facilities are not readily available, WHO recommends the use of clinical signs delineating Possible Serious Bacterial Infection (PSBI) which are highly sensitive for sepsis. Clinical signs of PSBI include; any one of the following; difficulty feeding, convulsions, movement only when stimulated, respiratory rate of 60 or more per minute, severe chest retractions or temperature of greater than 37.5 or less than 35.5 Celsius(6).

Neonatal infection is broadly classified into early or late-onset neonatal sepsis. Early-onset sepsis (EOS) is defined as sepsis occurring within 48–72 hours of birth (some define it as within <7 days). The main routes of infection are vertical transmission from the mother trans placentally or by ascending vaginal routes, and postnatally from the environment. Late-onset infection in neonates is defined as infection becoming clinically evident more than 72 hours after birth (some use time after 7 days as defining) (7). Late onset infections are typically acquired after delivery from the hospital or community (8). The implication of the above classification is the difference in causative organisms involved in early and late onset neonatal sepsis.

Regional variation occurs in the organisms commonly seen in neonatal sepsis and this further alters over time. A systematic review of data from developing countries revealed that *Klebsiella spp, Escherichia coli (E. coli)* and *Staph aureus (Staphylococcus aureus)* cause the majority of early onset infections. *Staph aureus, Group B streptococcus (GBS), Streptococcus pneumoniae* and *non-typhoidal Salmonella* were reported to be more common in late onset sepsis infections(9) . Antibiotic prescribing practices are one of the main factors influencing this variability.

The diagnosis of neonatal sepsis has for long posed a challenge since the clinical signs of infection tend to be nonspecific in this age group yet the laboratory diagnosis on the other hand is costly and time consuming. This therefore necessitates the immediate initiation of

empirical antibiotic treatment in order to reduce the morbidity and mortality associated with neonatal sepsis. However, empiric therapy is not without consequence as it has been associated with increasing antibiotic resistance (10). It is very critical for neonatal units to routinely survey the profile of causative organisms and their susceptibilities to guide effective antimicrobial treatment since microbial pathogens and their antimicrobial sensitivity patterns change over time and vary between countries(7).

Despite blood culture being the gold standard test for diagnosis of neonatal sepsis, its sensitivity is not as high as the specificity. In the neonatal age group, the yield from blood culture is further reduced due to inadequate blood volume collection as well as antibiotic treatment prior to sampling(11).

According to a 2016 World Health Organization report, there is limited data on the etiological pathogens involved in neonatal sepsis from low- and middle-income countries particularly from rural and community-based studies. Nonetheless, data from the available systematic reviews points out that the commonest causes of neonatal bacteremia are: *S. aureus*, *E. coli* and *Klebsiella spp* (6) .

Antimicrobial resistance has been a growing threat to the effective treatment of an ever-increasing range of infections for many decades. It causes reduced efficacy of antimicrobial agents for example antibacterial drugs, making it troublesome, expensive and sometimes impossible to treat patients. The magnitude of the problem is amplified by the lack of new antimicrobials to replace those lost through resistance. Large gaps in antimicrobial resistance surveillance exist worldwide particularly in resource limited settings (12).

LITERATURE REVIEW

Neonatal sepsis is a major cause of morbidity and contributes significantly to neonatal mortality. Proper antibiotic use is therefore of paramount importance in reducing deaths attributable to neonatal sepsis with the knowledge of locally implicated pathogens and their antimicrobial susceptibility patterns. Furthermore, it is crucial that antimicrobial profiling is undertaken from time to time in order to keep up with the evolving microbial trends and their sensitivities to antimicrobial agents.

Antibiotic resistance develops as a result of evolution; however, this is accelerated by the selective pressure exerted by widespread use of antibacterial drugs. Resistant strains can thrive and propagate where there is non-compliance with infection prevention and control (12).

Patterns of pathogens

According to a WHO report, in High Income Countries (HIC), frequent causes of EONS include; GBS and E. coli with the remainder of causes being *Staph aureus*, *Coagulase Negative Staphylococcus (CoNS), Listeria monocytogenes,* as well as other gram-negative bacteria. As for late onset sepsis, CoNS contributes to nearly half of the episodes while other implicated organisms include; E. coli, *Klebsiella spp, Candida spp* and less commonly *Staph aureus, Enterococcus spp* and *Pseudomonas aeureginosa* (6) .

Zaidi et al in 2007 studied the pathogens associated with sepsis in newborn and young infants in developing countries by a systematic review of 63 studies including 22 from Africa with 13 of these providing data on community acquired infections (2 from Kenyan studies). The study noted that within the 1st week of life, *Klebsiella spp*, E. coli and *Staph aureus* made up 25%, 15% and 18% of isolates in neonatal sepsis respectively. Unlike in HIC, GBS was relatively uncommon in early onset sepsis at 7%. Late onset sepsis was predominated by Staph aureus 14%, *non-typhoidal Salmonella spp* 13%, GBS and *Streptococcus pneumoniae* each accounted for 12% of the isolates. Gram negative organisms were noted to be predominant among home delivered infants, making up to 77% of neonatal sepsis cases. This study revealed regional variations in the distribution of pathogens for example in Africa, GBS, *Streptococcus pneumoniae, Streptococcus pyogenes* and

Staph aureus were the most implicated in neonatal sepsis and there was an overall gram-positive predominance.

However, the above study excluded CoNS as contaminants despite noting its importance in hospital-based studies and this could have affected the results. More so, inconsistencies in age cut offs for early and late onset sepsis between countries could have resulted in differences between organisms isolated in either type of sepsis(9).

A systematic review and meta-analysis by Okomo et al studied the etiology of invasive bacterial infection and antimicrobial resistance among neonates in Sub-Saharan Africa. The study included 151 studies, most being hospital based and collected data from 84,534 neonates from 26 countries between 2008 and 2018. Using the STROBE-NI (Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection) checklist, it was found that *Staph aureus, Klebsiella spp* and E coli accounted for 25%, 21% and 10% respectively of culture positive bacteremia or sepsis. Group B Streptococcus was predominant in meningitis resulting in 25% of cases followed by *Streptococcus pneumoniae* 17% and Staph aureus 12 % (13). In the local context, *Klebsiella spp* is likely to account for considerably high proportions of neonatal sepsis isolates as there have been outbreaks within the newborn unit at Kenyatta National Hospital.

Musoke et al in 1997 reported on the emerging gram-negative resistant organisms and the therapeutic implications at the newborn unit of Kenyatta National hospital. The study was designed predominantly as retrospective with a short prospective arm, documented an overall sepsis rate of 16.9%. The gram-negative sepsis rate was 11% accounting for 18% of the mortality during the study period. *Klebsiella spp* accounted for the highest proportion of Gram-negative isolates at 38% followed by *Citrobacter, Enterobacter, E. coli* and *Salmonella typhimurium* at 26%, 19%, 4% and 2% respectively. Gram-positive organisms were cultured in 32 of the 121 positive cultures with *Staphylococcus aureus* making up 9%, *S. epidermidis* 19% and Enterococcus 4%(14)

Sixteen years later, Cheruto et al in 2013 studied the bacterial isolates and their patterns of susceptibility at the NBU of Kenyatta national hospital. This was a retrospective study that sampled 226 culture positive results. There was an overall Gram-negative predominance of 51.3% and common organisms isolated included CoNS 30.1%, *Enterobacter spp* 19.9%, *Citrobacter spp* 12.8% and *Klebsiella spp* 11%(15). Less common isolates included *Enterococcus spp* 8.8%, *E.*

coli 7.1%, *Staph aureus* 3.5% and *Proteus spp* 0.9% (15). These results demonstrated a 40% rise in Gram-negative sepsis rate over the years compared to the previous study by Musoke et al.

A cross sectional study done by Ateka et al in western Kenya between September 2017 and July 2018 at Moi teaching and referral hospital, the 2nd largest referral hospital in Kenya, found *Klebsiella spp* to be predominant in neonatal sepsis at 46%, followed by *CoNS* at 27.8%. Less common isolates identified included; *Acinetobacter spp*. 6.6%, *Staphylococcus aureus* 4.7%, *Enterococcus fecalis* 3.3% and *Escherichia coli* 2.6%(16). Since the study was cross-sectional in design with blood samples having been taken by aseptic technique, it is possible the proportion of *CoNS* sepsis could be true. However, we still entertain the likelihood of skin contaminants since quality control measures for example taking a 2nd sample for comparison were not mentioned. More so since *CoNS* accounted for a large proportion of the total isolates obtained.

Regional differences in isolates are illustrated in a study by Tumuhamye et al on neonatal sepsis at Mulago hospital, Uganda's national referral hospital in 2018. The study was cross-sectional and 359 neonates were recruited, 46 of these had positive cultures with *Staphylococcus aureus* predominating at 63%, *E. coli* and *Klebsiella pneumoniae* accounted for 15.2% and 10.9% respectively. The study however excluded neonates who presented to the facility from 6pm till 8am as well as those who came in over the weekend. There were no clear reasons given for theses exemptions, which would have been important since the site of recruitment was operational on a 24-hour basis. This could have introduced bias and also affected the results obtained(17).

Antimicrobial susceptibility

The 2014 global surveillance report on antimicrobial resistance noted that the African region shares the worldwide trend of increasing drug resistance (12).

The systematic review by Okomo et al reported high resistance rates to WHO recommended beta lactam antibiotics at 68% and amino glycoside resistance at 27%. It is important to note, however, that insufficient details were reported using most of the STROBE-NI items therefore raising concerns regarding the data quality from these studies(13)

Downie et al in a systematic review, described resistance and reduced susceptibility to penicillin, gentamicin combination and 3rd generation cephalosporins in more than 40% of cases in

community acquired neonatal bacteremia within developing countries (18). Most studies describing antimicrobial susceptibility have however been carried out in tertiary hospitals and are likely to overestimate resistance rates(6) .

