

**MAKERERE**



**UNIVERSITY**

**ANTIMICROBIAL RESISTANCE PROFILE OF PATHOGENIC  
ISOLATES AMONG OUT-PATIENTS RECEIVING HEALTH CARE  
SERVICES AT GULU REGIONAL REFERRAL HOSPITAL, GULU –  
UGANDA**

**BY**

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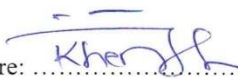
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**A RESEARCH PROJECT REPORT SUBMITTED TO THE COLLEGE OF  
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DEGREE OF BACHELOR OF BIOMEDICAL LABORATORY  
TECHNOLOGY OF MAKERERE UNIVERSITY**

**JULY, 2024**

**DECLARATION**

I **Akena Stephen**, declare that the information presented in this research report is original and has never been used before for any award of any category, where work of other people were used, it was quoted in the reference.

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## **DEDICATION**

I dedicate this report to my beloved parents, family and friends.

## **ACKNOWLEDGEMENT**

I thank the Almighty God who has enabled me this far. Special appreciation to my supervisor Mr. Nathan Musisi Lubowa, my family and friends.

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## OPERATIONAL DEFINITION

**Antimicrobial resistance:** This refers to the ability of the bacteria to grow in the presence of antibiotics at a concentration that would otherwise inhibit their growth

**Isolates:** This refers to the bacteria that are recovered in the samples.

**Risk factors:** This refers to the associated reasons that escalate the development and spread of antimicrobial resistance.

**Pan resistance:** This refers to the ability of the organism to resist all the antibiotics sensitivity will be set.

**Antibiotic pattern:** This refers to the measurable zone of inhibition when set on a drug sensitivity test by minimum inhibition concentration technique.

**Multiple antibiotic resistance index:** This is the ratio of the antibiotic that had a resistance to the total number of antibiotics set.

**Minimum Inhibitory Concentration:** This refers to the lowest concentration of the antimicrobial drug that inhibited the visible growth of the isolates after a 24 hours incubation at 37 °C

## ABBREVIATIONS AND ACCRONYMS

AMR	Antimicrobial resistance
ARG	Antimicrobial resistance gene
CLSI	Clinical and laboratory standard institute
C/S	Culture and sensitivity
DM	Diabetes mellitus
GDP	Growth Domestic Products
GLASS	Global Antimicrobial Resistance Surveillance system
GRRH	Gulu Regional Referral Hospital
HIV	Human-immuno deficiency virus
ICU	Intensive care unit
MDR	Multidrug resistance
MHA	Muller Hinton agar
MOH	Ministry of Health
MARI	Multiple antibiotic resistance index
MIC	Minimum inhibition concentration
OTC	Over the counter
WHO	World Health Organization
°C	Degree Celsius
TSI	Triple sugar ion
%	Percentage

## ABSTRACT

Antibiotic resistance poses a profound threat to human health with associated increased mortality rates and the odds ratio of readmission for resistant infections is as high as 1.492 (49.2%). It has increasingly caused high mortality and morbidity especially in low and middle-income communities with limited access to proper diagnosis, antibiotic prescription and use inadequately regulated. In Uganda alone; there was 30,700 associated deaths with 7,100 directly caused by antimicrobial resistance. The study aimed at determining antimicrobial resistance profile of pathogenic isolates among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda. This was a cross-sectional study conducted between January 2024 to April, 2024 on 101 out-patients receiving health care services at Gulu Regional Referral Hospital employing non-probability convenience sampling technique. Structured questionnaire was used to collect the socio-demographic factors and risk factors. The samples were cultured and antibiotic test set for the isolates. Out of the 101 patients, females 55 (54.46%) had a higher level of antibiotic resistance. The most affected age group was 15 to 24 (40.59%) with the mean age of 33.62 ( $\pm$ 19.13). Secondary level of education was the most predominant level at 52 (51.49%), higher antibiotic resistance was amongst peasant farmers at 40 (39.60%) than other occupation. Higher antibiotic resistance was amongst family size of 5-7 (51.49%). Purchasing the drugs over the counter and self-medication were the most likely direct associated risk factors to antibiotic resistance. *S. aureus* was the most predominant bacteria at 24 (23.76%), followed by *E. coli* at 23 (23.77%), Coagulase negative staphylococcus 18 (17.82%), with the least being *S. agalactiae* at 2 (1.98%). The others were *P. mirabilis* at 9 (8.91), followed by *K. pneumoniae* at 6 (5.94%). *Acinetobacter* spp, *E. faecalis*, *P. vulguris* and *S. pyogenes* were all at 4 (3.96%) respectively, *P. aeruginosa* (2.97%). The highest antibiotic resistance was recorded in Streptomycin (S) at 100% followed by Nalidixic acid (NA) at 95.24%, Ampicillin (AMP) at 94.87%, Cefuroxime (CXM) at 83.72%. There was complete susceptibility to Amikacin and Imipenem. *S. aureus* and *E. coli* continues to be the most widely spread organisms with the purchasing of drugs over the counter and self-medication being the most associated risk factors.

