Antimicrobial Susceptibility Patterns of Bacterial Isolates in Patients at Moi County Referral Hospital, Voi

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DECLARATION OF ORIGINALITY:

This dissertation is my original work. It has not been presented for examination or any degree. It is well understood that any false claim in this work shall result in disciplinary action in accordance with University of Nairobi policy.

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DEDICATION

This work is dedicated to my family & the UNITID community.

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I thank the Almighty God, for His grace, love, and faithfulness.

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LIST OF ACRONYMS

AMR- Antimicrobial Resistance

AST- Antimicrobial Susceptibility Testing

BSI- Blood Stream Infections

CNS-Central Nervous System

CRE- Carbapenem-Resistant Enterobactericeae

CSF- Cerebrospinal Fluid

ERC- Ethics Review Committee

ESBL- Extended-Spectrum Beta-Lactamases

HIV- Human Immunodeficiency Virus

KNH- Kenyatta National Hospital

MRSA- Methicillin-resistant *Staphylococcus aureus*

MDR- Multi-drug resistant

NDM-1- New Delhi Metallo-beta-lactamase

PPL- Priority Pathogen List

SSA- Sub Saharan Africa

UoN- University of Nairobi

XDR- Extensively drug-resistant

UTI- Urinary Tract Infection

VRSA- Vancomycin-resistant *Staphylococcus aureus*

OPERATIONAL DEFINITIONS

Antimicrobial resistance- This is the capability of bacteria and other microorganisms to withstand the effects of antibiotics to which they were once sensitive.

Antimicrobial susceptibility testing- This is a procedure that is used to determine whether an isolated pathogen is resistant or susceptible to particular antimicrobial agents.

WHO priority pathogens- This is a list of pathogens identified by the World Health Organisation that are resistant to antibiotics. This list is based on the urgency of research and development of newer antimicrobials. The pathogens were categorized into 3 tiers; critical, high and medium priority.

The critical organisms include; *Acinetobacter baumannii* (carbapenem-resistant *A. baumannii*), *Pseudomonas aeruginosa* (carbapenem-resistant *P. aeruginosa*), and Enterobacteriaceae (Carbapenem-resistant Enterobacteriaceae, Extended spectrum beta-lactamase).

High priority organisms include; *Staphylococcus aureus*- (methicillin-resistant *S. aureus,* vancomycin-resistant *S. aureus*), *Enterococcus faecium* (vancomycin-resistant enterococcus), *Helicobacter pylori* (clarithromycin-resistant), *Campylobacter spp* (fluoroquinolones-resistant), *Salmonella spp* (fluoroquinolones-resistant) and *Neisseria gonorrhea* (cephalosporin- resistant, fluoroquinolones-resistant).

The medium priority pathogens are *Streptococcus pneumoniae* (penicillin non-susceptible), *Haemophilus influenzae* (ampicillin-resistant) and *Shigella spp* (fluoroquinolones-resistant).

Extended-spectrum beta-lactamases- These are enzymes produced by some bacteria that provide resistance to beta-lactam antibiotics. These include penicillins, cephalosporins, and monobactam aztreonam.

New Delhi Metallo-beta-lactamase 1- This is an enzyme coded for by the NDM-1 gene that confers resistance to beta-lactam antibiotics.

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ABSTRACT

Background

Antimicrobial resistance (AMR) is an increasing worldwide concern to global health. Infections caused by these resistant organisms increase morbidity, mortality and treatment costs. This study was conducted at Moi County Referral Hospital, Voi. The aim of this study was to bridge the knowledge gap in antimicrobial resistance in order to influence better patient management. The data available in the country mainly focuses on antimicrobial surveillance within the national referral hospitals and Nairobi County private facilities. This study focused on a government facility located in Taita-Taveta County that has been carrying out culture and sensitivity on both pus and urine samples.

Objective

The aim of this study was to identify bacterial isolates cultured from patient samples and their corresponding antimicrobial susceptibility patterns and to describe the WHO antibiotic resistant 'priority pathogens' identified in the study population.

Methodology

This was a retrospective study carried out at Moi County Referral Hospital, Voi. It involved a review of patient laboratory records for bacterial isolates cultured from pus and urine samples carried out in the years 2015-2018 and their antimicrobial susceptibility patterns. Information on the patient's age and sex, bacteria isolated, antimicrobial susceptibility, duration of admission and the patient outcome was extracted and entered into a data abstraction tool. Statistical analysis was done on IBM SPSS statistics software.

Results

A total of 1098 cultures were carried out in pus and urine samples from the years 2015-2018, 296 were positive. Isolates with complete records that were included in the study were 250, 46 of them were excluded due to missing data. The samples were obtained from both inpatients and outpatients. Pus samples were (176/250, 70.4%), urine samples were (74/250, 29.6%). Samples from the outpatient department were (197/250, 78.8%) and (53/250, 21.2%) from the inpatient department. Gram negative bacteria were predominantly isolated 149/250, 59.6% and gram positive bacteria were (101/250, 40.4%). The only gram positive isolate was

S. aureus. Gram negative bacteria isolated included *E. coli* 28%, *Klebsiella spp* 16%, *Pseudomonas spp* 10%, and *Proteus spp* 6%. Resistance was observed with commonly used first-line antimicrobials such as penicillins, macrolides, sulfamethoxazole-trimethoprim and 3rd generation cephalosporins.

Conclusion

There's a rising resistance to first-line antibiotics and noted emerging resistance to second line antimicrobials. This shows a need for antimicrobial surveillance and antibiotic stewardship in order to combat this rising surge in antimicrobial resistance.

Chapter 1: INTRODUCTION

1.1 Background

Microbial infections are a common cause of morbidity and mortality in Sub- Saharan Africa and the developing world. Kariuki et al emphasize that drug-resistant infections are known to increase morbidity and mortality and lead to an increase in the cost of treatment in the population (1).

The World Bank 2018 final report on drug resistance describes that AMR is seen to occur when pathogens undergo an evolutionary change that enables resistance to antimicrobials. In recent decades the inappropriate widespread use of antimicrobials has resulted in resistance. As a result, antimicrobials are rapidly losing their ability to treat infections globally. With this continuing trend, the world may experience a setback in crucial public health wins of the past seven decades (2).

According to the WHO 2018 fact sheet, antimicrobial resistance is an expanding threat to global health. It endangers the fundamentals of modern medicine and the sustenance of an efficient global public health response to the threat from bacterial infection (3).

Worldwide incidence of AMR continues to rise, as evidenced by various studies around the world (4). The world is in desperate need to establish guidelines on appropriate antimicrobial therapy to improve the management of patients. Proper guidelines will only be established with proper surveillance on susceptibility and resistance patterns in our setting, which in turn will effectively decrease the morbidity and mortality in the population.

Laxminarayan et al have elucidated that the causes of AMR are complex. There has been a rise in resistance evident in the decrease in efficiency of antibiotics in the treatment of common infections. In recent years we have observed a rise of carbapenem-resistant Enterobacteriaceae. They further mention that developed countries have high antimicrobial use within the hospitals, the community and the agricultural sector which have attributed to selection pressure that is sustaining these resistant strains in the environment resulting in a shift to the use of more costly broad-spectrum antibiotics. In low and middle-income countries antimicrobial use is increasing with the rise in incomes, resulting in higher rates of hospital admission and an increasing prevalence of nosocomial infections, noted in our urban population (5).

Laxminarayan et al further describe that the developing resistance from mutations in bacteria and the selection pressure from antibiotic use is facilitated by sub-optimum doses during treatment. The genes associated with resistance are found on chromosomal and transmittable extra-chromosomal genetic elements, with resultant resistant clones like; MRSA (Methicillin resistant *Staphylococcus aureus*), ESBL (extended spectrum beta-lactamase), CRE (carbapenem resistant Enterobacteriaceae) and others. Antibacterial resistant Enterobacteriaceae are now globally distributed. The spread of resistant genes is increased by gene transmission, poor hygiene and inadequate sanitation in hospitals and the community. The increased global travel and trade which has facilitated disease transmission has also fueled the spread of these drug-resistant species. This selection pressure that has resulted from the use of antibiotics over the last seven decades has facilitated AMR (5).

In a meta-analysis by Kimang'a et al; a situational analysis of antimicrobial drug resistance in Africa, they identified a total of a hundred and three works of literature. The meta-analysis concluded that resistant bacteria are on the rise, and our actions are fueling the rise in resistance. The haphazard use of antibiotics in human health and agriculture, wrong and sub-standard prescriptions by unqualified medical personnel together with poor diagnosis or lack of it are all fueling antimicrobial resistance. They concluded that antimicrobial stewardship programs are crucial in the war against AMR (6).

Major achievements in modern medicine in surgery, organ transplantation, management of preterm babies, and the treatment of cancer would not be feasible without access to effective antibiotics. The consequences of infection with resistant bacteria can be severe leading to longer duration of illness, increase in mortality, long hospital stay and consequently an increase in health care cost.

Previous studies have noted that infection with drug-resistant infections especially in children is associated with a more than doubled increase in morbidity and mortality, likelihood of admission, longer hospital stay, and higher health care costs in comparison to drug-susceptible infections (7).

Keiji Fukuda, the Assistant Director-General for Health Security in the WHO in his speech on 27th May 2015 declared that AMR is one of the most significant global health menaces of the modern world with the world on the brink of a post-antibiotic age.

In efforts towards addressing the rising global resistance to antimicrobial agents, the WHO on 27th of February 2017 published a list of antibiotic-resistant 'priority pathogens'. This list has three categories based on the urgency for new antibiotics (8).