Antibiotic resistance to gram negative isolates described by Musoke et al revealed highest rates to amoxicillin/ampicillin at 66.3%, followed by cefuroxime at 21.3%, ceftazidime at 19%, and amoxil/ clavunate at 13.5%. When resistance was implied by special tests the rates were higher for the three antibiotics with ranges of 27-50% and gentamicin resistance was at 20.2%. The prospective arm of the study scrutinized the use of antibiotics showing that as many as 73% of participants were treated for more than 2 weeks with antibiotics. Combinations of antibiotics were noted to be fairly standard although 28.6% were deemed unjustified from a review of clinical notes(14).Being a largely retrospective study, some of the limitations included; missing data from files prompting some potential participants to be left out, missing culture bottles sometimes therefore higher infection rates could not be ruled out and having no control over the specimen collection for culture which could have also influenced the results. Furthermore, the objectivity of justifying antibiotic use using a retrospective study design is questionable.

High resistance rates to commonly used antibiotics were also reported in the NBU at Kenyatta National Hospital by Cheruto et al in 2013. Gentamicin resistance was 65.6%, ampicillin 72.6%, penicillin 75%, ceftriaxone 75.2%, cefuroxime 72.6% and ceftazime 67.1%. Some of the antibiotics with high sensitivities included; vancomycin 100%, piperacillin-tazobactamm 89.7%, meropenem 90.8%. Antibiotic susceptibility to these commonly used antibiotics with respect to some isolates revealed resistance rates of CoNS to ampicillin, gentamicin, ceftriaxone and ceftazidime to be up to 50-59%, *Klebsiella spp* ranges of 89.5-100% and *Enterobacter spp* rates of 71.4-100% were noted. The high culture rates of CoNS however raise concern of possible contaminants although this was not addressed by the study which had no control over the blood sampling methods (15).

Ateka et al found that *Klebsiella spp*. was sensitive to meropenem (OR=3.298; 95% CI: 2.219-4.902), amikacin (OR=1.116; 0.920-1.354) and cefepime (OR=1.157; 0.167-8.002). However, there was high resistance to vancomycin (OR=2.455; 1.888-3.192, $p<0.001$). Interestingly, CoNS

was sensitive to vancomycin (OR=5.710; 3.478-9.374) and amikacin (OR=1.497; 0.884-2.535), but resistant to meropenem ($p<0.001$)(16).

In the Mulago study by Tumuhamye et al, 73.9% of isolates were resistant to ampicillin, 23.9% to gentamicin and 8.7% to ceftriaxone. *MRSA (Methicillin Resistant Staphylococcus Aureus)* was isolated from the blood specimens of 19 (5.3%) of the 359 neonates sampled, while 3 (0.8%) grew extended spectrum beta lactamase producers(17). Resistance rates to ampillicin are similar however much lower in the case of gentamicin and ceftriaxone when compared to the study by Cheruto et al at Kenyatta national hospital in 2013(15).

A pertinent question whether penicillin and gentamicin are still the appropriate 1st line antibiotics of choice can therefore be raised from the above studies. More so since good protocol adherence rates were reported by Tank et al in the NBU, KNH where 97.8% of neonates with signs and symptoms of sepsis were initiated on appropriate antibiotics at admission (19). In light of new antimicrobial resistance data, WHO in its 2016 evidence update on antibiotic use for sepsis in neonates and children, pointed out the possible need to revise the existing WHO guidelines and has instituted reviews to this effect(6).

The aim of this study was to identify the current pathogens culpable in neonatal sepsis and their antimicrobial susceptibility patterns at the Newborn unit within a tertiary facility in Kenya. The study also described some clinical characteristics and outcomes of culture positive neonatal sepsis.

The need for ongoing antimicrobial surveillance cannot be over emphasized if we hope to win the battle against increasing antibiotic resistance. This is especially true since the last new class of drugs was discovered in the 1980s and we race against rising global trends in drug resistant microbial agents.

“Surveillance that generates reliable data is the essential basis of sound global strategies and public health actions to contain antimicrobial resistance, and is urgently needed around the world” (12).

STUDY JUSTIFICATION

Worldwide, antimicrobial resistance is rising at alarming rates and Africa is no exception to this trend. The prevalence of neonatal sepsis and spectrum of causative organisms varies over time hence the need for routine surveillance through antimicrobial susceptibility testing. The 2018 Kenya national newborn guidelines recommend empirical treatment for neonatal sepsis using benzyl penicillin, gentamicin combination and cephalosporins as 1st and 2nd line treatment respectively (20). These agents have previously registered high resistance rates in neonatal sepsis within our newborn unit at Kenyatta national hospital. It is of paramount importance to carry out routine bacterial and antibiotic surveillance in our tertiary referral facility in order to inform treatment choices.

This study provides information on local bacterial pathogens involved in neonatal sepsis as well as their susceptibility patterns to guide patient treatment. It also describes patients with culture proven sepsis by; organisms implicated in early or late onset sepsis; pathogens common to hospital born versus referred patients as well as patient outcomes. The study also puts into perspective the issue of antimicrobial resistance in the local Kenyan setting and this evidence is imperative for emphasizing the importance of antibiotic stewardship. The findings from this research study will be disseminated to benefit other facilities within the country which refer patients for care at the national referral hospital.

RESEARCH QUESTION

What bacterial pathogens are implicated in neonatal sepsis from the Newborn Unit at Kenyatta National Hospital and what are their susceptibility patterns?

STUDY OBJECTIVES

Primary objectives

1. To describe the bacterial pathogens implicated in culture positive neonatal sepsis at the newborn unit, KNH from January to June 2019.

2. To outline the susceptibility patterns of pathogens identified in culture positive neonatal sepsis at the newborn unit, KNH.

Secondary objective

To determine the clinical characteristics of patients with culture positive neonatal sepsis for example; birth weight, gestation, place of delivery (KNH or referred), early or late onset sepsis and patient outcome.

METHODOLOGY

STUDY DESIGN

This was a retrospective cross-sectional study

STUDY AREA/ SETTING

The study area was Kenyatta National Hospital located in Nairobi, (the country's capital) which is a tertiary national referral hospital and the largest in the country. It is also a teaching hospital for the University of Nairobi as well as the Kenya Medical Training College among other institutions.

This research study involved the microbiology laboratory, records department and the newborn unit of the hospital.

1. Newborn unit

Physical setup

The Newborn unit has a total bed capacity of 60 beds including the Neonatal Intensive Care Unit (NICU). There are number of subunits for patient stratification as a means to reduce in-hospital infections as well as to aid in delivery of care. These subunits include; the NICU which has a six-bed capacity, the neonatal High Dependency Unit (HDU) with a five-bed capacity, three preterm infant rooms, an isolation room, a nursery for clinically stabilized infants and lastly the Kangaroo Mother Care (KMC) room with ten bed capacity. Since the numbers greatly outweigh the bed capacity of the unit, congestion is a major challenge with sometimes up to 3-4 babies sharing a cot.

Patient admissions

Despite having a limited bed capacity, the actual number of patients in the NBU averages 130 at any given time. Monthly admissions to the unit approximate 300 patients of which about 30% are referrals from lower-level facilities and the rest are deliveries from the hospital's labor wards, theatres and postnatal wards.

A number of infection control measures are in place to reduce infections within the unit aside from patient stratification. These include; emphasis on hand washing, use of alcohol hand rubs, personal protective equipment, training staff members on infection control and reinforcement of this information through Continuous Medical Education (CME), sterilization of equipment and use of uniform linen provided by the hospital within the unit.

2. Laboratory

The microbiology laboratory at Kenyatta National Hospital adheres to international standards and has a standard operating procedure for handling and preparing blood culture specimens.

The BACTEC 9050 series of blood culture instruments which are designed for rapid detection of microorganisms in clinical specimens is used. Blood sample volumes of about 2ml inoculated into BACTEC 9050 vials are entered into the BACTEC instrument for inoculation and periodic reading. Alternatively, using conventional (ordinary) blood cultures bottles, the daily appearance, readings and date are documented on the laboratory request form. Observation is made for visible signs of bacterial growth for example; turbidity, hemolysis, surface pellicle, gas bubbles and a clot. Identified microorganisms and their antimicrobial susceptibilities are reported on the laboratory request form and blood culture log book.

STUDY POPULATION

Neonates who had a blood culture sample taken during the period from 1st January to 30th June 2019.

TARGET POPULATION

Patients with positive blood culture results registered between January to June 2019.

INCLUSION CRITERIA

- Neonates admitted in the NBU.
- All neonates who had blood culture positive laboratory results reported between January and June 2019.

EXCLUSION CRITERIA

- Infants whose blood culture samples were taken while in a ward besides the NBU during the study period.

SAMPLE

All individuals who met the inclusion criteria within the study period were incorporated into the study since this was a retrospective study and the data was available from the microbiology records.

CASE DEFINITION

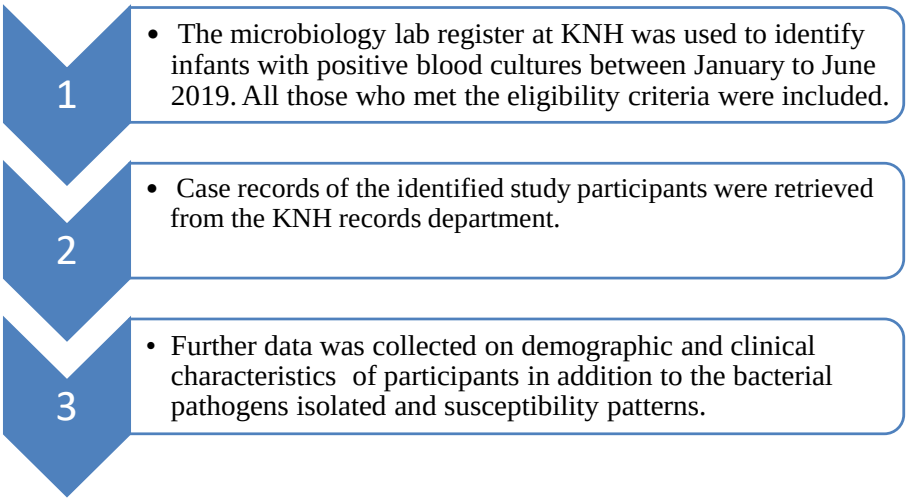
Blood culture positive- the isolation of a micro-organism or microorganisms from a blood sample cultured in a suitable growth medium under controlled laboratory conditions.