**Key words:** Antimicrobial resistance, pathogenic isolates, resistance, susceptibility, out-patients

# **CHAPTER ONE**

## **INTRODUCTION**

### **1.1 Background**

Alexander Fleming anticipated the global burden with a renowned sentence, “There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring resistance” (Aleksun MN, 2007). Over the decade, antibiotic resistance was recognized as global health problem but now has been escalated by WHO as one of the top health challenges facing the 21<sup>st</sup> century. (Levy, 2011)

Antimicrobial resistance (AMR) is therefore defined as the inherited or acquired ability of the microorganism to stop the antimicrobial drug from working against it to an extent it cannot be used any longer, hence, rendering the drugs less effective/ ineffective leading to persistence and spread of the resistant organism causing infections (WHO, 2021) which is characterised by measuring the minimum inhibitory concentration of a particular antibiotic wherein resistant bacteria are able to multiply (Markus Huemer, 2020).

It can be classified as either natural or acquired resistance mechanism; the natural mechanism by the ability of the organism to block the antibiotic function as a result of inherent structure or functional characteristics. In contrary, acquired mechanism is based on gaining new functions and it becomes resistant to the drug (s) it would have been otherwise susceptible to. (Rosini R, 2020).

The main mechanism employed by the bacteria to tackle the antibiotics are based on; inactivation of drug by hydrolysis or structural modification, preventing access to the target by reducing membrane permeability or over expression of efflux pump and changes in the antibiotic targets by mutation or post-translational modifications (Rosini R, 2020)

The presence of antimicrobial resistance genes is the most cause of bacterial resistance. Pathogenic bacteria usually acquire the ARGs through plasmid exchange at the gene level and develops strong resistance. The ARG-carrying plasmids, integrons (In) and transposons (Tn) in bacteria can undergo horizontal gene transfer (HGT) among strains of the same and different species. Even after death, the resistance gene exist in the environment under the protection of enzyme deoxynucleotide (Zonghui J, 2021)

The possible mechanisms for the transmission of genetic determinants involved in AMR and in the generation of superbugs includes transduction, conjugation, transformation and other mobile genetic materials (transposons and integrons) (Rosini R, 2020)

There has been an increase in the number of bacteria becoming multidrug resistant (Markus H 2020). Some microorganisms such as *S. aureus* are naturally susceptible to almost every antibiotic that has been developed but are well known for quickly acquiring antibiotic resistance by means of obtaining specific genetic modifications such as mutations or horizontal gene transfer (Markus Huemer, 2020)

To further mention, *Staphylococci* spp appeared to become more readily drug resistant than most other bacteria as followed by the introduction of various antibiotics into general use. The resistant has been associated with resistant plasmid (R-plasmid) that has the ability to mediate the production of drug inactivated enzymes such as B- lactamase and other related functions (Musa Y. Tula, 2016)

Species such as *Acinetobacter* are presenting with high emergence of multiresistant strains, some of which are pan-resistant to antibiotics, which are suddenly causing outbreak of infection involving several patients in clinical units (Raminder Sandhu, 2016)

It is important to note that the relationship between antibiotic use and resistance has been a complex phenomenon as a major driving factor for AMR within both human and veterinary

medicine. (Osundiya OO, 2013). The rise in Growth Domestic Products (GDP) as well as living standards in Low and middle-income countries showed a correlation with antibiotic consumption by increased animal protein consumption, which may require more antibiotics to be added to their (animals), feed. (Dadgostar, 2019)

It normally occurs when bacteria become able to adapt and grow in the presence of medications that once impacted them (Dadgostar, 2019) frequently resulting in delayed adequate antibiotic treatment, increasing morbidity and mortality (Markus Huemer, 2020).