These organisms as seen on the WHO priority pathogen list (WHO PPL) include (8);

Critical organisms-

1. *Acinetobacter baumannii* (carbapenem-resistant .*A. baumannii*- CRAB)
2. *Pseudomonas aeruginosa* (carbapenem-resistant *P. aeruginosa*- CRPA)
3. Enterobacteriaceae (carbapenem-resistant Enterobacteriaceae-CRE, extended spectrum beta-lactamases-ESBL)

High priority-

1. *Staphylococcus aureus*- (methicillin-resistant *S. aureus*-MRSA, vancomycin-resistant *S. aureus*-VRSA)
2. *Enterococcus faecium* (vancomycin-resistant enterococcus-VRE)
3. Clarithromycin-resistant *Helicobacter pylori*
4. Fluoroquinolones-resistant *Campylobacter spp*
5. Fluoroquinolones-resistant *Salmonella spp*
6. *Neisseria gonorrhea* (cephalosporin-resistant, fluoroquinolones-resistant)

Medium priority

1. Penicillin non-susceptible *Streptococcus pneumoniae*
2. Ampicillin-resistant *Haemophilus influenzae*
3. Fluoroquinolones-resistant *Shigella spp*

This study focused on identifying antimicrobial susceptibility of all pathogens isolated and to determine the critical and high priority resistant pathogens that are prevalent amongst the study population.

1.2 LITERATURE REVIEW

1.2.1 Anti-microbial resistance crisis

Bacterial infection, which encompasses a large proportion of infectious disease in man, is the main cause of illness and death in Sub- Saharan Africa (SSA) and the developing world. The causes, antimicrobial susceptibility patterns, and the clinical outcomes differ between countries. Multiple health facilities in the developing world lack sufficient laboratory capacity for diagnosis of infectious diseases resulting in empiric management of patients. Better surveillance and more recent data on aetiology and susceptibility will contribute to better patient management. In SSA with the high disease burden from other infectious diseases like HIV/AIDS, malaria, multi-drug resistant (MDR) and extensively drug-resistant (XDR) tuberculosis and malnutrition, treatment of any febrile illness will often target multiple aetiologies without a definitive diagnosis (9).

1.2.2 Factors contributing to antimicrobial resistance

As reported by Michael et al the AMR crisis has led to a rise in the global incidence of infectious diseases. Ventola et al identify three major factors that are considered to contribute to this crisis (10);

- Increase in the number of AMR phenotypes among microorganisms which is an evolutionary reaction to the improper use and overutilization of antibiotics.
- Global travel and interaction facilitate the transfer of pathogens from any environment to a larger population across the globe.
- There is an unnecessary use of antibiotics by man which has provided the strong selective pressure that has facilitated evolutionary forces in the environment.

In the above, population growth is the most unlikely to change. However, the other factors can be manipulated; offering an opportunity to manage this crisis. The evolution of microbes may be halted by reducing this selection pressure (10).

Although AMR is primarily a result of the selection pressure on microbes a number of social and regulatory factors have been seen to contribute to AMR. These can be generally characterized as patient and health care provider factors. They include;

1.2.2.1 Healthcare provider

Prescription of inappropriate antibiotics even in the absence of a confirmed bacterial infection is a practice noted worldwide, where patients are treated empirically. Such physician practices are often cultivated by diagnostic uncertainty, poor patient follow up, lack of sufficient insight on alternative therapies and patient demand. Accuracy of diagnosis is crucial in the competent management of bacterial infections. This will, in turn, decrease the empiric management of patients. In developing countries, antibiotics are readily available and are readily bought over the counter without prescriptions. The presence of multiple unsanctioned providers has contributed to the inappropriate use of antibiotics (11). This is evident and widely noted in the Kenyan urban setting.

1.2.2.2 Patient factors

Self-medication is widespread in the Kenyan setting due to easy access to pharmacies and health care providers who readily dispense antibiotics. Self- medication which is associated with insufficient, untimely dosing and poor compliance of these antibiotics has resulted in a rise in AMR. In the developing world, the issues of compliance and self-treatment are marked due to the below par and fraudulent antibiotics accessible in unlicensed pharmacies. There is also a multiplicity of health facilities, with patients seeking health care from different facilities for the same problem leading to a loss to follow up. Co-morbidities like HIV infection and immune suppressive conditions are also associated with higher rates of resistance. Of note is that in SSA HIV/AIDS, malaria, sickle cell disease, viral infections, and parasitic infections still make up the bulk of the disease burden (11,12).

1.2.2.3 Hospital factors

Nosocomial infections are infections that are acquired from the hospital environment. The cut off time to differentiate between nosocomial and community-acquired infections is 48 hours after admission.

The commonest nosocomial infections in children are BSI, ventilator-associated pneumonia, urinary tract infections (UTI), skin and surgical site infections. Nosocomial infections have the highest rates of AMR noted worldwide.

A study by Agaba et al on nosocomial infections and their antimicrobial susceptibility among patients in Ugandan ICUs was carried out. They assessed a total of 111 adult patients in the ICU. They found that 30% of the isolates were *K. pneumoniae*, *Acinetobacter spp* were 22% and *S. aureus* was 14%. Prevalence of multi-drug resistant bacterial species was 58%. *Acinetobacter spp* were extensively drug resistant. Imipenem was seen to be the antibiotic with the highest susceptibility rates across most of the bacterial species. The presence of ventilator support and severe traumatic brain injury were associated with the development of nosocomial infections (13).

Multiple factors in the hospital environment have attributed to this. Hospitals provide a fertile environment for the growth of resistant microbes. Large hospitals deal with large numbers of patients who are in close proximity to each other, and on comprehensive and lengthened antimicrobial treatment. There is ease in the transmission of drug-resistant bugs amongst patients, through the airborne route, from contact with a contaminated environment, or fomites. Transmission is also seen from contact with health care providers who don't practice infection control measures like hand washing (11,12).

1.2.3 Situational analysis of antimicrobial resistance

The WHO in 2014 published a list of priority pathogens in order of urgency for the need for new antibiotics. They were categorized into three tiers, critical, high priority and medium priority organisms.

Organisms associated with most infections in the Kenyan setting include gram-negative bacteria like Enterobacteriaceae and gram-positive bacteria like *Staphylococcus aureus*. Enterobacteriaceae- ESBL was categorized as a critical pathogen while MRSA was categorized as high priority.

1.2.3.1 Gram-negative bacteria

Gram-negative bacteria have been associated with the highest rates of AMR. These comprise a number of bacteria, including *Neisseria gonorrhea*, Enterobacteriaceae (*E. coli, K. pneumoniae, Proteus spp*, and *Enterobacter spp.* There has been a significant rise in resistance seen in gram-negative pathogens that cause severe nosocomial infections. These include *A. baumannii, P. aeruginosa* and Enterobacteriaceae(14). *A. baumannii* was previously thought to be avirulent; it is increasingly noted to be a cause of ventilator-

associated pneumonia. Enterobacteriaceae produce the enzymes, ESBL which inactivate the beta-lactam ring in antibiotics conferring resistance to these antibiotics. Enterobacteriaceae are a leading cause of both community-acquired and nosocomial infections. They are the commonest causes of UTI, which accounts for most paediatric outpatient visits. *P. aeruginosa* is an invasive gram-negative bacterium that is responsible for severe nosocomial infections; these include pneumonia, UTI and bacteraemia. Studies done across the world have made this evident.

Further studies across Africa have shown a rise in antimicrobial resistance notably in gram-negative bacteria with a predominance of Enterobacteriaceae, these studies have assessed the aetiology and antimicrobial susceptibility of the pathogens identified in cultures. In a South African hospital, a retrospective review by Dramowski et al on BSI in children collected 17001 blood cultures over a 6 years period. They noted a total of 864 BSI episodes with a predominance of gram-negative bacteria at 60%. *K. pneumoniae* was 14% and *E. coli* at 11% and both were noted to be most prevalent. Hospital-acquired BSI was at 47%. The rate of AMR was at 70% in nosocomial infections and 25% in the community-acquired BSI. Resistance was noted to be associated with younger age at <1year, HIV co-infection and Gram-negative BSI(15). Noted in this particular study was the predominance of gram-negative bacteria, dominated by *E. coli*. Nosocomial infections amounted to the largest number of BSI. High rates of resistance were also noted in these nosocomial infections, gram-negative BSI, HIV co-infection and young age.

Enterobacteriaceae are the predominate cause of urinary tract infections. UTIs as previously mentioned account for most paediatric outpatient visits. Njiru et al while assessing antimicrobial susceptibility patterns of Enterobacteriaceae in urine samples in KNH, found a significantly higher incidence of Enterobacteriaceae noted in females at 56%. The predominant Enterobacteriaceae isolated included *E. coli* at 46%, and *Klebsiella spp* at 19.5%. Most of the bacteria isolated were susceptible to carbapenems. There was a high ESBL production >60%. *E. coli* was resistant to most antibiotics and susceptible to carbapenems. *Proteus spp* were noted to be prevalent ESBL producers. *Acinetobacter spp* showed high resistance to carbapenems (16). This study showed a predominance of Enterobacteriaceae, especially *E. coli* which is a known normal commensals of the urogenital tract, with noted resistance to other beta-lactam antibiotics and susceptibility to carbapenems.

Gram-negative bacteria are associated with higher numbers of nosocomial infections and also high rates of AMR as reported by Maina et al who executed a study assessing the microbial spectrum and the susceptibility patterns at a private teaching hospital in Kenya. They focused on organisms causing bacteraemia within 72hrs of admission. *E. coli* was the commonest. Gram-negative bacteria were the commonest in blood culture isolates. Noted were the low MRSA rates. *E. coli* susceptibility to cephalosporins was 55% and 43% for quinolones. It was noted that 15% of the *Klebsiella spp* showed susceptibility to third generation cephalosporins. Susceptibility to oxacillin was seen in 94% of *S. aureus* isolates. There was an 87% and 84% susceptibility to erythromycin and tetracycline. All the *E. faecalis* were susceptible to ampicillin. While susceptibility to gentamycin and streptomycin was in 57% and 71% (17).

There are over 1000 resistance related beta-lactamases that confer resistance to antibiotics; this is a ten-fold increase in the last two decades. The New Delhi-Metallo-beta-lactamase (NDM-1) coded for by the NDM-1 gene, is a carbapenemase beta-lactamase enzyme that confers resistance to beta-lactam antibiotics. A study by Walsh et al was carried out in Central New Delhi to assess the dissemination of AMR and ESBL in the environment. They collected 171 seepage samples and 50 tap samples from New Delhi, and 70 sewerage samples from Cardiff. The NDM gene was not found in any sample from Cardiff. NDM-1 containing bacteria were found in 12 of the 171 samples, and 2 of the drinking water samples. This included 11 species not previously reported in New Delhi. The study concluded the presence of these bacteria in the environment in New Delhi has a great impact on the population that is solely relying on the public water and sanitation program. The evolution and spread of resistance have been clearly seen in the case of beta-lactamases. New Delhi Metallo-beta lactamase (NDM) enzymes were first reported in 2008. AMR surveillance reports have shown that they are now found worldwide. The worldwide distribution of resistance genes such as ESBL, NDM-1 and *Klebsiella pneumoniae* carbapenem resistance (KPC) shows the spread and impact of these resistant genes (18).