Early onset neonatal sepsis- onset of sepsis within the first 72 hours after birth(21).

Late onset neonatal sepsis- onset of sepsis occurring 3 to 90 days after birth(21).

STUDY PROCEDURE

FLOW CHART OF PROCEDURE



The study involved three key locations for data collection namely; microbiology laboratory, records department of Kenyatta National Hospital and the Newborn Unit.

1. Starting from the microbiology lab, using the blood culture results dispatch register, patients from the newborn unit who had a positive blood culture result between January and June 2019 were identified.

Those identified were recorded using a numbered case record form which captured the patient's unique hospital number, date of results release, organism grown and any additional information such as possible contamination.

2. Using the case record form for identified participants, patient files were retrieved from the records department of the hospital by the records officer. This was done using the patients' hospital numbers.

3. Further information about the study participants was collected from their admission records in the retrieved files, including;

- Sex
- Gestation
- Birth weight
- Early or late onset sepsis
- Place of delivery (home, Kenyatta National Hospital or referral from peripheral facility)
- Duration of treatment on antibiotics, 1st or 2nd line
- Clinical and laboratory features
- Clinical outcome whether discharged home or died

4. The study tool attached in the appendix was used for complete data collection and here patient names were excluded for confidentiality.

Handling discrepancies and missing data

Using the blood culture register at the laboratory, patients' names and inpatient hospital numbers were collected for comparison to ease patient file retrieval more so when discrepancies existed in the numbers recorded at the laboratory and records departments.

In case of missing data from patient files, attempts were made to get this information from the newborn unit registers for example; the newborn admission register and the mortality book kept in the unit.

DATA MANAGEMENT

Data collected from the laboratory and newborn registers and patient files were recorded on case record forms (attached) and thereafter transferred onto Microsoft EXCEL and data verification and cleaning and coding was done before transfer for analysis on STATA version 13 package. This data was password protected to restrict access and ensure confidentiality. Data was routinely backed up in three copies; locally, on an external drive, and google drive. Following completion of the study, this study will be uploaded onto the university server as well as repository.

DATA ANALYSIS

Data generated on Microsoft EXCEL was coded then transferred to STATA version 13 for analysis following cleaning. Continuous data like birth weight and categorical data for sex, place of birth was used to describe the study population. This data is presented as a table.

Bacterial isolates are presented as bar graphs and pie chart and categorized into either gram negative or gram-positive organisms. Antibiotic susceptibilities of the isolated bacteria are tabulated with columns as either; sensitive, resistant or intermediate and percentages are indicated.

Tests of association were used to compare clinical characteristics such as; clinical features, outcome, duration of stay, antibiotics used as well as their duration of use with bacterial isolates obtained. Comparison tests also sought associate pathogens with both early or late onset (within and after 3 days respectively) neonatal sepsis and their antibiotic susceptibilities.

DATA DISSEMINATION

The study results will be disseminated to the University of Nairobi repository as well as the department of Pediatrics and Child Health, the Newborn Unit and archives at Kenyatta National Hospital. The study will also be submitted for publishing in a medical Journal.

ETHICAL CONSIDERATIONS

1. Research approval was obtained from the University of Nairobi/KNH ethical review committee prior to proceeding with data collection.
2. A waiver of informed consent was also obtained from the above committee in light of this being a retrospective study.
3. No research funding was obtained to facilitate the research study.
4. Accessibility of the sources of research data such as the laboratory and Newborn registers as well as patient's admission records were limited to the principal investigator and this ensured confidentiality.
5. Furthermore, case records were filled into a password locked computer for security and to reinforce confidentiality.

BENEFITS

This study had no direct benefit to its participants however; information derived from it will inform and enhance future patient treatment especially through the collection of demographic and clinical characteristics of culture proven neonatal sepsis cases.

RISKS

The study conferred no risk and no safety issues were encountered concerning study participants.

STUDY STRENGTH

All eligible individuals within the study period were included. This ensured fairly accurate representation of common bacterial isolates and their susceptibilities as recorded within that period.

STUDY LIMITATIONS

Like most retrospective studies, there was missing patient information during data collection. In order to address this problem, additional data was got from the laboratory, NBU and records departments of the hospital. Complete data was obtained on the isolates and their susceptibility patterns however, clinical information was incomplete for 37 participants and unavailable for 34 participants.

There was no control over the method of sample collection for the blood culture results obtained in this study. The implication is that the lack of standardization of sample collection procedures could have influenced the results we obtained.

RESULTS

During the study period of January to June 2019, the total number of blood culture samples received in the microbiology laboratory from the Newborn Unit at Kenyatta National Hospital were 357. Of these, 158 isolates were obtained as positive cultures giving a culture positivity rate of 44.2%. Complete data was obtained on the isolates and their antibiotic susceptibilities for all the 158 positive cultures. This data was got from patient file and for those whose files were not traced or for whom results were not filed, the microbiology laboratory duplicate filed results were used to fill in missing data.

Using the patient details obtained from the blood culture logbook in the laboratory, 87 files out of the 158 culture positive cases were traced from the records department at Kenyatta National Hospital as shown in table 1.

Table 1. **Summary of data**

Total number of blood culture samples received in the lab during the study period	357
Total number of isolates	158
Number of files retrieved from KNH records department	87
Culture positivity rate	44.2%

Of the 71 participants whose files were not traced, data was sourced from the records department for 37 cases on birthweight, outcome whether discharged or died in hospital, sex, date of admission and duration in hospital. This information was obtained by extracting electronic records of death for patients during the study period under the diagnosis of either prematurity or neonatal sepsis. Therefore, data for the remaining 34 cases was missing.

SOCIO- DEMOGRAPHIC CHARACTERISTICS

The socio-demographic characteristics of the study population are described in table 2.

Table 2. **Socio-demographic characteristics**

Variables	**Characteristics**	**Frequency (%) (n=87)**
Gestational age in weeks	<37 weeks	46(52.8)
	≥37 weeks	41(47.2)
Age at admission	1-7 days	72(82.8)
	8-28 days	15 (17.2)
Age at the onset of symptoms	1-3	40(46)
	>3	47(54)
Sex	Males	52(59.8)
	Female	35(40.3)
Birth weight in grams	<2500	48(55.2)
	≥2500	39(44.8)
Referral from another facility	Yes	41(47.1)
	No	46(52.9)
Place of delivery	Home	2(2.3)
	Peripheral facility	41(47.1)
	KNH	44(50.6)
Mode of delivery	SVD	41(47.1)
	C/S	46(52.9)

More than half of the cases were preterm infants accounting for 52.8% with the remainder being term and 55.2% were born with low birth weight less than 2500grams. The majority of cases, 82.8% were admitted within the first week of life, and male infants were predominant at 59%. Regarding the place of delivery, 50.6% were born at Kenyatta National Hospital, 47% were born at a peripheral facility and only 2% accounted for home deliveries. Similarly, 52.9% were admitted from the maternity and post-natal wards at KNH whereas 47.1% were referred from other facilities. Neonates with early onset sepsis who presented with symptoms within the first three days of life made up 46% while late onset sepsis accounted for the greater proportion of 54%. Caesarean births were more than those by spontaneous vaginal delivery represented by 52.9% and 47.1% respectively.

OBJECTIVE 1. PATHOGENS IMPLICATED IN CULTURE POSITIVE NEONATAL SEPSIS

Gram negative isolates were predominant in this study at 58% whereas gram positives were 42% of all bacterial isolates. Table 3 describes the organisms isolated

Table 3. **Organisms isolated**

	Isolates	n=158(%)
1.	*Klebsiella pneumoniae*	43 (28.9)
2.	*Staph epidermidis*	27(18.1)
3.	*Pantoea agglomerans*	18(12.1)
4.	*Serratia marcescens*	15(10.1)
5.	*Enterococcus faecalis*	11(7.4)
6.	*Staph haemolyticus*	8(5.4)
7.	*Micrococcus species*	4(2.7)
8.	*Coagulase negative staph*	4(2.7)
9.	*E. coli*	3(2.0)
10.	Other bacteria	15(9.3)

The top five bacterial isolates cultured were; *Klebsiella pneumoniae* 28.9%, *Staphylococcus edipermidis* 18.1%, *Pantoea agglomerans* 12.1%, *Serratia marcescens* 10.1%, and *Enterococcus faecalis* 7.4%. *Escherichia coli* accounted for 2% of the isolates and other less common isolates contributed 10% including; *Klebsiella oxytoca*, *Staphylococcus aureus, Acinetobacter baumani*, *Pseudomonas luteola* and *Staphylococcus lentus* each contributing 2(1.3%) isolates while *Enterococcus cloacae*, *Enterococcus gallinarum*, *Staphylococcus hominis* and *Aerococcus viridans* each accounted for 1(0.7%) of the isolates. What is worth noting is that fungal pathogens although not the focus of this study, represented 10 of the total isolates obtained during the period

Early and late onset sepsis

Isolates obtained in early and late onset neonatal sepsis are shown in figure 1.