## **1.2 Problem statement**

Antimicrobial resistance has increasingly caused high mortality and morbidity especially in low and middle-income communities with limited access to proper diagnosis, antibiotic prescription and use inadequately regulated (WHO, 2021). Globally, drug resistance claimed 4.95 million lives with 1.27 million directly caused by AMR in 2019. In Uganda alone; there was 30,700 associated deaths with 7,100 directly caused by AMR (IHME, 2023 ). There has been irrational use of antibiotics as seen in over the counter prescriptions, in animal and poultry breeding, self-medication and antibiotic residue in water, which has likely aggravated development and spread of the super bugs. This has been greatly shown by an increasing trend of Antimicrobial resistance in Uganda (MOH, 2022). By observation and clinical records, patients have repeatedly been coming back to the hospital to seek medical attention with similar bacterial diagnosis over a short period time at Gulu regional referral hospital. AMR burden is very crucial and if left unchecked and unattended to, it could make many bacterial pathogens more lethal than they are today. Since a number of studies and reports on AMR has been done in Kampala, the researcher seeks to compare the urban findings to another, hence Gulu city. This research is therefore out to ascertain the AMR profile of commonly isolated pathogens from patients receiving health care services at Gulu Regional Referral Hospital.

### **1.3 Objectives**

#### **1.3.1 General objective**

To determine antimicrobial resistance profile of pathogenic isolates among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda.

#### **1.3.2 Specific objectives**

- i. To identify the risk factors of AMR among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda.
- ii. To identify the common pathogenic bacterial isolates among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda.
- iii. To ascertain the pattern of antibiotic resistance to the commonly used drugs among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda.

#### **1.3.3 Research questions**

- i. What are the risk factors of AMR among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda?
- ii. What are the common pathogenic bacterial isolates among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda?
- iii. What is the pattern of antibiotic resistance to the commonly used drugs among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda?

### **1.4 Significance**

There is a continuous increase trend of AMR due to the numerous gaps in knowledge since the statistics and particulars about the use of different antibiotics in both health care and animal production not well gathered worldwide (Dadgostar, 2019).

Only few reports and publications were available on the problem of AMR on the continent with only three main review articles that were published since 2001. Only nine African countries were able to report the AMR data in the Global Antimicrobial Resistance Surveillance system (GLASS) over 2016 to 2018, which clearly indicated inadequate coordination and implementation of policies to assess and monitor the situation of AMR in Africa. (WHO, 2021).