Infectious disease is the leading cause of mortality and morbidity in SSA. Diagnostic precision is lacking, with most health practitioners resulting in the empiric management of patients. The disease burden from other infectious diseases like malaria and HIV contributes to complexities in patient management. This is also contributing to AMR and a rise in fatal outcomes. A study by Aiken et al on the risk and causes of nosocomial bloodstream infections in Kilifi District Hospital amongst the paediatric patients was carried out. Data

from 33,188 admissions through a 7 year period were reviewed. The study showed that there was an overall risk of hospital-acquired BSI during the 7 year study period. The risk was 40 times higher than that seen in community-acquired BSI in the setting. The mortality rate from nosocomial BSI was 52% while the community acquired BSI had a mortality rate of 24%. *K. pneumoniae*, *E. coli, S. aureus, Acinetobacter spp,* group D streptococci, and *P. aeruginosa* contributed to about 75% of the hospital-acquired bacteraemia. There was an association of nosocomial bacteria with malnutrition (19). There are higher mortality rates from nosocomial BSI in comparison to community-acquired infections.

A systematic review by Salma et al on AMR in gram-negative bacteria in developing countries was carried out. It included 15 studies carried out in Africa. This review showed that 50% and 11% of *E. coli* isolates were resistant to ampicillin and gentamicin respectively. *K. pneumoniae* isolates showed a 30% resistance to ceftriaxone. Gram-negative pathogens causing nosocomial infections showed higher rates of resistance; these include *Acinetobacter spp*, *P. aeruginosa*, and ESBL. Resistant strains were associated with a longer hospital stay, higher treatment costs and higher mortality rates (14).

Studies have been done further, to assess the factors that are associated with AMR. A study was done by Blomberg et al in Tanzanian children on the incidence of BSI and the associated risk factors for death in 1828 patient admissions. BSI were13.9% of admissions, though 67% of the population had received antibiotics prior to cultures. The commonest pathogens isolated were *Klebsiella spp, Salmonella spp, E. coli, Enterococcus spp* and *S. aureus*. 34.9% of the patients with BSI died. Mortality from the gram-negative bacteria was 43.5%. This more than doubled mortality seen from malaria which was at 20.28%, the gram-positive BSI was at 16.7%. The risk factors associated with mortality included; inappropriate antibiotics due to AMR and infection with resistant bacteria, other co-morbid infectious diseases like HIV/AIDS, protein-energy malnutrition and BSI by Enterobacteriaceae (20).

There has been a noted increase in resistance to last resort carbapenems noted in *Klebsiella pneumoniae* which have spread worldwide, WHO fact sheet 2018. *K. pneumoniae* is a major cause of nosocomial infections like pneumonia, BSI, neonatal infections and predominant in intensive care patients. Resistance of *E. coli* to fluoroquinolones has become widespread. There are countries where this treatment no longer works in more than half the population (3).

A newly emerging resistance is being seen to antibiotics that were previously considered last resort medications. De Maio Carrhilho et al in 2016 reported that colistin which is the last recourse for life-threatening carbapenem-resistant Enterobacteriaceae infections has shown treatment failure. Antimicrobial resistance to colistin has been seen in several countries and regions across the world (21).

1.2.3.2 Gram-positive bacteria

The resistance profile differs in different regions of the world. Surveillance of resistance patterns is crucial in all regions. This will assist to tailor the treatment guidelines according to the local resistance patterns in a particular region. A study by Babay et al in King Khalid University Hospital, Saudi Arabia focused on BSI in paediatric patients over a one year period. BSI was seen in 220 patients, 32.2% were from ICU. A total of 95.4% had a single blood culture isolate. Gram-positive bacteria were cultured in 78.6% of the isolates. These included *Staphylococcus epidermis* at 55.4%. *S. aureus* cultured was 9.5% of which 14% were MRSA. *S. pneumoniae* was 4.5%, of which 40% were penicillin resistant and *E. faecalis* at 4%. Gram-negative bacteria were 20% and comprised of *E. coli* and *K. pneumoniae* 3.6%. There were 3 isolates of *K. pneumoniae* and one of *P. aeruginosa* isolates which were multidrug resistant. Nosocomial infections were 184 (88%) of BSI isolates. Most of the patients presented with fever of which a total of 26% had a positive blood culture with no foci of infection. Respiratory, cardiac, renal, gastrointestinal, malignancy and surgical cases were associated with clinical disease in BSI in paediatric patients. Patients with immune-suppression with BSI had culture isolates of bacteria like *salmonella spp, S. pneumoniae,* and *P. aeruginosa*. Mortality was associated with underlying medical conditions and they also had associated risk factors such as longer duration of admission, intensive care admission, the presence of indwelling catheters, mechanical ventilation and previous use of antibiotics (22). This particular study showed a predominance of gram-positive bacteria, with high rates of nosocomial BSI infections. A percentage of MRSA was seen. Co-morbidities contributed to fatal outcomes, longer hospital stay and transfer to higher level of care.

An additional study done in KNH showed a predominance of gram-positive bacteria. The aim of the study was to determine the antimicrobial susceptibility patterns amongst patients with blood culture positive sepsis at the casualty department in Kenyatta National Hospital by Ochieng' et al. They collected 232 blood samples for culture. Pathogenic bacteria were grown in 6.5% of the samples. The commonest was gram-positive bacteria. Coagulase-

negative *Staphylococcus aureus* was commonest at 40%. Most of the bacteria were susceptible to commonly used antibiotics, with minimal resistance against carbapenems and 3rd generation cephalosporin. The highest resistance noted was against penicillin and macrolides (23). This study identified *S. aureus* as the common pathogen with resistance to penicillins and macrolides noted.

The escalation of resistance is seen when antibiotics that are reserved for second, third and fourth line treatment in resource-rich settings are widely used to manage simple infections and in the treatment of viral and parasitic illnesses without diagnostic accuracy. This is evident is the Kenyan setting with an ease of accessibility to these over the counter treatments.

1.3 Problem statement

Infant and maternal mortality is still a worldwide problem, the WHO fact sheet reports more than 300,000 women and 400,000 new-borns dies annually from infections, with most of these seen amongst the low-income population. With AMR there are more than 200,000 new-born deaths annually from antimicrobial resistant infections. Data from larger hospitals where microbes are more likely to develop AMR has shown that an estimated 40% of infections in neonates are resistant to standard antimicrobial treatment (9).

AMR surveillance is not routinely carried out in Kenya; there is a need to develop an antibiogram that will influence our treatment guidelines tailor-made to our resistance patterns. There is a need to attain baseline data, to curb the mortality rate from the infectious disease. This can only be achieved when data on the prevalent pathogens within the general population and the susceptibility patterns have been established and used as a guide in formulating policy.

In SSA, a question of access versus excess has risen. This is dependent on region or factors to do with urbanization. Within the urban region, there is a problem of excesses or better defined as a ‘tragedy of the commons’. This is seen when the population squanders a resource that is limited; in this case antibiotics. The rural regions have a problem of access, with a noted poor access to proper healthcare and antibiotics. Saving the lives of mothers, infants and children will require addressing the problem of access and excess. In summary, those in

need of life-saving antibiotics must have access to them and those who do not need antibiotics, should not use them.

This study is based in the rural setting, antimicrobial surveillance has mostly been carried out in the urban setting, which is plagued by antibiotic excesses, the urban majority have access to over the counter medication, are middle-income earners with the financial access to more expensive medications. The urban setting has a multiplicity of health care providers, leading to higher rates of outpatient visits, and access to antibiotics. In comparison, the rural setting has less access to these over the counter antibiotics in comparison to the urban setting. This study will facilitate a comparison on AMR in the urban setting from the studies previously done and this particular study.

1.3.1 Challenges in fighting antimicrobial resistance

AMR is a public health calamity; in SSA it's a handicap to the provision of basic health care to the local population. The lack of information and absence of effective surveillance and reporting make it difficult to understand the magnitude of antimicrobial resistance in SSA. There is proof of resistance seen particularly in incidents of treatment failure seen in bacterial infections, and undesired outcomes such as longer duration of treatment and death.

There are many challenges in SSA that have affected the war against AMR. These include the high disease burden from infectious disease, poor access to proper healthcare and laboratory for diagnostics, inappropriate use of antibiotics and economic and provider incentives for antimicrobial use amongst healthcare providers. By addressing these challenges, there will be a positive impact in the war against AMR.

The existing measures to regulate the use of antimicrobials and to educate the population on the AMR crisis are useful but have not in whole addressed the desperately needed overall decrease in the inappropriate use of antibiotics.

Michael et al proposed that in addition to the current measures and research into new antimicrobials and diagnostics, a fully comprehensive program is required to educate the population in order to change the communal paradigm of antibiotic use, from a 1st line treatment to that of the last option when all others have failed (10). Public health education takes the bulk of managing the AMR crisis. A number of factors can be taken into consideration;

- There’s a need for public health education and awareness on infection control and the importance of seeking care from a qualified health practitioner.
- Educate the public on hand hygiene and proper sanitation which is crucial to infection control.
- Educate the public on over the counter self-medication, compliance to medication and the importance of proper dosing of medication.
- Promote maternal and child health, proper antenatal care, and immunization to reduce infant mortality from infectious disease.
- There is a need for prescription control of antibiotics by discouraging over the counter dispensing of medications.
- Health care providers need to avoid on demand prescription of antibiotics and educate the patients on the impact of inappropriate use of antibiotics.
- Prophylactic antibiotics should only be reserved in extreme cases like post-surgery and conditions like sickle cell disease.
- Antimicrobial therapy should be tailored according to resistance patterns in a particular region.
- Antimicrobial stewardship is an important factor in managing the AMR crisis (24).