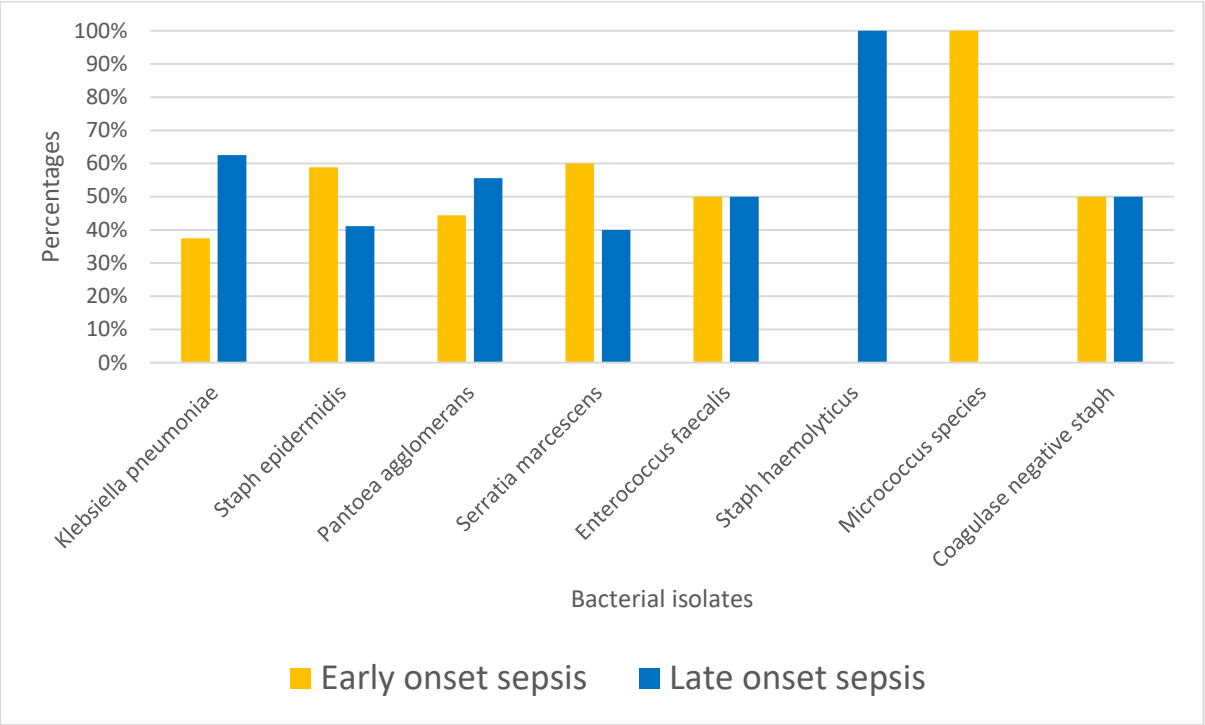


Figure 1. **Isolates by age of onset of symptoms**

The predominant organisms observed in early onset neonatal sepsis were; *Micrococcus species* (100%), *Serratia marcescens* (60%) and *Staphylococcus epidermidis* (59%) whereas in late onset sepsis; *Staphylococcus hemolyticus* (100%), *Klebsiella pneumoniae* (63%) and *Pantoea agglomerans* (56%) were most common. *Enterococcus faecalis* and *CoNS* were equally distributed in early and late onset sepsis.

Isolates by place of delivery

Bacterial pathogens isolated among neonates born at KNH and those referred from peripheral facilities are shown in figure 2.

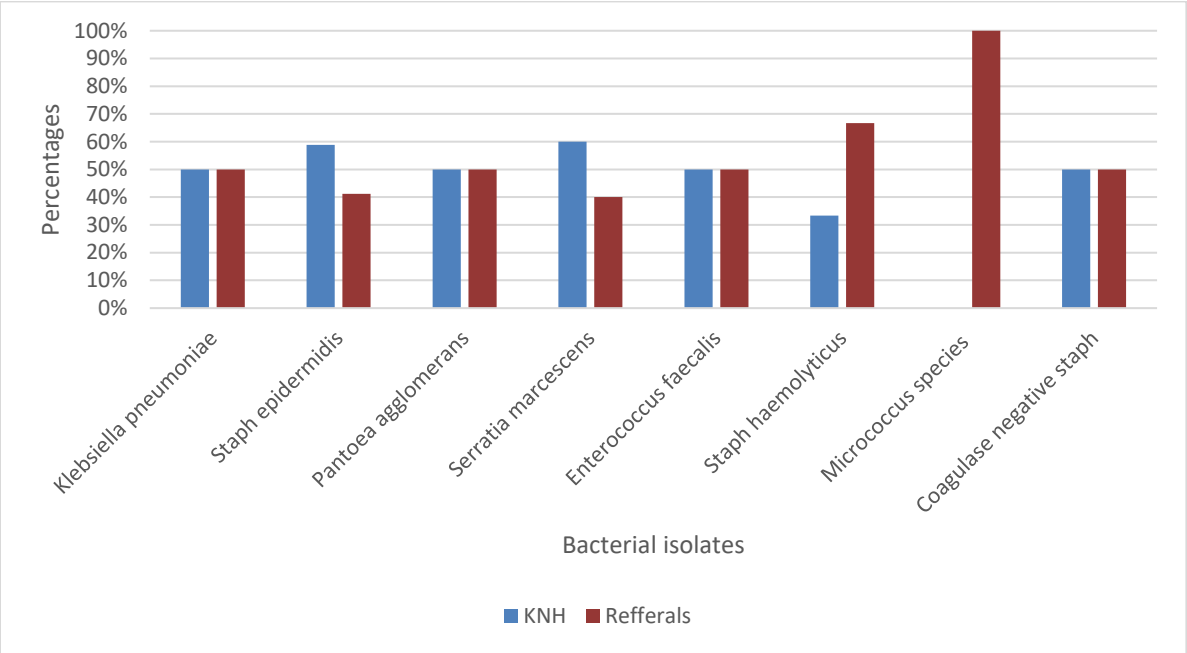


Figure 2**. Isolates by place of delivery**

Among the participants born within KNH, *Serratia marcescens* and *Staphylococcus epidermidis* were predominant accounting for 60% and 59% respectively relative to those born in peripheral facilities at 40% and 41% respectively.

Of those referred from peripheral facilities, *Micrococcus species* and *Staphylococcus hemolyticus* were predominantly isolated at 100% and 67% respectively as compared to 33% of *Staphylococcus hemolyticus* obtained among those delivered within Kenyatta National Hospital.

Klebsiella pneumoniae, *Pantoea agglomerans*, *Enterococcus faecalis* and *CoNS* were isolated in equal proportions among participants born within KNH and those referred from peripheral facilities.

OBJECTIVE 2. ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF PATHOGENS

The antibiotic susceptibility patterns of various bacterial organisms are shown in the figure 3.

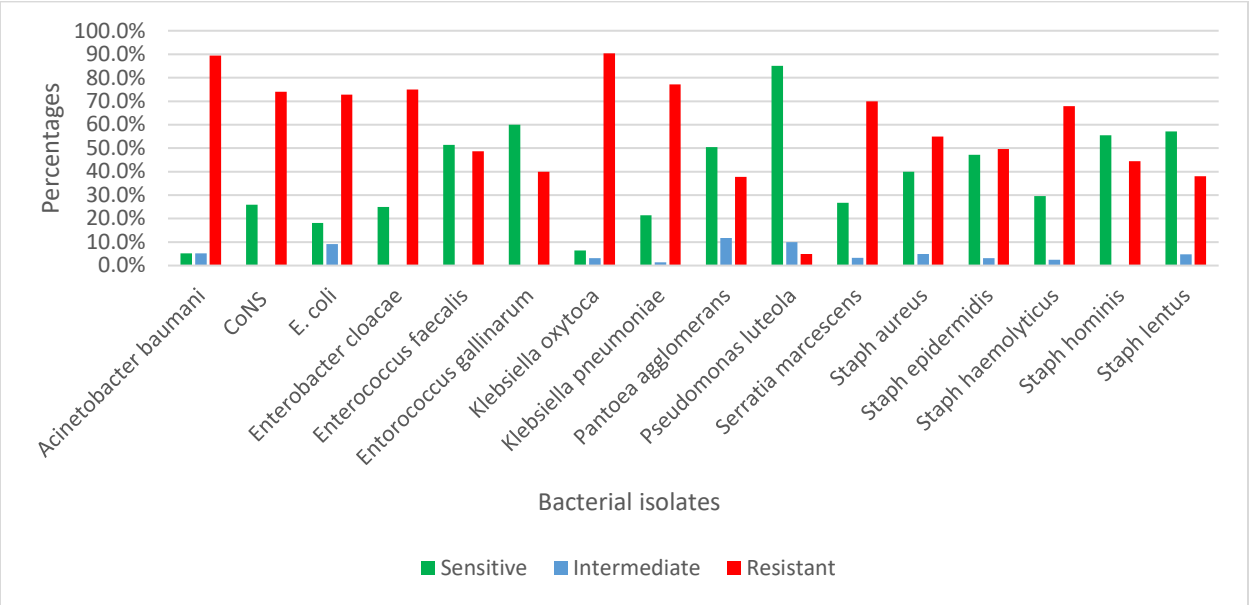


Figure 3. **summary of the antibiotic susceptibility profile of bacterial isolates**

Organisms showing high resistance rates against antibiotics they were tested against included *Klebsiella oxytoca* which was 90.3% resistant and only 6.5% were sensitive, *Acinetobacter baumani* 89.5% resistant to antibiotics with only 5.3% being sensitive. *Klebsiella pneumoniae* had a resistance rate of 77.1% with 21.5% being sensitive to antibiotics. E. coli was 72.7% resistant to antibiotics and 18.2% sensitive. Half of the *Pantoea agglomerans* 50.4% were sensitive to antibiotics while 37.8% were resistant. *Serratia marcescens* resistance to antibiotics stood at 69.9% while 26.8% were sensitive. *Pseudomonas luteola* showed high sensitivity to antibiotics, 85% whereas only 5% were resistant. Other bacteria that were sensitive to antibiotics included; *Enterococcus gallinarum*, *Staph lentus*, *Staph hominis* and *Enterococcus faecalis* with sensitivity rates of 60%, 57%, 55.6% and 51.4% respectively.

1. Klebsiella pneumoniae

The antibiotic susceptibility profile of *Klebsiella pneumoniae* is shown in figure 4.