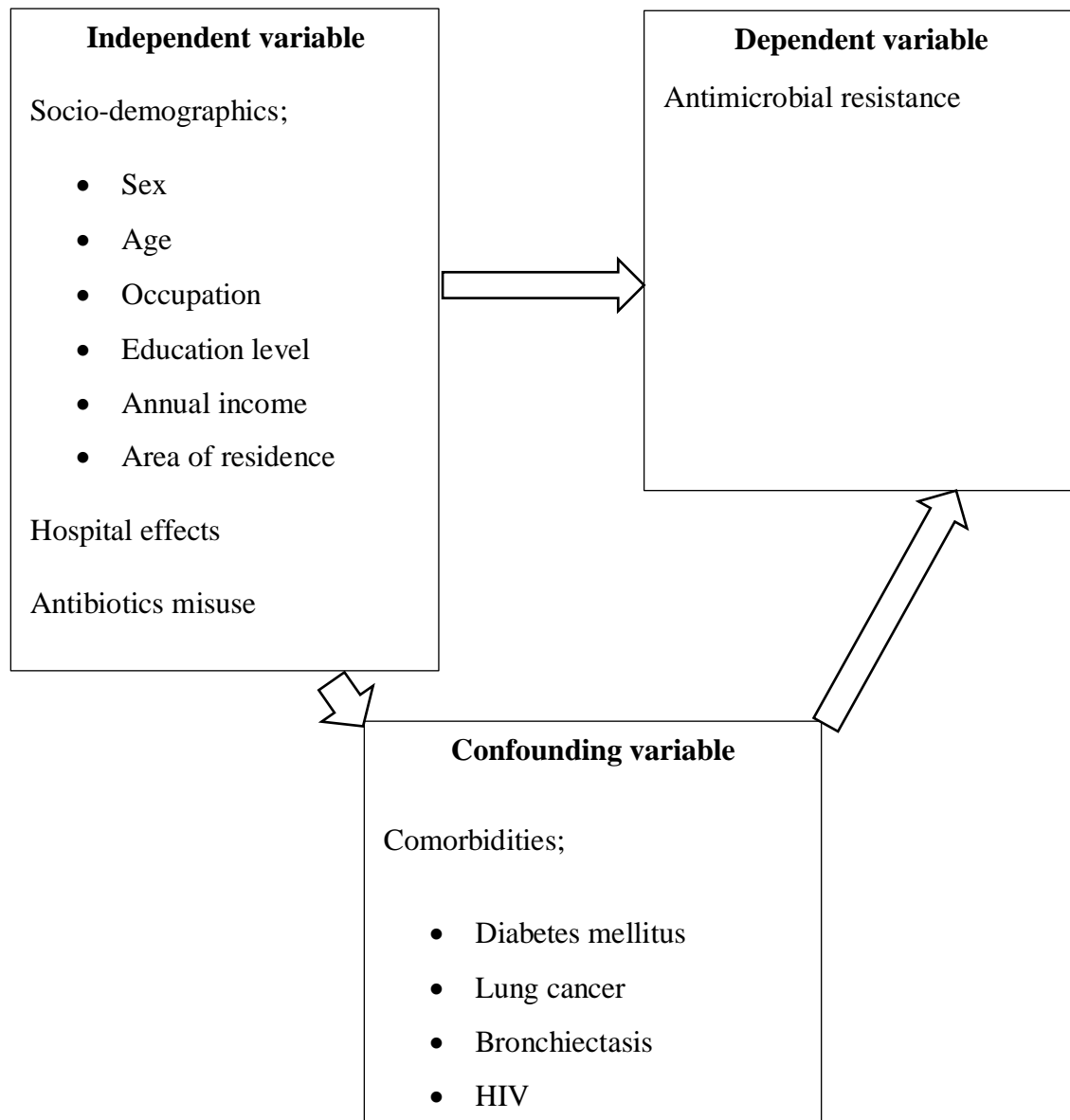
This study has therefore provided more information/ data on AMR at Gulu Regional Referral Hospital that can be adopted in Uganda, Africa and for global implementation, hence, closing the knowledge gap.

It has provided a benchmark upon which other researchers can develop more research ideas related to AMR enhancing good implementation guidelines on antibiotic use.

### **1.5 Scope**

The study findings greatly impact the approach and view of AMR in Gulu district, Uganda, Africa and causing a global effect. The study was conducted at Gulu Regional Referral Hospital, Gulu – Uganda. Gulu is located in the northern part of Uganda, approximately 340 km away from the capital city, Kampala. GRRH is approximately 500 meters away from Gulu district headquarters from the east. It was conducted between January 2024 to April 2024.

## 1.6 Conceptual frame



## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Antibiotic resistance and Multiple antibiotic resistance index

Increasing numbers of bacteria are becoming resistant to multiple antibiotics currently in use resulting into multidrug resistant bacteria. This is characterized by the ability of the organism to be able to grow in a high concentration of antibiotics, which is measured by minimum inhibition concentration (Markus Huemer, 2020). This have been attributed by the time required by the bacteria to develop resistance becoming shorter (Rosini R, 2020).