1.4 JUSTIFICATION

AMR has been seen in both hospital-acquired and community-acquired infections across the world. Guidelines for the management of common bacterial infections are dependent on information that is available on aetiology and AST. Sub-Saharan Africa lacks optimum diagnostics and AMR surveillance. SSA lacks extensive antibiotic surveillance strategies compared to other regions in the world; there are also poor infection control and prevention programmes. According to the WHO PPL 2018 report only 15% of WHO Africa member states actually carry out AMR surveillance and there is a deficiency of quality assurance of laboratory procedures (2).

As mentioned by Laxminarayan et al the consequences of infection with antimicrobial-resistant microorganisms can be dire; these include a higher mortality rate, long illness duration, and treatment with a longer hospital stay, a loss of protection for patients on chemotherapy, post-surgery and other medical procedures (5).

Hospitals have resulted in the use of last resort medications that are expensive and often unavailable and unaffordable in most low-income countries. There is a rapid development of resistance through the overuse of antibiotics. Some of the common illnesses can no longer be managed with older more easily available antibiotics. Poor prescription habits and over the counter dispensing of antibiotics leads to this misuse and overuse.

Health care workers are key enactors in the preservation of antimicrobials. The failure to preserve antimicrobials has been seen through irresponsible prescription & dispensing, a lack of up to date information, yielding to patient pressure to prescribe unnecessary antibiotics &/or sale and supply of medicines for personal benefit. A regulation of prescription and dispensing of antibiotics will prove crucial in the fight against antimicrobial resistance.

Chapter 2: RESEARCH DEFINITION

2.1 Research questions

1. What are the bacterial isolates in urine and pus cultures obtained at the Moi County Referral Hospital, Voi from 2015 to 2018?
2. What is the antimicrobial susceptibility profile of bacterial isolates cultured during the study period?

2.2 Main objective

To describe the antimicrobial susceptibility patterns of bacterial isolates cultured from patient samples at Moi County Referral Hospital, Voi over the study period, 2015-2018.

2.3 Specific objectives

1. To describe the bacterial isolates obtained from cultures of urine and pus samples at the Moi County Referral Hospital, Voi during the study period 2015-2018.

2. To determine the antimicrobial susceptibility profile of bacterial isolates obtained from urine and pus cultures at Moi County Referral Hospital, Voi from 2015 to 2018.

3. To assess the clinical outcomes of patients with drug-resistant bacterial isolates from the study population.

Chapter 3: STUDY DESIGN AND METHODOLOGY

3.1 Study design

This was a retrospective study of bacterial isolates cultured from urine and pus samples, and corresponding susceptibility data from 2015-2018 at Moi County Referral Hospital, Voi.

3.2 Study setting

Moi County Referral Hospital, Voi, is a government hospital located in Voi constituency in Taita- Taveta County. The hospital provides a number of services including; ante-natal care, basic emergency obstetric care, curative in-patient services, integrated management of childhood illnesses, prevention of mother to child transmission, Tuberculosis treatment. It is a 112-bed facility with a fully functional laboratory. The laboratory through a public-private partnership model; a collaboration between the County government of Taita Taveta and Pathologists Lancet Kenya Ltd has facilitated running of laboratory procedures and especially in this case pus and urine cultures. Over the study period, the laboratory carried out approximately 1098 cultures, with 296 positive tests.

The microbiology laboratory at Moi County Referral Hospital, Voi measures approximately 12 by 14 feet. Equipment includes microscopes, incubator, biosafety cabinet, fridge, genexpert machine. The microbiology laboratory mainly focuses on microscopy, cultures, and sensitivity of urine and pus samples, sputum for acid-fast bacilli and genexpert, with the exception of blood, cerebral spinal fluid, and tracheal aspirates.

3.3 Study population

Retrospective data was abstracted from the microbiology laboratory records of patients with culture-positive urine and pus samples during the study period, 2015-2018.

3.4 Study procedure

This study focused on the isolate results of all culture-positive urine and pus samples analyzed in the Moi County Referral Hospital, Voi Microbiology laboratory and their susceptibility results. Retrospective data was collected and reviewed in order to record an antibiogram report from 2015 to 2018. The laboratory results and their corresponding

antibiotic susceptibility patterns were identified and retrieved from the hard copy laboratory records.

Relevant patient demographics and clinical information significant to this study were extracted. These included;

- Patient sex
- Patient age
- Date of hospital admission/sample collection and department
- Date of discharge
- Patient diagnosis
- Pre-existing co-morbidities
- Previous use of antibiotics
- Specimen type (urine, pus)
- Duration of hospital stay prior to specimen collection

3.5 Sample size calculation

Moi County Referral Hospital, Voi over the study period, carried out an approximate number of 1098 cultures with 296 sensitivity tests over the study period. The total population of positive cultures done per year at the Moi County Referral Hospital, Voi, averaged about 80.

Sample size estimation was done using Fisher's formula. (Fishers 1991)

$n=Z^2 P (1-P)/m^2$

n- Minimum sample size

Z- Constant, standard normal deviation (1.96 for 95% Confidence interval)

P- A prevalence rate of 50% was assumed since the prevalence of some of the outcome variables was unknown.

the m= Acceptable margin of error.

Z= 1.96

P= 0.5

Q= 0.5

m= 0.05

n= $(1.96)^2$ *0.5(1-0.5)/ $(0.05)^2$

n= 384 (minimum sample size).

The total number of positive urine and pus cultures was less than the minimum sample size, thus the finite population correction factor was used.

N is the population size while n is the Fisher's estimate.

$$n = \frac{n}{1 + \frac{n-1}{N}}$$

N= 296, n= 384
Adjusted sample size= 167

3.6 Sampling

Due to the small number of isolates attained from the number of samples, a census of all positive cultures that met the inclusion criteria was carried out. No sampling techniques were employed, but instead all isolates were included. A census was appropriate because the population was small, less than 300. The advantage of a census is that it provides a narrow confidence interval thus higher accuracy at a given confidence level.

3.7 Inclusion criteria

Culture positive isolates obtained from all patients from 2015 to 2018.

3.8 Exclusion criteria

Isolates with incomplete data records was excluded; an example was missing antimicrobial susceptibility test results or any mismatched patient records.

3.9 Study variables

Many factors contribute to antibiotic resistance, and they vary in terms of patient, clinician and hospital factors. The study focused on relevant patient characteristics obtained from hospital records.

3.9.1 Dependent variables

- Bacterial isolates- Genus/species, antimicrobial susceptibility, priority resistance pathogens, duration of hospital stay and clinical outcome.

3.9.2 Independent variables

- Age, sex, diagnosis, previous antimicrobial use, and co-morbidities (HIV, sickle cell disease, diabetes). Specimen type (urine and pus).

3.10 Data collection

Data was retrieved from the Moi County Referral Hospital, Voi microbiology laboratory records. Patient demographics, clinical outcomes, and co-morbidities were extracted from the patient files.

3.11 Data management and analysis

Data was retrieved from the laboratory records, entered into MS Excel, and exported to SPSS for statistical analysis. The data was analyzed for demographic characteristics, isolate outcomes, clinical outcomes and antimicrobial susceptibility patterns. Data collected was entered into a database in a password protected computer and a backup. Patient identifying information such as name and patient numbers were excluded from the data collection tool. Study data was collected and analyzed whilst maximizing on patient confidentiality. Data abstraction tool was used for systematic data collection. The tool used was a modification of previous data abstraction tools used in similar studies by Njiru, Bwisa, and Wangai (16,25,26).

The univariate analysis was employed using frequencies and proportions for categorical variables, such as *patient sex, prior antibiotic use, instrumentation.* Measures of central tendency (mean, mode, median) were used for continuous variables such as *age, duration of*

admission. Nominal variables such as number of organisms isolated were compared with the antibiotics using pivot tables. This enabled to determine the sensitivity of each organism to each antibiotic.

3.12 Study instruments

- A data abstraction tool was used for systematic data collection.
- Personnel: Principal investigator and 1 assistant (lab technologist).
- Facility: Moi County Referral Hospital, Voi.
- Supplies: Stationery and software.

3.13 Ethical considerations

Ethical approval was sought from KNH/UON ethics and research committee. Permission to extract data from the laboratory records was sought from the medical superintendent, Moi County Referral Hospital, Voi. Confidentiality was maintained by keeping the patient identifiers out of the records. For confidentiality, patient records were only accessed within the confines of the hospital. This was a retrospective study which involved a review of medical records; thus a minimal risks study with no direct patient involvement. There was no active participation of the patients in this study. Thus, there was no risk to patients. A waiver of informed consent was sought from the KNH/UON ethics committee. Raw data that was filled in the data abstraction tool in a password protected computer and the backup were destroyed at the end of the study.

3.14 Quality assurance

Quality medical laboratory service provision is important in order to enhance diagnostic value. The Moi County Referral Hospital, Voi laboratory has existing internal and external checks in quality control through the implementation of the laboratory quality management system established through the intergration between the WHO Lyon office for National Epidemic Preparedness and Response, the United States of America Centres for Disease Control and Prevention (CDC) Division of Laboratory Systems and the Clinical and Laboratory Standards Institute (CLSI)(27).

Chapter 4: RESULTS

4.1 Study profile

A total of 1098 cultures, yielding 296 positive cultures were carried out in Moi County Referral Hospital, Voi from 2015 to 2018. Out of 296 isolates, 46 were excluded from the study due to missing data and erroneous entries. Examples of missing entries included isolates listed without antibiotic susceptibility profiles.

In compliance to the clinical and laboratory standards institute (CLSI) guidelines, all duplicate isolate entries were excluded so as to remain with one isolate per patient per admission period (28). The final number of bacterial isolates included into the retrospective dataset was 250(29).

Of the 250 isolates that were analysed, 104 (41.6%) were from male patients and 146 (58.4%) were from female patients. Isolates collected in the year 2015 were (25/250, 10%), 2016 were (76/250, 30.4%), 2017 were (48/250, 19.2%) while majority were collected in 2018 (101/250, 40.4%).