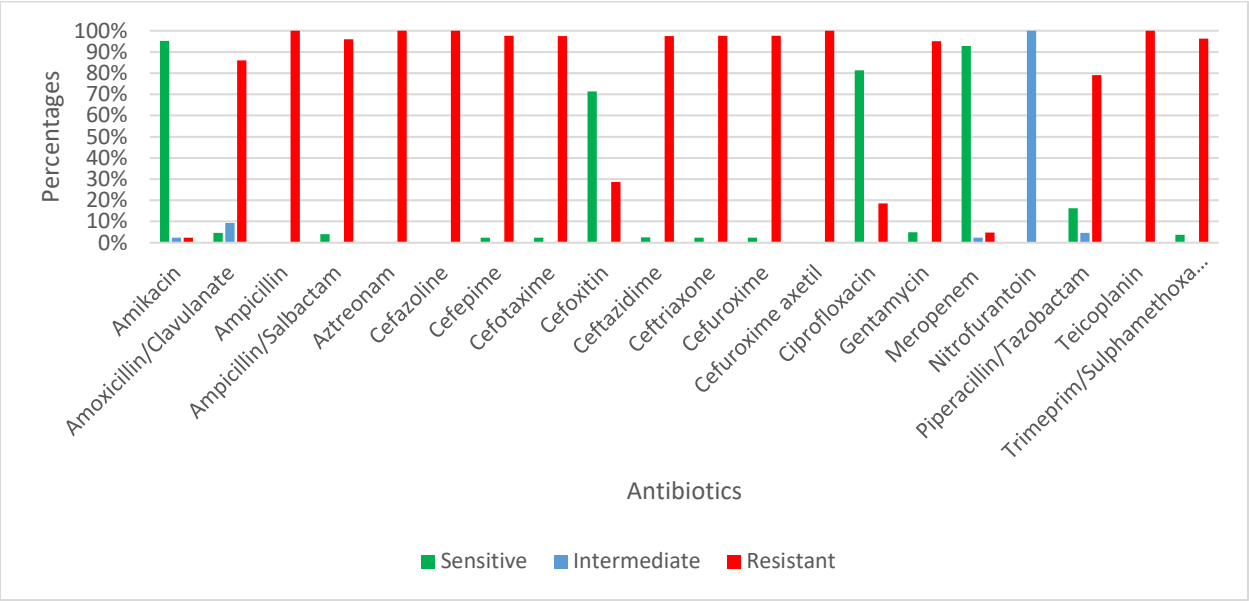


Figure 4. ***Klebsiella pneumoniae*** **antibiotic sensitivity profile**

Klebsiella pneumoniae, which accounted for the majority of isolates obtained, was highly sensitive to amikacin and meropenem at 95% and 93% respectively. *Klebsiella pneumoniae* was however highly resistant to the majority of antibiotics it was tested against with 100% resistance to ampicillin, aztreonam, cefazoline, cefuroxime axetil and teicoplanin. Resistance levels of 98% were noted to cefepime, ceftriaxone, cefuroxime and cefotaxime. Resistance to ceftazidime was 97%, that to ampicillin/sulbactam and trimethoprim/Sulfamethoxazole was 96% and resistance to gentamicin was 95%.

2. Staphylococcus epidermidis

The antibiotic susceptibility profile of *Staphylococcus epidermidis* is shown in figure 5.

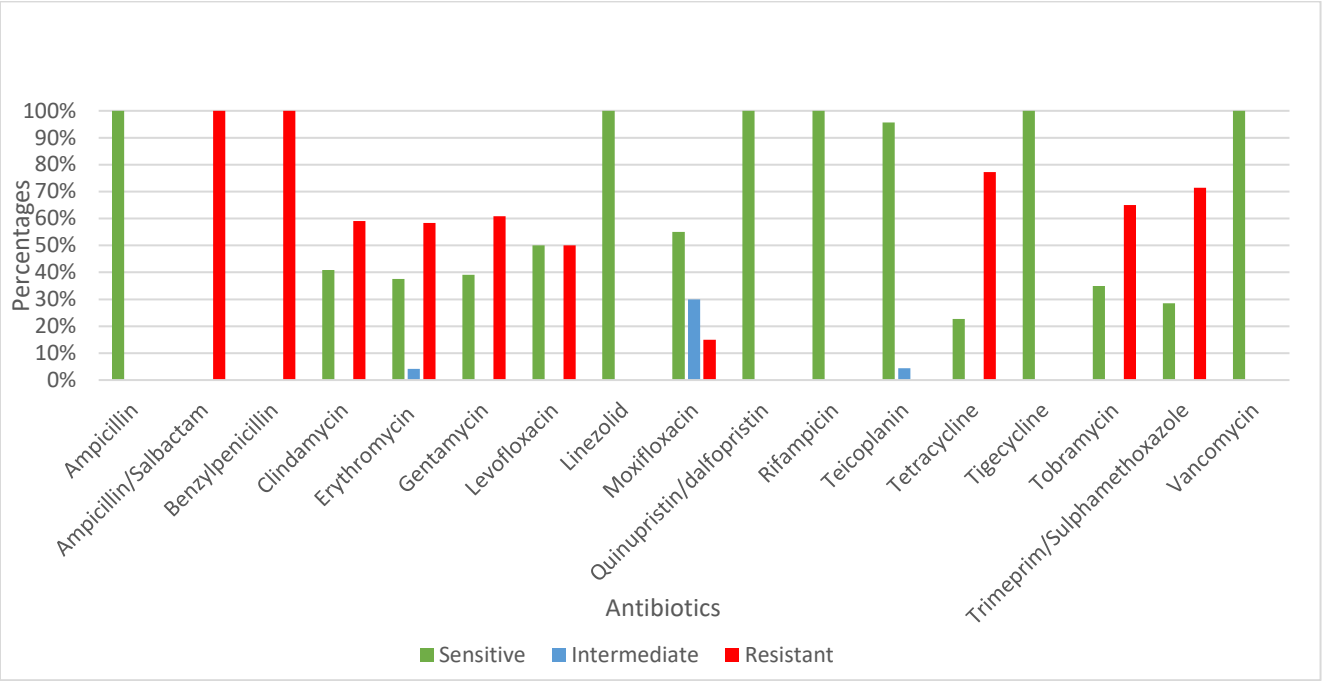


Figure 5. ***Staphylococcus epidermidis*** **antibiotic sensitivity profile**

Staph epidermidis, the 2nd most common isolate showed high sensitivity of 100% to ampicillin, linezolid, quinupristin/dalfopristin, rifampicin, tigecycline and vancomycin with 96% sensitivity to teicoplanin. Resistance was 100% to ampicillin/sulbactam and benzylpenicillin with lower resistance rates of 77% and 71% to tetracycline and trimethoprim/sulfamethoxazole respectively.

3. Pantoea agglomerans

The antibiotic susceptibility profile of *Pantoea agglomerans* is shown in figure 6.

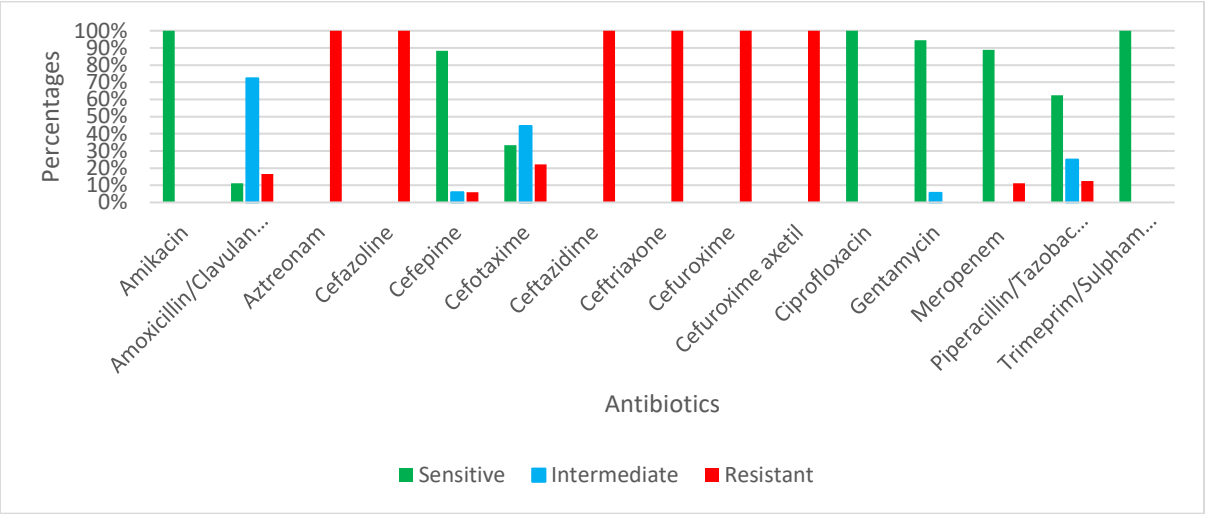


Figure 6. ***Pantoea agglomerans*** **antibiotic sensitivity profile**.

Pantoea agglomerans had 100% sensitivity to amikacin, ciprofloxacin and trimethoprim/ sulfamethoxazole. Sensitivity to gentamicin, meropenem and cefepime were also high at 94%, 89% and 88% respectively. The isolate had very high resistance of 100% to aztreonam and cephalosporins; ceftriaxone, ceftazidime, cefuroxime and cefazoline.

4. Serratia marcescens

The antibiotic susceptibility profile of *Serratia marcescens* is shown in figure 7.

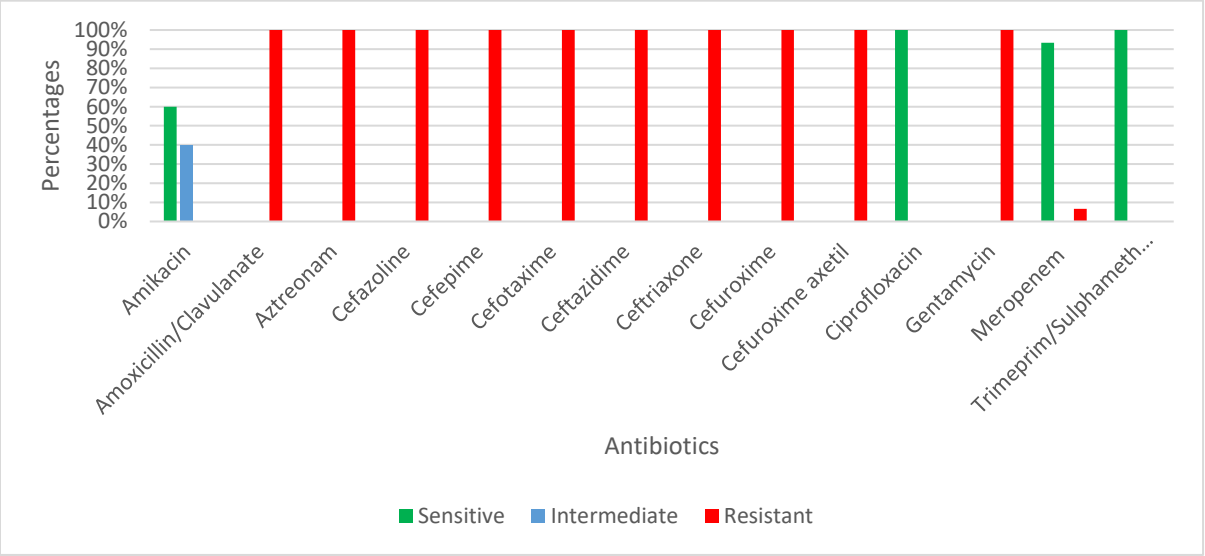


Figure 7. ***Serratia marcescens*** **antibiotic sensitivity profile**

Serratia marcescens was 100% sensitive to ciprofloxacin and trimethoprim/sulfamethoxazole and also highly sensitive to Meropenem 93% and 60% sensitive to Amikacin. It was however highly resistant to the cephalosporin; cefazoline, cefepime, cefotaxime, ceftazidime, cefuroxime as well as amoxicillin/clavulanic acid, aztreonam and gentamicin at 100%.

5. *Enterococcus faecalis*

The antibiotic susceptibility profile of *Enterococcus faecalis* is shown in figure 8.

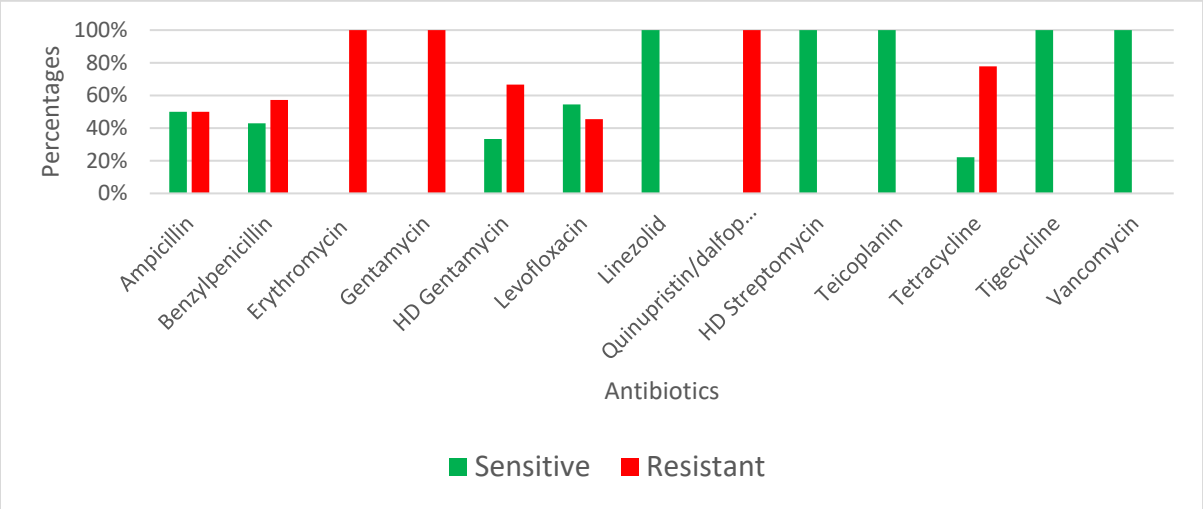


Figure 8. ***Enterococcus faecalis*** **antibiotic sensitivity profile**

Enterococcus faecalis, a common cause of nosocomial infections was 100% sensitive to linezolid, high dose streptomycin, teicoplanin, tigecycline and vancomycin however, sensitivity was low to benzylpenicillin and tetracycline at 43% 22% respectively. There was 100% resistance to erythromycin, gentamicin and quinupristin/dalfopristin.

Antibiotic susceptibility profile

The susceptibility profile of commonly used antibiotics in our newborn unit are shown in table 4.

Table 4. **Susceptibilities of commonly used antibiotics**

	Antibiotic	Totals isolates	Sensitive (%)	Intermediate (%)	Resistant (%)

1	Amikacin	83	73(88)	9(11)	1(1)
2	Amoxicillin/Clavulanate	82	4(5)	18(22)	60(73)
3	Benzylpenicillin	46	3(7)	0(0)	43(93)
4	Ceftazidime	81	2(2)	1(1)	78(96)
5	Ceftriaxone	85	2(2)	1(1)	82(96)
6	Cefuroxime	80	1(1)	0(0)	79(99)
7	Ciprofloxacin	86	75(87)	0(0)	11(13)
8	Clindamycin	36	13(36)	0(0)	23(64)
9	Erythromycin	52	10(19)	1(2)	41(79)
10	Gentamycin	124	36(29)	1(1)	87(70)
11	Meropenem	85	74(87)	3(4)	8(9)
12	Piperacillin/Tazobactam	70	21(30)	7(10)	42(60)
13	Trimethoprim/Sulfamethoxazole	91	35(38)	0(0)	56(62)
14	Vancomycin	48	43(90)	1(2)	4(8)

Of the commonly used antibiotics in our setting, the highest sensitivities by the various isolates were to; vancomycin 90%, amikacin 88%, ciprofloxacin and meropenem at 87% as in table 4. The highest antibiotic resistance levels were to; cefuroxime 99%, ceftriaxone and ceftazidime 96%, and benzylpenicillin 93%. Gentamycin resistance stood at 70%.

Antibiotic susceptibilities of other antibiotics used are shown in table 5 below.

Table 5. **Susceptibilities of other antibiotics**

Antibiotic	**Sensitivity testing**		
	Sensitive (%)	**Intermediate (%)**	**Resistant (%)**
Ampicillin	4(8)	0	50(92)
Ampicillin/Sulbactam	1(3.2)	0	30(96.8)
Aztreonam	0	0	45(100)
Cefazoline	2(4.8)	0	40(95.2)
Cefepime	18(21.7)	1(1.2)	64(77.1)
Cefotaxime	9(10.8)	8(9.7)	66(79.5)
Cefoxitin	5(71.4)	0	2(28.5)
Cefuroxime axetil	0	0	48(100)
HD Gentamycin	1(33.3)	0	2(66.6)
Levofloxacin	24(47)	0	27(53)
Linezolid	47(100)	0	0
Moxifloxacin	13(43.3)	10(33.3)	7(23.3)

Nitrofurantoin	0	1(100)	0
Oxacillin	0	0	1(100)
Quinupristin/dalfopristin	1(33.3)	0	2(66.7)
Rifampicin	3(100)	0	0
HD Streptomycin	3(100)	0	0
Teicoplanin	44(93.6)	1(2.1)	2(4.3)
Tetracycline	8(18.6)	0	35(81.4)
Tigecycline	5(83.3)	0	1(16.7)
Tobramycin	9(29)	0	22(71)

Outcomes

More than half of patients from whom clinical information was obtained were discharged alive from hospital accounting for 53% whereas the remainder, 47% died as shown in figure 9.

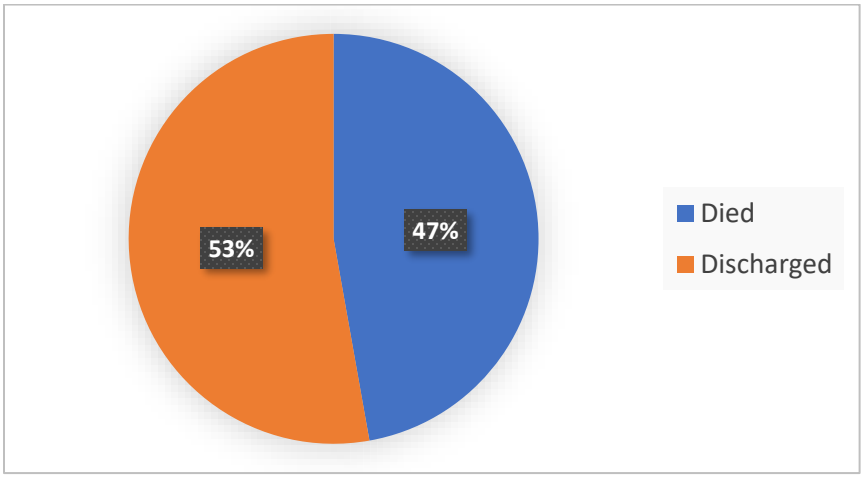


Figure 9. **Clinical outcomes**

Medians were used to describe bacterial isolates and their outcomes in terms of duration of stay. Since the data distribution was skewed, the wilcoxon rank sum test was used for association. Table 6 shows the median duration of hospital stay among the variables.

Table 6. **Duration of hospital stay**

Variables	Median [P25%, P75%] duration of hospital stay in days	p-values
Gram staining		
Gram positive	15[12,39]	0.075
Gram negative	23[5,34]	
Clinical outcome		
Discharged	25[11,39]	**0.0069***
Dead	13[6,18]	

Gram-negative sepsis was associated with longer median duration of hospital stay of 23 days compared to 15 days by those who had gram-positive sepsis. Those who were discharged (53% of patients), stayed longer in hospital with median duration of 25 days compared to 13 days spent by those who died while in hospital. There was statistical significance p=0.0069, in the median duration of hospital stay among those who were discharged compared to those who died in hospital. However, no statistical significance was found in median duration of hospital stay among the gram negative and gram-positive isolates (p=0.075).