Table 1: Specimen type, organism and year collected.

		n	%
Prior antimicrobial use	Yes	34	65..4
	No	18	34.6
Specimen Type	Pus	176	70.4
	Urine	74	29.6
Organism	*E. coli*	69	27.6
	Klebsiella spp	39	15.6
	Proteus spp	16	6.4
	Pseudomonas spp	25	10.0
	S. aureus	101	40.4
Year	2015	25	10.0
	2016	76	30.4
	2017	48	19.2
	2018	101	40.4

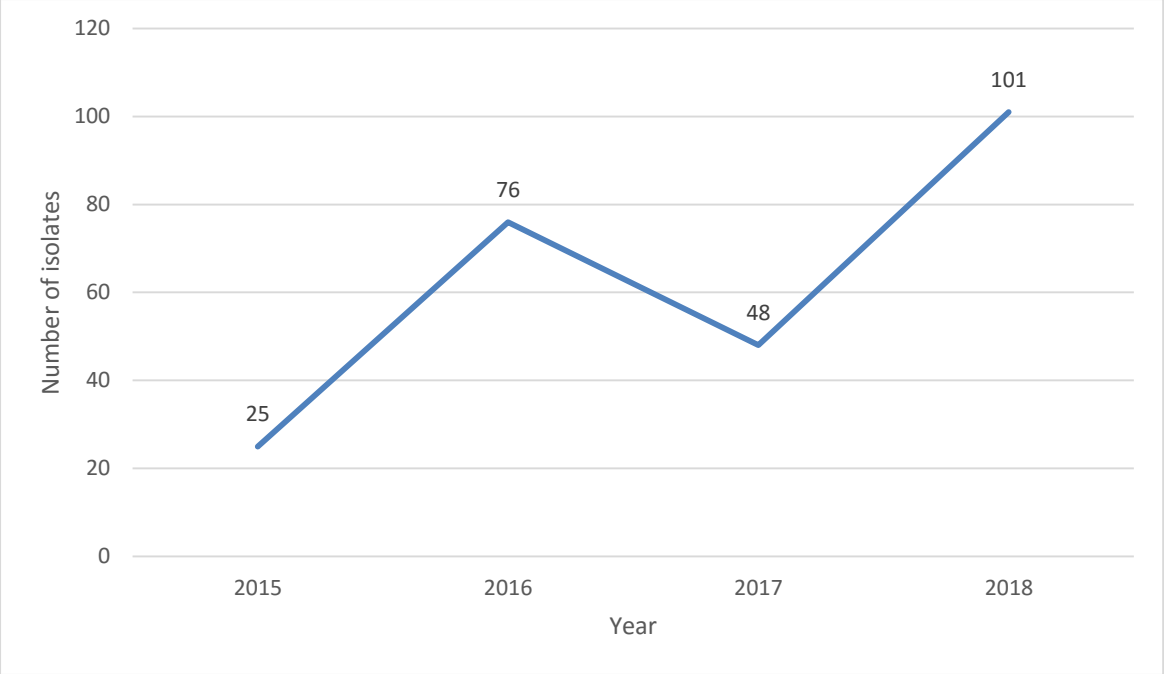

Figure 1: Number of isolates per year

Organism/Year	2015	2016	2017	2018
S. aureus	10	28	20	43
E. coli	8	19	9	33
Klebsiella	7	10	8	14
Pseudomonas	0	8	7	10
Proteus	0	11	4	1

Figure 2: Number of organism isolated each year

The number of isolates per organism isolated per year was noted to increase with each consecutive year, with 2018 seen to have the highest number of isolates. This could partly be due to improved laboratory capacity over the years. Resistance patterns with both *S. aureus* and *E. coli* were noted to increase over the years as with the numbers isolated.

Patient age ranged from 2 weeks to 98 years with a mean of 37.9 years, standard deviation of 20.1 and a median of 35years. Patients below the age of 1 year were 3.2%. Patients below 10 years were a total of 22 (8.8%), 10years and above were 228 (91.2%)

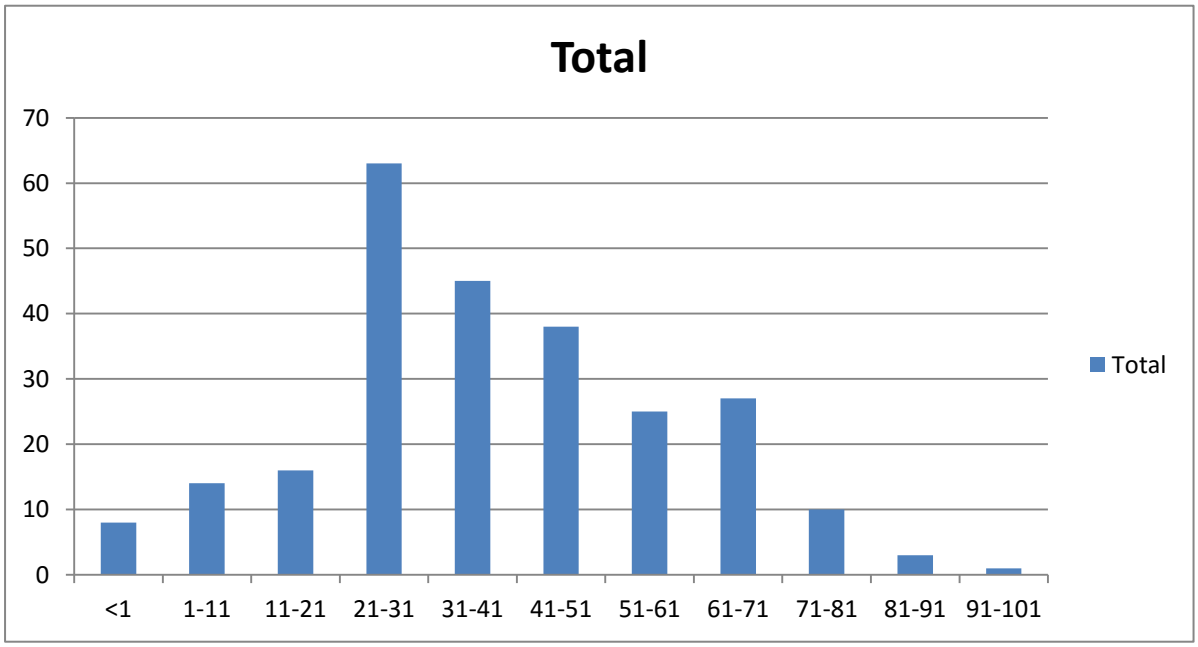

Figure 1: Age distribution of the study population

Majority of the samples were collected from the outpatient department (197/250, 78.8%), while (53/250, 21.1%) samples were collected from the inpatient department. Inpatient samples were distributed amongst the various hospital wards; the female medical ward were (17/250, 6.8%) patients, male ward were (6/250, 2.4%), while (11/250, 4.4%) were from the maternity ward, (12/250, 4.8%) from the paediatric ward and (7/250, 2.8%) from the surgical ward.

		n	%
Sex	Male	104	41.6
	Female	146	58.4
Location	Inpatient(Female ward)	17	6.8
	Inpatient(Male ward)	6	2.4
	Inpatient(Maternity ward)	11	4.4
	Inpatient(Pediatrics ward)	12	4.8
	Inpatient(Surgical ward)	7	2.8
	Outpatient department	197	78.8

Table 2: Gender distribution and patient location

4.2 Specimen type

The bacteria isolated were cultured from both pus and urine samples over the study period, 2015-2018. Pus samples were (176/250, 70.4%) out of the total 250 sample types while urine samples were (74/250, 29.6%).

4.3 Isolate species

Overall, gram negative bacteria isolated were the most predominant bacteria. Gram negative bacteria were 149/250 (59.6%), while gram positive bacteria were 101/250 (40.4%). The only gram positive isolate was *S. aureus* 101/250 (40.4%), which was also the most frequently isolated bacterium. Gram negative bacteria isolated included, *E. coli* (28%), *Klebsiella spp* (16%), *Pseudomonas spp* (10%), and *Proteus spp* (6%).

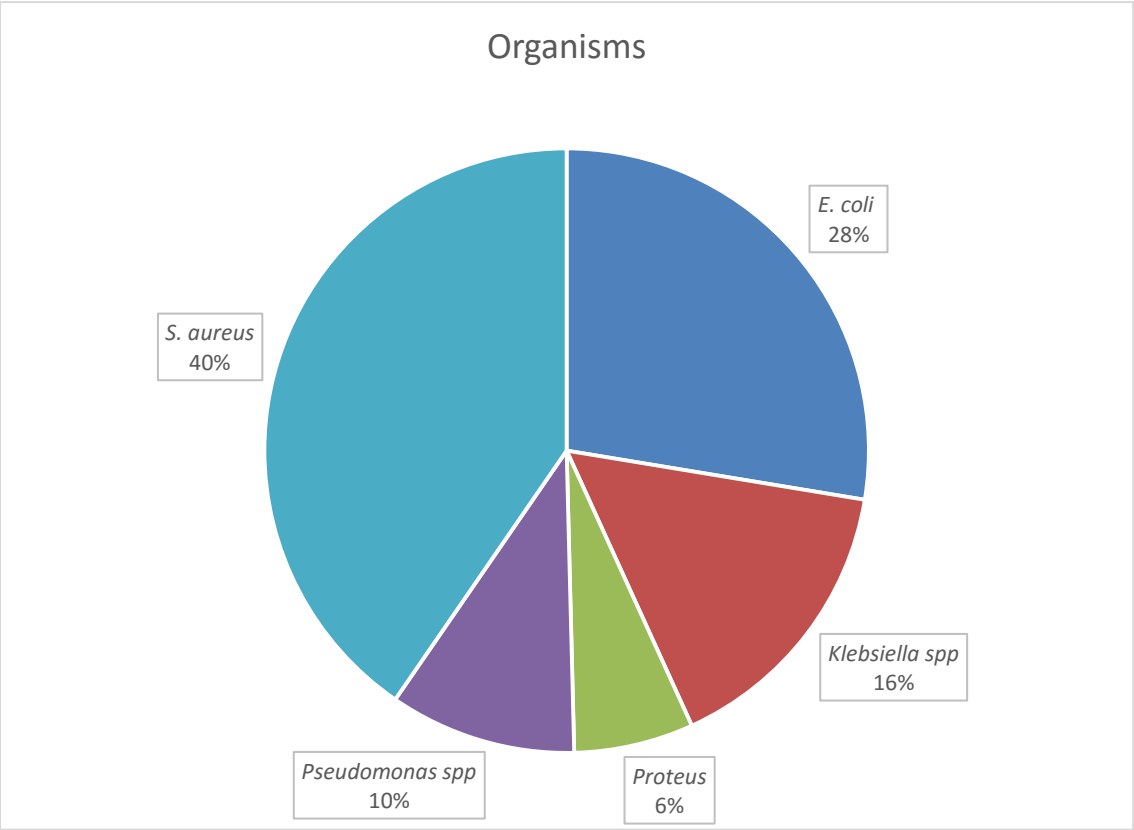

Figure 2: Bacterial isolates of the study population

4.4 Antimicrobial susceptibility testing (AST)

There was overwhelming resistance to penicillins; resistance to ampicillin was 90.8%, 90.9% to methicillin and 83.7% to amoxicillin. Resistance to cephalosporins was 0-63.8%, aminoglycosides 27-52.9% and the resistance to macrolides was 69%. Resistance to trimethoprim-sulfamethoxazole was high at 93%. Susceptibility to chloramphenicol was seen at 73.4%. Resistance to fluoroquinolones was 44.5%-56.9%. There was moderate resistance to nalidixic-acid (66.2%).