The outcomes of cases based on the type of neonatal sepsis is shown in table 7.

Table 7. **Clinical outcome by the type of sepsis**

Variable	Outcome		p-values
	Discharged	Died	
Onset			
Early onset sepsis	30(75%)	10(25%)	0.862
Late onset sepsis	36(76.6%)	11(23.4%)	
Gram staining			

Gram negative isolates	31(43.7%)	40(56.3%)	**0.006**
Gram positive isolates	32(69.6%)	14(30.4%)	

Outcomes in mortality were comparable among cases of early and late onset neonatal sepsis. Mortality was higher among those with gram negative sepsis at 56.3% compared to 30.4% among those with gram positive sepsis with statistical significance p= 0.006 using the chi square test.

Antibiotics prescribed

The antibiotics prescribed during the study period are shown in table 8 below.

Table 8. **Frequency of antibiotics prescribed**

Antibiotics list	Frequency	Percentage
Gentamycin	66	20.63
X pen	66	20.63
Ceftazidime	57	17.81
Amikacin	46	14.38
Meropenem	27	8.44
Erythromycin	19	5.94
Metronidazole	14	4.38
Ceftriaxone	10	3.13
Amoxicillin	3	0.94
Vancomycin	3	0.94
piperacillin/tazobactam	3	0.94
Amoxicillin/clavulanic acid	2	0.63
Flucloxacillin	2	0.63
Ciprofloxacin	1	0.31
Trimethoprim/sulfamethoxazole	1	0.31

The most frequently prescribed antibiotics were; gentamycin and X-pen at 20.63% followed by ceftazidime, amikacin, meropenem and erythromycin at 17.81%, 14.38%, 8.44% and 5.94% respectively. Those least prescribed were trimethoprim/sulfamethoxazole and ciprofloxacin at

0.31%, flucloxacillin and amoxicillin/clavulanic acid at 0.63% and amoxicillin, vancomycin and piperacillin/tazobactam at 0.94%.

Associations

Multi variate analysis done with poor clinical outcome of death as the dependent variable against several independent variables, yielded statistical significance for gram negative sepsis with p=0.007 and clinical signs of fever(p<0.001) and Jaundice (p=0.001) as shown in tables 9- 11. Variables associated with death following regression analysis were; gram negative isolates p=0.007, fever (p<0.001), convulsions (p=0.041) and tachypnea (p=0.021) as shown in table 12.

Table 9. **Association between bacterial isolates and clinical outcomes**

Bacteria A	**Odds Ratio (95% CI)**	P-value
Klebsiella pneumoniae	2.05(0.927-4.546)	0.076
Staph epidermidis	0.5(0.187-1.34)	0.168
Serratia marcescens	1.8(0.6-5.402)	0.295
Pantoea agglomerans	0.72(0.239-2.15)	0.553
Micrococcus species	2.28(0.201-25.82)	0.505
Staph hemolyticus	1.13(0.218-5.801)	0.888
CONS	1.12(0.153-8.232)	0.909

Table 10. **Association between clinical outcome and gram staining**

Gram staining	Outcome		**Odds Ratio (95% CI)**	P-value
	Died	Discharged		
Gram negative	40	31	2.95(1.346-6.458)	**0.007***
Gram positive	14	32		

Table 11. **Associations between signs and symptoms and clinical outcomes**

Variables	**Odds Ratio (95% CI)**	**p-value**
Desaturation	6.88(0.821-57.697)	0.075
Tachypnea	2.38(0.849-6.661)	0.099
Chest in drawing	2.91(0.978-8.646)	0.055
Nasal flaring	3.38(0.674-16.968)	0.139
Respiratory distress	1.09(0.432-2.743)	0.857
Irritability	3.74(0.406-34.466)	0.244
Tachycardia	1.20(0.258-5.617)	0.813

Jaundice	6.875(2.206-21.426)	**0.001**
Neck retraction	1.81(0.16-20.518)	0.631
Pallor	1.77(0.61-5.119)	0.294
Grunting	0.89(0.172-4.584)	0.888
Cyanosis	2.76(0.28-27.304)	0.385
Reduced reflexes	2.58(0.984-6.75)	0.054
Hypothermia	1.81(0.16-20.518)	0.631
Fever	**13.75(3.901-48.486)**	**<0.001**
Apnea	0.89(0.121-6.53)	0.909
Dehydrated	3.74(0.406-34.466)	0.244
Difficulty in breathing	1.42(0.535-3.748)	0.483
Vomiting	1.53(0.35-6.699)	0.572
Coffee aspirates	2.46(0.727-8.298)	0.148
Convulsion	3.33(0.871-12.76)	0.079
Abdominal distension	0.29(0.029-2.84)	0.286

Logistics regression analysis was done to determine the independent determinant of poor clinical outcome (mortality). Forward stepwise analysis was used to determine the parsimonious model and the variables with p-values <0.1 recruited in the model.

Table 12**. Logistic regression analysis**

Variable	S.E.	Odds Ratio	95% CI. for EXP(B)		p-value
			Lower	Upper	
Gram negative isolates	0.522	4.049	11.236	1.456	**0.007**
Fever	0.755	15.607	3.551	68.597	**<0.001**
Jaundice	0.696	3.548	0.906	13.893	0.069
LCWI	0.733	2.579	0.613	10.854	0.196
Desaturate	1.292	11.462	0.912	144.124	0.059
Reduced reflexes	0.699	0.632	0.161	2.489	0.512
Convulsion	0.866	5.858	1.074	31.964	**0.041**
Tachypnea	0.667	4.647	1.257	17.180	**0.021**
Klebsiella pneumoniae	0.632	1.008	0.292	3.481	0.990

The following variables were found to be independent determinants of mortality; gram negative sepsis, fever, Convulsions and tachypnea. The neonates with gram negative sepsis were 4 times more likely to die than those with gram positive sepsis (95% CI:11.2-1.5) and was statistically significant (p 0.007).

DISCUSSION

This study set out to describe the bacterial pathogens and susceptibility patterns of isolates in neonatal sepsis from the NBU at Kenyatta National hospital within a six-month period. It also aimed to determine the patients' characteristics and clinical outcomes of neonates with culture positive sepsis. Neonatal sepsis continues to be a major challenge due to the constantly changing trend in bacterial pathogens and their susceptibility patterns.

The culture positivity rate in this study was 44.2% however after excluding possible contaminants or normal skin flora (48 isolates), the rate was 31%. The gram-negative bacteria predominated at 58% compared to gram positive isolates 42%. These findings are similar to those reported in previous studies by Musoke et al in 1997 and Cheruto in 2013 which both had high gram-negative sepsis rates of 73.6% and 51.3% respectively at the newborn Unit of Kenyatta National Hospital(14)(15). The persistently high gram-negative sepsis rates are likely attributable to hospital acquired infections like Klebsiella pneumoniae whose isolation rate continues to be high in our newborn unit.

Klebsiella pneumoniae was the top isolate obtained from this study 43/149(28.9%) which is a comparable finding to that by Musoke et al where *Klebsiella pneumoniae* was the top isolate at 31.4%(14). *Klebsiella pneumoniae* continues to pause a challenge in our Newborn unit and has occasionally been associated with infection outbreaks. Cheruto et al however found the commonest isolate to be *Coagulase negative Staphylococcus* at 30% followed by *Enterobacter species at* 20%. *Citrobacter species* and *Klebsiella species* accounted for 12.8% and 11% respectively(15). Mohsen et al also found Klebsiella pneumoniae to be the predominant isolate in both blood and endotracheal aspirates at 42% and 41% respectively at a major referral neonatal intensive care unit in Egypt(22).

Late onset neonatal sepsis accounted for a higher proportion of infections during this study, 47/87(54%) while early onset was represented by 40/87(46%) of the cases. Phillay et al, found a similar but higher rate of late onset sepsis accounting for 86.8% of infections in a retrospective study done on neonatal sepsis at a tertiary unit in South Africa(19). In the study by Musoke et al, there was equal distribution of infection rates between early and late onset sepsis each with 20 isolates. These studies are all retrospective and therefore rely on documentation of symptoms

which when poor or not done can affect results and these findings might have been different if done as prospective studies.

This study revealed the commonest organisms in early onset neonatal sepsis to be *Micrococcus species, Serratia marcescens* and *Staphylococcus epidermidis* at 100%, 60% and 59% respectively when compared to proportions in late onset sepsis. Late onset sepsis was predominated by *Staphylococcus hemolyticus, Klebsiella pneumoniae* and *Pantoea agglomerans* at 100%, 63% and 56% respectively compared to proportions in early onset sepsis. The finding of *Klebsiella pneumoniae* as a common isolate in late onset neonatal sepsis is similar with that by Phillay et al although the rate of isolation was lower at 12.4% and 6.7% in late and early onset neonatal sepsis respectively(23).

Klebsiella predominance in late onset neonatal sepsis is not surprising particularly in our setting since it is likely attributable to hospital acquired infections given the large number of patients that our newborn unit holds in excess of its intended bed capacity. This encourages overcrowding within the unit with babies having to share cots and increasing the patient to nurse ratio which reduces quality of care and adherence to infection prevention strategies. Ngugi S et al documented a low compliance rate of 15% to hand hygiene practices in our newborn unit and found that more than half, 52% of healthcare workers were unaware of the 5 moments of hand hygiene(24). Under staffing makes the situation even worse with increased workload in health facilities being linked to higher infection rates as well as poor outcomes(25).