4.4.1 Antimicrobial susceptibility of gram positive organisms

S. aureus was the only gram positive organism isolated. *S. aureus* met the threshold for antibiogram reporting (more than 30 isolates per species) (29). The results recorded a poor susceptibility to penicillins. Resistance to benzyl-penicillin was high and seen at 91.5%. It showed poor susceptibility to amoxicillin at 19.4% and ampicillin at 11%. *S. aureus* susceptibility to amoxicillin-clavulanic acid was 53.2%. Moderate susceptibility was seen with cefuroxime at 64.2%. Susceptibility to ciprofloxacin was seen at 44.2%, ofloxacin

susceptibility was at 41.5%. There was poor susceptibility to macrolides 28.8-38.5%. Isolates susceptibility to the aminoglycoside streptomycin were 18/26 and 20/26 to gentamicin. However only 6 isolates were tested for amikacin sensitivity, 2/6 of these isolates were sensitive to amikacin. Susceptibility to nitrofurantoin was seen in 14/21 of the isolates. The susceptibility to chloramphenicol was at 81.1%, seen in 47/58 of the isolates. Only 9.1% isolates were susceptible to trimethoprim-sulfamethoxazole were sensitive.

Table 3: Antimicrobial susceptibility of *S. aureus*

S. aureus	**Sensitivity n (%)**
Ampicillin	6/51(11.8%)
Amoxicillin-Clavulanic	25/47(53.2%)
Amoxicillin	7/36(19.4%)
Amikacin	2/6(33.3%)
Chloramphenicol	47/58(81%)
Ceftazidime	21/30(90%)
Ciprofloxacin	19/43(44.2%)
Cefuroxime	34/53(64.2%)
Erythromycin	15/52(28.8%)
Cefixime	28/42(66.7%)
Nitrofurantoin	14/21(66.7%)
Gentamicin	20/26(76.9%)
Ofloxacin	17/41(41.5%)
Benzyl penicillin	4/47(8.5%)
Piperacillin	14/41(34.1%)
Sulfamethoxazole-trimethoprim	4/44(9.1%)
Tetracycline	36/62(58.1%)
Streptomycin	18/26(69.2%)
Kanamycin	5/10(50%)
Minocycline	8/8(100%)
Nalidixic acid	7/16(43.8%)
Azithromycin	15/39(38.5%)

4.4.2 Antimicrobial susceptibility of gram negative organisms

E. coli and *Klebsiella spp* met the threshold for antibiogram reporting (more than 30 isolates per species). Although *Pseudomonas spp* and *Proteus spp* did not meet the threshold, results will be discussed due to their clinical significance.

A total of 28 *E. coli* isolates were tested for susceptibility to ampicillin, only 2 isolates were sensitive to ampicillin. Ceftazidime sensitivity was seen in 11 out of 18 isolates while

sensitivity to cefuroxime was seen in 15/28 isolates. Susceptibility to the aminoglycoside streptomycin was (12/31, 38.7%), (29/48 60.4%) to gentamicin while 22/26 isolates were sensitive to amikacin, 9/22 isolates were sensitive to kanamycin. Thirteen out of 16 isolates were susceptible to chloramphenicol. There was moderate to good susceptibility to fluoroquinolones 66.7% to 73.1%. Susceptibility to nitrofurantoin was (22/30, 66.7%), while 8/12 isolates were susceptible to the monobactam, aztreonam.

Table 4: Antimicrobial susceptibility of *E. coli*

E. coli	**Sensitivity n (%)**
Ampicillin	2/28(7.1%)
Amikacin	22/26(84.6%)
Chloramphenicol	13/16(86.7%)
Ceftazidime	11/18(61.1%)
Ciprofloxacin	17/24(70.8%)
Clindamycin	10/18(55.6%)
Ceftriaxone	7/11(63.6%)
Cefuroxime	15/28(53.6%)
Cefixime	6/16(37.5%)
Nitrofurantoin	20/30(66.7%)
Gentamicin	29/48(60.4%)
Norfloxacin	14/21(66.7%)
Ofloxacin	19/26(73.1%)
Sulfamethoxazole-Trimethoprim	1/19(5.3%)
Tetracycline	14/32(43.7%)
Streptomycin	12/31(38.7%)
Kanamycin	9/22(40.9%)
Nalidixic acid	7/16(43.8%)
Aztreonam	8/12(66.7%)

A total of 39 isolates of *Klebsiella spp* were obtained. This number met the threshold for antibiogram reporting. Three of the 8 isolates tested for ampicillin susceptibility were sensitive. Susceptibility of the isolates to gentamicin was (23/35, 65.7%), 14/25 isolates were sensitive to amikacin, 9/10 to kanamycin, 8/18 to streptomycin. Susceptibility to ceftriaxone was seen in 7/13 isolates, 6/14 to ceftazidime and 11/18 to cefuroxime. Susceptibility to fluoroquinolones was seen at 6/15 to ciprofloxacin, 5/14 to norfloxacin and 6/18 to ofloxacin. Susceptibility to aztreonam was a seen in 4/13 isolates tested.

Table 5: Antimicrobial susceptibility of *Klebsiella spp*

Klebsiella	**Sensitivity n (%)**
Ampicillin	3/18(16.7%)
Amikacin	14/15(93.3%)
Ceftazidime	6/14(42.9%)
Ciprofloxacin	6/13(46.2%)
Clindamycin	5/13(38.5%)
Ceftriaxone	7/13(53.8%)
Cefuroxime	11/18(61.1%)
Nitrofurantoin	9/21(42.9%)
Gentamicin	23/35(65.7%)
Norfloxacin	5/14(35.7%)
Ofloxacin	6/18(33.3%)
Tetracycline	9/15(60%)
Streptomycin	8/13(61.5%)
Kanamycin	9/10(90.0%)
Nalidixic acid	5/21(23.8%)
Aztreonam	4/13(30.8%)

Only 16 isolates of *Proteus spp* and 25 isolates of *Pseudomonas spp* were obtained, this did not attain the threshold for antibiogram reporting, but due to the clinical significance each of these will be discussed.

All the 12 isolates of *Proteus spp* tested for ampicillin susceptibility were resistant, 3/5 were susceptible to chloramphenicol. Susceptibility to amikacin was seen in 2/4 isolates, 9/14 to gentamicin, 2/10 to streptomycin and 2/6 to kanamycin. Only 3 out of the 4 samples were sensitive to ofloxacin while 2/7 isolates of *Proteus spp* were susceptible to cefuroxime. None of the 10 isolates tested for susceptibility to sulfamethoxazole-trimethoprim were sensitive, 3/5 isolates tested were sensitive to chloramphenicol, susceptibility to tetracycline was seen in 3/11 of the isolates tested and only 4/6 seen to nalidixic acid.

Table 6: Antimicrobial susceptibility of *Proteus spp*

Proteus	**Sensitivity n (%)**
Ampicillin	0/12(0%)
Amikacin	2/4(50%)
Chloramphenicol	3/5(60.0%)
Cefuroxime	2/7(28.6%)
Nitrofurantoin	0/7(0%)
Gentamicin	9/14(64.3%)
Ofloxacin	3/4(75.0%)
Sulfamethoxazole-Trimethoprim	0/10(0%)
Tetracycline	3/11(27.3%)
Streptomycin	2/10(20.0%)
Kanamycin	2/6(33.3%)
Nalidixic acid	4/6(66.7%)

A total of 6/12 isolates of *Pseudomonas spp* were sensitive to amikacin, 8/18 sensitive to gentamicin, 1/7 sensitive to streptomycin and all the 5 isolates tested for kanamycin susceptibility were resistant. Only 2/6 isolates tested for chloramphenicol susceptibility were sensitive. 3/12 isolates were sensitive to ceftriaxone, 1/12 to cefixime, and 3/12 to Cefotaxime. Fluoroquinolones susceptibility was seen in 7/14 isolates tested for ciprofloxacin susceptibility, 7/12 to norfloxacin and 8/16 sensitive to ofloxacin. 1/12 isolates were susceptible to nitrofurantoin 3/12 isolates tested were sensitive to aztreonam. 8/12 isolates were susceptible to tetracycline and 2/15 to nalidixic acid.

Table 7: Antimicrobial susceptibility of *Pseudomonas spp*

Pseudomonas	**Sensitivity n (%)**
Amikacin	6/12(50%)
Chloramphenicol	2/6(33.3%)
Ceftazidime	2/10(20%)
Ciprofloxacin	7/14(50.0%)
Ceftriaxone	3/12(25.0%)
Cefuroxime	0/15(0%)
Cefixime	1/12(8.3%)
Cefotaxime	3/12(25.0%)
Nitrofurantoin	1/12(8.3%)
Gentamicin	8/18(44.4%)
Norfloxacin	7/12(58.3%)
Ofloxacin	7/16(43.8%)
Tetracycline	8/10(80.0%)
Streptomycin	1/7(14.3%)
Kanamycin	0/5(0%)
Nalidixic acid	2/15(13.3%)
Aztreonam	3/12(25%)

4.5 Resistance profiles

The antibiotic categories tested included aminoglycosides, penicillins, cephalosporins, fluoroquinolones, lincomycins, macrolides, the monobactam aztreonam and nitrofurantoins.

A total of (216/250, 86.4%) of the isolates showed resistance. In this study a total of (168/250, 67.2%) of isolates were multi drug-resistant, (48/250, 19.2%) were drug resistant. The rest of the samples were sensitive to all drugs tested.

4.6 Co-morbidities

Patient data on co-morbidities was obtained from inpatient records, 47 of the total 53 had no co-morbidities, 3 (5.7%) were diabetic, and 3 (5.7%) had both diabetes and hypertension.

Table 8: Patient co-morbidities

		n	%
CO-MORBIDITIES	None	47	88.6
	Diabetes	3	5.7
	Hypertension	0	.0
	Diabetes/Hypertension	3	5.7

4.7 Duration of admission

Inpatients were 53 of the total, duration of admission amongst the inpatients ranged from a minimum of 3 days to a maximum of 34 days of admission. The three patients who were diabetic and hypertensive were admitted for three, seven and eight days respectively. The three patients with diabetes were admitted for five, seven and nine days respectively. Four of the patients with co-morbidities had *S. aureus* isolates obtained from the pus specimen. All had drug resistant organisms.

4.8 Previous antimicrobial use

Information on previous antimicrobial use was only available in the inpatient files. The outpatient register did not contain this information. A total of 53 patients out of the total 250 were inpatients, (34/53, 64%) of patients had prior use of antibiotics, either obtained by self-

prescription, or prescribed by a medical practitioner. The rest had no recorded prior antimicrobial use.

Of the 19 patients who had no recorded prior use of antimicrobials, 16 samples had multi drug-resistant isolates and 3 had drug resistant isolates. However, amongst the patients who had a recorded history of prior antimicrobial use, 29 had multi drug-resistant isolates and 4 had drug-resistant isolates. One sample was sensitive to all drugs tested.

4.9 Methicillin Resistant Staphylococcus aureus

There was a total of 101 *S. aureus* isolates, of this total only 11 isolates were tested for methicillin resistance. Methicillin resistant *S. aureus* were 10/11 (90.9%). This level was suspiciously high and contrary to studies reflected in SSA on MRSA and begs the question; was there misidentification of *S.aureus*, how did this affect the assessment of MRSA in this population, and how will this be addressed in AMR surveillance to facilitate accuracy in surveillance?

Chapter 5: DISCUSSION

5.1 Antimicrobial susceptibility patterns

The aim of this study was to describe the antimicrobial susceptibility patterns of bacterial isolates from pus and urine samples obtained from patients at the Moi county referral hospital, Voi.

There were a total of 1098 cultures, with 296 positive cultures carried out in pus and urine samples in this study, the number of isolates obtained were 250, 46 samples were excluded due to missing data, and these were from both inpatients and outpatients. Pus samples were the majority, 70.4%. Samples from the outpatient department were 78.8%.

Gram negative bacteria were predominant at 59.6%. The only gram positive isolate was *S. aureus* of which 10/11 isolates were reported as MRSA. Gram negative bacteria isolated included *E. coli* 28%, *Klebsiella spp* 16%, *Pseudomonas spp* 10%, and *Proteus spp* 6%. Resistance to commonly used first-line antimicrobials such as penicillins, macrolides, sulfamethoxazole-trimethoprim and third generation cephalosporins was observed. The number of isolates obtained over the years was noted to be on the rise, probably due to the better quality control and established laboratory procedures. Resistance patterns were also noted to be on the rise. This was seen with more isolation of more antibiotic resistant organisms. This shows a progression of resistant organisms in the population, a point of concern.

The results of this study showed a predominance of three bacterial agents, namely *S. aureus, E. coli* and *Klebsiella spp*. This has also been reflected in previous studies seen locally and internationally(26). Whereby, there are high rates of resistance with first-line antibiotics. These three agents are of greatest concern in the war against antimicrobial resistance, with rates of resistance noted to be increasing world over and in SSA. Locally this was similar to a study by Wangai et al in KNH on antimicrobial susceptibility of patient samples, which showed a predominance of gram negative organisms at 67% and high rates of resistance with 1^{st} line antibiotics(26).

This study showed that *S. aureus* was susceptible to most cephalosporins, with a 90% susceptibility seen to Ceftazidime while susceptibility to cefuroxime was seen at 64.2%. Taking into consideration that *S. aureus* was the most predominant of all the agents isolated;

it's poor susceptibility to penicillins and excellent susceptibility to cephalosporins. This information is dire to a clinician in this setting, taking into account that standard treatment guidelines in our region recommend first- line treatment with penicillins. Therefore guidelines tailor-made for this region can be guided by this information. This will facilitate proper management of patients. This was similar to a study by Gitau et al in KNH, which showed high susceptibility of *S. aureus* to first line antimicrobials(30).

Resistance to first line antimicrobials has also been well documented in a study by Kiman'ga et al on the situational analysis of antimicrobial resistance in Africa, with a rise in antimicrobial resistance seen to commonly used antibiotics(6).

There was an overwhelming resistance seen in this study in Moi County hospital, Voi, to sulfamethoxazole-trimethoprim, this could be due to the prophylactic use for *Pneumocystis jirovecii* in HIV-AIDS patients', while taking into account the burden of disease in Africa. Various case studies have been published on the resistance of bacteria to sulfamethoxazole-trimethoprim. The most outstanding being a noted resistance of *P. jirovecii* to the drug. Mutations have been seen in the dihydropteroate synthase gene in *P. jirovecii*. This consequently affects the management and prevention of opportunistic infections in HIV/AIDs patients thus increasing the disease burden, mortality and morbidity in Africa from infectious diseases(31).

5.1.1 Methicillin-resistant *Staphylococcus aureus*

As noted in the Voi study a total of 11 samples were tested for methicillin resistance. Of these, 10 isolates were resistant to methicillin. These numbers were notably variable to prevalence rates of MRSA seen in SSA. This therefore begged the question, was there misidentification of MRSA in this population, how does it affect diagnostic accuracy and in turn patient management?

MRSA infection is one of the main causes of hospital acquired infections and is commonly associated with significant morbidity, mortality, length of hospital stay and cost of treatment. Methicillin resistance in *S. aureus* has occurred due to a mutation in a penicillin-binding protein, a chromosome encoded protein.

The history of MRSA dates as far back as the 1960s. Since then, rates of MRSA have been seen to increase world over. The prevalence of MRSA has been seen to be on the rise in

Africa, as demonstrated by Falagas et al, who was assessing MRSA in Africa by reviewing articles published in 2005 or later that were reporting the prevalence of MRSA among *S. aureus* clinical isolates. They included 32 studies carried out across Africa. A conclusive review of these studies showed that the prevalence of MRSA was lower than 50% in most of the African countries, although it appeared to have significantly risen since the year 2000 in many African countries, except South Africa. In South Africa, the prevalence decreased from 36% in 2006 to 24% from 2007 to 2011(32).

Larger numbers of isolates are required to assess MRSA in this population. This would facilitate identification of the rates of MRSA true to this population and provide a comparison in MRSA rates among the urban and rural populations. Falagas et al while assessing MRSA in Africa noted that among the low human development index countries, that is; Madagascar, Senegal, Cameroon, Niger, Eritrea and Tanzania there were low prevalence rates of MRSA, varying from 6%-16% during the period 2001-2009(33).

Okamo et al, on the prevalence and antimicrobial susceptibility profiles of *S. aureus* nasal carriage in pre-clinical and clinical medical students in a Tanzanian university reported an overall MRSA nasal carriage rate of 0.3%(34). The low rates of MRSA in these low human development index countries could be attributed to the underutilization of antibiotics compared to high income countries, thus leading to comparatively lower selection pressure for MRSA.

There has been a noted rise in the prevalence of MRSA in SSA. The prevalence rates of MRSA in a population are always dependent on proper identification of the pathogen. There are various studies in regards to the misidentification of MRSA in SSA. One is a paper published by Ahmed et al on misidentification of methicillin-resistant *Staphylococcus aureus* in hospitals in Tripoli, Libya which reported that of the 170 isolates examined, 51% were confirmed as MRSA (i.e. 49% were misidentified as MRSA)(35).

Furthermore Wangai et al also published a paper in 2019, querying the misidentification of MRSA in East Africa. They noted that out of a total 187 *S. aureus* isolates, antimicrobial susceptibility data revealed that the overall MRSA prevalence was 53.4%. They noted that the prevalence rate was higher in comparison to the tertiary private facilities in the region. The reasons for these are multifactorial. Some of the factors being that there are marked socio-demographic differences among the population, differences in antimicrobial

accessibility; differences in the hospital environment as well as infection control measures(36).

Isolate identification varies markedly in laboratories and can influence MRSA reporting. Molecular methods are noted to demonstrate lower rates of misidentification in comparison to phenotypic identification. This is especially true of phenotypic misidentification of coagulase- negative *Staphylococcus* (CoNS). This can lead to over reporting of methicillin resistance. CoNS are mostly normal flora found on the skin and mucous membranes (37,38). The Moi County referral Hospital, Voi lab utilizes phenotypic methods of identification of MRSA, which can result in a misidentification of CoNS. A review on laboratory methods of identification is required for diagnostic accuracy.

5.1.2 Enterobacteriaceae

This study showed a predominance of gram negative organisms at 59.6%, with *E.coli* at 27.6%, *Klebsiella spp* at 15.6%, *Proteus spp* at 6.4% and *Pseudomonas spp* at 10%. This was similar to a study carried out in Tanzania on the predominance of multi-drug resistant bacterial pathogens causing surgical site infections at Muhimbili National hospital. Out of a total of 147 pathogenic bacteria isolated, 77.5% were gram negative organisms. Most predominant gram negative organism was *P. aeruginosa* (16.3%) (39).