Similar to the above findings, Essel V et al demonstrated the role of overcrowding in outbreaks of health-care associated blood stream infections (HCA-BSI) in a multi-sectoral investigation of a neonatal unit outbreak of *Klebsiella pneumoniae* bacteremia, at a regional hospital in South Africa. The study conducted from January 2017 to August 2018, had a culture positivity rate of 11% which was lower than that of our study (44.5%) and 52% were HCA-BSI predominated by *Klebsiella pneumoniae* at 32%. In our study *Klebsiella pneumoniae* was the overall top isolate at 28.9%. The South African study registered an average bed occupancy rate of 118% and this increased to 121% during the outbreak period. The study also highlighted inadequate infection prevention and control practices including sub-optimal adherence to aseptic technique and hand hygiene at 57% in the

NICU(26). These findings are likely similar in our newborn unit in light of the excess admissions since Kenyatta hospital is a national referral tertiary facility.

During this study, Fungal isolates constituted 10/159 (6.2%) of the total positive blood cultures obtained as an incidental finding as the study was set on bacterial pathogens. However, the finding gives important information about the scope of causative organisms and so clinicians ought to anticipate these alternative causes of neonatal sepsis in order to provide adequate treatment. Phillay et al documented a 4.5% fungal sepsis rate which is comparable to that in our study (23). In this present study, the fungal species isolated included the *Candida species*; *albicans, parapsilosis* and *pelliculosa.* Fortunately, all these isolates were sensitive to the antifungal agents they were tested against which included; Fluconazole, Voriconazole, Caspofungin, Micafungin, Amphotericin B and Flucytosine.

In comparison to previous studies, gram negative sepsis was noted to be associated with increased mortality (P= 0.007) as well as increased morbidity in terms of prolonged hospital stay at 23 days median which was 8 days more than that of neonates with gram positive sepsis. This difference in duration of hospital stay was however not statistically significant (p=0.075) perhaps owing to the low numbers of participants studied. Mortality rate among those with gram negative sepsis was almost twice those with gram positive sepsis, at 56.3% and 30.4%% respectively. In the study by Musoke et al, the case fatality rate among those with gram negative sepsis was 41%(14). These findings are not usual because gram negative pathogens are notorious for causing severe infections and pose a medical challenge in treatment owing to their inherent virulence factors. The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, fatal shock, and other destructive events (27). In the same light, our study revealed that patients who were discharged alive had stayed much longer in hospital with a median duration of 25 days compared to 13 days (almost 2 times longer) spent by neonates who died in hospital, p=0.0069. This further illustrates the burden of neonatal sepsis in terms of costs and the challenges faced in treatment. It is estimated that 5.29–8.73million DALYs (disability adjusted life years) are lost annually in Sub Saharan Africa due to neonatal sepsis with the annual economic burden ranging from $10 billion to $469 billion(28).

Bacterial susceptibility to antibiotics has changed over the years in our setting just like on the regional and global scale. Vancomycin and Amikacin have retained relatively high overall sensitivities, 90% and 88% respectively that of Ciprofloxacin and Meropenem is 87%. It is important to note however that vancomycin and meropenem sensitivities have dropped from 100% and 91% respectively while amikacin and Ciprofloxacin sensitivity has remained consistent at 87% and 86% respectively since 2013 in the study by Cheruto et al(15). Piperacillin/ tazobactam sensitivity remarkably dropped from 90% in the 2013 study to 30% in this current study. Very high resistance rates were recorded against commonly used antibiotics particularly to 3rd generation cephalosporins; ceftriaxone, ceftazidime, cefazoline and cefuroxime with rates exceeding 95%. Benzyl penicillin and Gentamicin also had high resistance rates of 93% and 70% respectively. Comparing with the 2013 study, there has been an increase in resistance of nearly 30% for each of these antibiotics with the exception of Gentamicin for which resistance has risen by only 5%. Mohsen et at had similar findings in a neonatal sepsis study done in Egypt where Gram negative organisms were most resistant to ampicillins (100%), cephalosporins (93%–100%) and with less resistance to aminoglycosides (36%–52%). Piperacillin-tazobactam resistance was much higher in their study at 99%(22) compared to 60% in our study.

A major contributory factor to the rise in antibiotic resistance is poor investigation of patients with signs and symptoms of neonatal sepsis while prolonging the duration on antibiotics. P. Tank et al found very low rates of investigations to confirm infection at the newborn unit of KNH with blood cultures done only in 4% neonates on admission, while complete blood count and C reactive protein were done in 70% and 62% of patients respectively. Although 97.8% of antibiotic were prescribed in accordance to the Kenya guidelines at admission, these were continued beyond 48-72 hours in 53.6% who had shown clinical improvement(19). In the study by Musoke et al, antibiotic use extended beyond 14 days in 73% of patients. The 2018 Kenya newborn guidelines recommend initiation of prophylactic antibiotics for patients with clinical signs or risk factors of infection for 48-72 hours while awaiting laboratory investigation results and subsequently reviewing the need for further antibiotics(20).

Currently the first line antibiotics in our Newborn Unit consists of a Penicillin and aminoglycoside whereas 3rd generation cephalosporins constitute 2nd line treatment (20). These recommendations have not changed over the years which is a cause for concern. Is it time to revise our empiric antibiotic choice? Yes, I believe so in light of the high resistance rates to our recommended antibiotics. There is also need to preserve aminoglycoside sensitivity as this class of drugs has proven resilient. This can be achieved through correct dosing as misuse can enhance antibiotic resistance(29). Aluvaala et al found drug doses in excess of recommendations by 20% in prescriptions of penicillin and gentamicin in an audit of neonatal care in 22 clinical training facilities in Kenya(30). It is also paramount to administer antibiotics in carefully chosen combinations for synergism while broadening bacterial coverage. The importance of preserving the efficacy of existing antibiotics to minimize the development and spread of resistance cannot be overemphasized since last completely new classes of antibacterial drugs were discovered during the 1980s (12).

The study had some limitations. Since it was retrospective, there was no control over blood sampling techniques and procedures which could have contributed to the high rates of possible skin contaminants including *Staphylococcus species*; *hemolyticus, epidermidis, hominis, lentus, CoNS* and *micrococcus species*. Although possible contamination could hold true, it is important to remember that some of these pathogens have been implicated in neonatal sepsis particularly *CoNS* in both early and late onset neonatal sepsis(6).

More than half of the cases in this study were discharged home alive 53% compared to 47% of those who died in hospital. This may however not be accurate because the outcomes of a quarter (34/158) of our study participants was unaccounted for and the files of nearly half (71/158) were not retrieved. This was another limitation and so outcome data was sourced from the hospital's electronic records for 37/71 of the unretrieved files. Death files were particularly a challenge to retrieve possibly due to poor storage within the records department and better handling of files is therefore recommended to enable easy retrieval for future studies.

CONCLUSIONS

Gram-negative sepsis rate remains high (58%) with increased morbidity as well as mortality (56%) and *Klebsiella pneumoniae* is still a major contributor, accounting for 29% of this sepsis burden.

There is rising resistance to commonly used antibiotics particularly very high resistance levels to cephalosporins, >95% and benzylpenicillin 93%were noted. Also of concern is the exponential increase in resistance to piperacillin/tazobactam which has for long been one of the reserve antibiotics in our newborn unit.

Many isolates from our Newborn Unit remain highly sensitive to vancomycin (90%), amikacin (88%), ciprofloxacin and meropenem (87%).

RECOMMENDATION

Due to very high resistance rates to commonly used antibiotics, there is need to review the antibiotics currently used in the treatment of neonatal sepsis.

A prospective study will possibly address the issue of skin contaminants when blood samples are taken under aseptic technique and also eliminate the challenge of missing data when collected in real time.

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APPENDIX 1

RESEARCH STUDY TOOL CASE NUMBER…

BACTERIAL PATHOGENS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG SEPTIC NEONATES AT KENYATTA NATIONAL HOSPITAL, NEWBORN UNIT.

DEMORGRAPHICS;

1. SEX …

2. DATE OF BIRTH…

3. DATE OF ADMISSION…

4. AGE AT ADMISSION…

5. BIRTH WEIGHT…

6. GESTATIONAL AGE (Tick where appropriate); 1, >37 WEEKS….2, <37 WEEKS……

7. WAS THE PATIENT REFERRED; YES/ NO

8. PLACE OF BIRTH; 1, KNH 2, HOME 3, PERIPHERAL FACILITY

9. LABORATORY NUMBER IN REGISTER……

CLINICAL INFORMATION

10. DURATION IN NBU AT BLOOD CULTURE SAMPLE COLLECTION …...

11. AGE AT ONSET OF SYMPTOMS…..

12. ORGANISMS ISOLATED;

A. BACTERIAL……..
B. OTHERS …

13. ANTIMICROBIAL SUSCEPTIBILITY

ANTIBIOTIC	SENSITIVITY	INTERMEDIATE	RESISTANCE

14. ANTIBIOTICS PRESCRIBED AND DURATION OF USE;

	ANTIBIOTIC	DURATION	AT TIME OF BLOOD CULTURE
1ST LINE			
2ND LINE			
3RD LINE			

15. CLINICAL FEATURES;

15.1. SIGNS AND SYMPTOMS PRESENT AT BLOOD SAMPLE COLLECTION;

A……..…B……..……..C……..…..D……..

E……..……F…….

15.2. LABORATORY FINDING;

PARAMETER	VALUE
TOTAL WHITE CELL COUNT	
HEMOGLOBIN CONCENTRATION	
PLATELET COUNT	
C REACTIVE PROTEIN	

16. CLINICAL OUTCOME;

DURATION OF HOSPITAL STAY (IN DAYS) …

DISCHARGED ……DIED IN HOSPITAL……

APPENDIX 2

BUDGET

Activity	**Item**	**Kshs**
Proposal Development	Printing costs	5,000
Data Collection	Printing Case record forms	5,000
	One research assistants @kshs 500/day for 30 days	15,000
	Stationary	1,000
Data Analysis	Statistician	40,000
Thesis Development	Printing costs	5,000
PI's Payment	Kshs 1000 per day for 30 days	30,000
	Contingency fund (10% of total budget)	10100
	TOTAL	**111,100**

APPENDIX 3

ETHICS APPROVAL.pdf