The gram negative organisms isolated in this study were susceptible to most third generation cephalosporins and aminoglycosides, with poor susceptibility to penicillins. In a similar study by Maina et al that was assessing the spectrum of microbial disease and resistance patterns in a private teaching hospital in Kenya and implications in clinical practice, they isolated *E. coli* as the commonest bacterial isolate, followed by *S. aureus.* Isolates of *E. coli* and *Klebsiella spp* that were cultured showed susceptibility of 47% and 24% to third generation cephalosporins respectively and 41% and 59% to quinolones. Among outpatient urinary isolates of *E. Coli* and *Klebsiella spp* from this study only 79% and 65% respectively were susceptible to cephalosporins(17). These findings were similar in our Voi study which showed a poor susceptibility of *E.coli* to penicillins at (0-7%).

5.2 Correlates of antimicrobial resistance

This study also included important patient data and clinical information. This was aimed at identifying patient factors that might influence antimicrobial resistance. These included:

5.2.1 Empiric antimicrobial use

Empiric antimicrobial use was assessed in the study population, this information was obtained from inpatient files; this was however not available in the outpatient data. A total of 53 patients out of 250 were inpatients, 34 of the inpatients were recorded to have previously utilised antibiotics, either obtained from a pharmacy by self-prescription, or prescribed by a medical practitioner. The rest had no prior antimicrobial use.

Most of the samples collected in the inpatients were collected after empiric antimicrobial treatment was started. The patients' whom antimicrobials were changed according to culture results had a good outcome with a noted reduction in period of admission. This enhanced patient management. This was also seen by Saravanan et al in a study in India on antimicrobial resistance patterns in a tertiary care hospital; where 64% of patients were started on antimicrobials before culture and sensitivity of the specimen was carried out, 36% of samples were collected before starting antimicrobials. Previous use of antimicrobials could only be established in 63% of the study subjects. The percentage of those without prior antimicrobial use was 20% and 10% had a prior history of antimicrobial use in the Saravanan study(40).

5.2.2 Co-morbidities

Patient co-morbidities were only recorded in the inpatient data, of the total 53 inpatients, 6 (11.4%) were diabetic and/or hypertensive. The other 47 (88.6%) patients had no co-morbidities. One patient who was diabetic and hypertensive had a multi-drug resistant isolate the rest of the patients with co-morbidities had isolates that were drug-resistant. In a similar study by Wangai et al on antimicrobial susceptibility in KNH; in the prospective arm of the study, patients with co-morbidities were noted to have some of the highest rates of resistance. Of these, 73% were drug-resistant, 73.4% were MDR, 68.8% were XDR and 66.7% were PDR(26).

Multi-drug resistance according to the European Centre for Disease Control (ECDC) and Centre for Disease Control and Prevention (CDC) is non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and pan-drug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories (41).

5.2.3 Patient outcomes

There has been a recorded correlation between antimicrobial resistance and its influence on patient outcome. This includes the duration of admission, mortality and economic implications(42). This study obtained information on the duration of admission and mortality. The economic impact of admission was not assessed. The median duration of hospital admission in this study was 7 days, minimum days were 3 and maximum was 34 days. Isolates that were MDR all showed a minimum of 5 days duration of admission. There were no outstanding differences on duration of admission and the resistance profiles in this study.

Mortality was recorded in two patients, with multi drug-resistant pathogens and co-morbidities, both patients were diabetic and hypertensive. Mortality could be attributed to both their condition and presence of MDR isolates. Other cases of mortality in this population were seen in one patient from the paediatric ward, and post-operative complications, one from the maternity ward, two from the surgical ward above the age of 65. This was seen in patients with drug resistant pathogens.

5.2.4 Specimen collection and laboratory testing

This study focused on urine and pus samples, since these are the samples tested in the Voi laboratory. Blood, CSF, pleural fluid and synovial fluid were not included. An inclusion of a wider scope of specimen type would facilitate proper identification of antimicrobial resistance.

Out of the total 296 positive cultures, 46 were excluded from the study, mainly due to missing data or erroneous entries. This is attributed to poor record entry and poor record keeping. Proper record keeping and record entry would facilitate proper data collection and thus facilitate the identification of AMR in the population. In this study only three bacterial pathogens attained the minimum thirty isolates per species threshold for antibiogram

reporting. Including a larger scope of specimen type and minimal errors in record keeping would lead to higher numbers of isolates which will yield larger numbers for antibiogram reporting and thus better results.

Quality control and laboratory procedures were validated but this study highlighted important areas that require addressing. This includes; specimen collection, pathogen identification and data entry in laboratory records.

Various studies across SSA have shown rates of misidentification of MRSA. The phenotypic misidentification of MRSA from CoNS has been recorded, especially taking into account that CoNS are normal commensals in the skin and respiratory tract. This is both influenced by specimen collection and the phenotypic identification of MRSA from other coagulase negative *Staphylococcus.*

5.2.5 Quality control

This is the total process by which the quality of laboratory reports can be ascertained. The term quality control covers that part of quality assurance which concerns the control of errors in the performance of tests and verifications of test results. Culture media must be tested for sterility and performance. Each laboratory must have standard operating procedures (SOPs). Quality assurance of pre-analytical, analytical and post-analytical stages of microbiological procedures should be incorporated in SOPs. Regrettably implementation of quality laboratory service in many resource poor countries has been unsystematic and is still questionable. Quality medical laboratory service provision is important in order to enhance diagnostic value. This is a major factor in East Africa and requires addressing to facilitate diagnostic capability and value. Enrolment into an external quality assessment (EQA) program allows for comparison of a laboratory's testing to an outside source. The Voi laboratory is enrolled into an EQA program.

Chapter 6: CONCLUSION

This study aimed to identify antimicrobial susceptibility patterns in a hospital setting outside the Kenyan capital, Nairobi. AMR surveillance is routinely carried out in tertiary hospitals in the capital, but data on AMR in the county hospitals is minimal, thus creating a knowledge gap. This study aimed to identify antimicrobial resistance patterns in a county referral hospital to better understand resistance patterns and formulate an antibiogram that will be utilised in the hospital, while at the same time bridging the knowledge gap in antimicrobial surveillance.

The results obtained revealed an astounding resistance to commonly used first-line antimicrobials, such as penicillins as seen in studies on antimicrobial susceptibility patterns across the country. This gives insight to a desperate need for routine antimicrobial surveillance that caters for each region. It also informs a need for cohesion amongst health facilities in regards to antimicrobial stewardship, with regular surveillance and involvement of the community to curb antimicrobial resistance.

Quality assurance implementation stood out as revealed in this study. It has been seen world over that in resource poor settings; quality laboratory service is unsystematic and still questionable. This affects diagnostic value.

Chapter 7: RECOMMENDATIONS

I. Antimicrobial susceptibility testing- We recommend routine antimicrobial susceptibility testing to include other specimen types like blood. This will broaden the scope of the pathogens isolated from culture, and facilitate development of an antibiogram, that tailor made for the region.

   Clinicians should be trained in proper specimen collection with an aim to increase the yield of pathogenic bacteria. Proper specimen collection and proper isolate identification will facilitate AST.

   We also recommend a wider scope of antimicrobial testing on all isolates obtained to better understand the rates of drug resistance.

II. Antibiotic stewardship- Educate clinicians on the proper use of antimicrobials so as to provide informed antimicrobial practices. This will facilitate adequate use of antibiotics, to curb the misuse and overuse of antimicrobials.

   Educate the general population on antimicrobial use, including discouraging over the counter self-prescription.

III. Continuous surveillance to monitor pathogens and antimicrobial susceptibility patterns both in the community and the hospital setting to guide empiric use of antibiotics. There's a necessity for continuous surveillance to monitor aetiology to facilitate the revision of current treatment guidelines and regular surveillance of pathogens and the antimicrobial patterns on a larger scale is recommended, both in the public and private domain.

IV. Quality medical laboratory service provision is crucial in order to enhance diagnostic value. It involves implementation of quality laboratory service and standard operating procedures. There is also a need to build the capacity of local microbiology laboratories to espouse molecular methods in resistance testing which would be ideal. This is greatly recommended to emend diagnostic accuracy and surmount discrepancies that arise in the laboratories. This study showed a probable misidentification of MRSA, this informs the needs to build laboratory capacity, with implementation of more accurate laboratory testing and reporting of significant isolates of concern. Though expensive, molecular assays improve accuracy, cheaper methods are more accessible in this setting. These cheaper methods involve the use of laboratory phenotypic methods of identification such as coagulase testing among

other biochemical techniques such as mannitol salt agar and deoxyribonuclease (DNase).

Chapter 8: STUDY STRENGTHS AND LIMITATIONS

Strengths of this study include:

I. The study was carried out in a county referral hospital located in Voi, a rural region in the country. Antimicrobial surveillance is carried out more routinely in tertiary institutions within the capital. Minimal data is available on surveillance within the rural facilities. This study bridges a gap in antimicrobial surveillance.

Limitations of this study include:

I. There were a few numbers of isolates in certain species, e.g. *Pseudomonas spp* and *Proteus spp* which did not attain the required numbers for antibiogram reporting. Other important species were equally lacking, like *Acinetobacter spp,* which are crucial and part of the WHO PPL. These two organisms were still included in the study due to the clinical significance of *Pseudomonas spp* and *Proteus spp.*
II. There was limited specimen profile. The sample types in this study were limited to pus and urine samples, which are the only cultures carried out in this facility. An inclusion of other specimen types, e.g. blood would widen the scope of pathogens and thus susceptibility patterns, facilitating antibiogram reporting. This will hopefully be included in future antimicrobial susceptibility testing in Voi.
III. There is a need to increase lab capacity in that; there was a lack of information on susceptibility to anaerobes. Patient outcome can be improved if laboratory processing of specimen would include procedures that will enable isolation of strict anaerobes.
IV. Poor quality control implementation with possible misidentification of MRSA, with other CoNS. This resulted in higher rates of MRSA recorded in this study.
V. Incomplete outpatient records. There were missing or illegible data in the outpatient laboratory records, this resulted to exclusion of these data in the study, limiting the number of isolates.
VI. Missing inpatient records. Some patient files could not be traced in the records department. Either missing or misplaced. These were omitted from this study.

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