The five main biochemical mechanisms of resistance include enzymatic modification or destruction of the antibiotics, modification of the antibiotic target site, mimicking the antibiotic target with similar biochemical functions or overproduction of the antibiotic target, decreasing the antibiotic penetration and elimination of the antibiotic from the cell by efflux. (WHO, 2021)

The presence of antimicrobial resistance genes is the most cause of bacterial resistance. Pathogenic bacteria usually acquire the ARGs through plasmid exchange at the gene level and develops strong resistance. The ARG-carrying plasmids, integrons (In) and transposons (Tn) in bacteria can undergo horizontal gene transfer (HGT) among strains of the same and different species. Even after death, the resistance gene exist in the environment under the protection of enzyme deoxynucleotide (Zonghui J, 2021)

Multiple resistance by microbes to antibiotics have been indicated by the presence of resistance factors such as R-plasmids containing one or more resistance genes, each encoding a single antibiotic resistance phenotype. This R-plasmid mediated resistance can spread in a population subjected to heavy antibiotic therapy (Musa Y. Tula, 2016).

Multiple antibiotic resistance indexing (MARI) has shown cost effectiveness and a valid method of bacteria source tracking. It is calculated as the ratio number of antibiotics used to

which the organism is resistant to total number of antibiotics, which the organism was exposed. MARI values greater than 0.2 indicates high-risk source of contamination where antibiotics are often used (Raminder Sandhu, 2016).

## **2.2 Risk factors associated with AMR**

In a systemic analysis by (Naghavi, 2022), in high income region by GDP, AMR was attributed to *S aureus* and *E coli* in contrast to Sub-Saharan Africa that was attributed to *S pneumoniae* and *K pneumoniae*. Vaccination uptake for the pathogens could play a crucial role despite the vaccine being available for only six leading pathogens. Increased antibiotic use both in human and in farming has been identified a potential contributor to AMR although the direct casual linkage remains controversial.

High rates of antibiotic resistance were observed in patients with lung cancer, DM as a comorbidity, hospitalized in ICU and patients with radiological bronchiectasis (Hüsnü Baykal, 2022).

Common misconceptions that antibacterial agents could cure viral diseases like common cold or flu, patient management predominantly on prescriptions due to deficiencies in proper diagnostic tools, prescription of antibiotics when not actually needed, insufficient enforcement of regulatory policies on prescribing medicines, poor quality antibiotics sold over the counter in the developing countries and high prevalence of over the counter for self-prescription were the accelerators of AMR (Dadgostar, 2019).

In a meta-analysis conducted in China, the risk factors included drug exposure; antibiotics against other drug exposures looking at current and previous medications too, admission to health care stings that was measured along invasive procedures and hospital admission history, patient clinical information that included the underlying diseases and severity the underlying

diseases, socio-demographic factors that included sex, age, annual income, education and residence. (Qi Chen, 2021).

Other factors identified included human practices involved the use of antibiotics in food production and animal husbandry with the intention to prevent infection; demographic factors included age; history of co-morbidities such as diabetes, HIV and other infections were found to be with colonization of AMR, history of antibiotic use was also associated with development of AMR, prolonged hospitalization. (Bugwesa Z. Katale, 2020).

### **2.3 Common pathogenic bacterial isolates associated with AMR**

The linkage between some specific bacteria; *Enterococcus* spp, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp and their tendency to progress to MDR has recently gained global attention (Qi Chen, 2021).

The most common and serious MDR pathogens have been encompassed within the acronym “ESKAPE” for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (Raminder Sandhu, 2016)

In 2019, six pathogens were each responsible for more than 250 000 associated deaths to AMR; *E. coli*, *Staphylococcus aureus*, *K pneumoniae*, *S pneumoniae*, *Acinobacter baumannii* and *Pseudomonas aeruginosa* with the same six being responsible for 929 000 of 1.27 million deaths attributable to AMR. Six more pathogens were responsible for between 100 000 and 250 000 deaths associated with AMR; *M tuberculosis*, *E faecium*, *Enterobacter* spp, *Streptococcus agalactiae* (group B Streptococcus), *S typhi* and *E faeculis*. For the attributable death, *E coli* was responsible for most deaths in 2019, followed by *K pneumoniae*, *S aureus*, *A baumannii*, *S pneumoniae* and *M tuberculosis* (Naghavi, 2022).

In East Africa, resistant genes were isolated from *E. coli*, *Klebsiella spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *salmonella spp* and *E. cloacae* complex. (Bugwesa Z. Katale, 2020)

#### **2.4 Pattern of the commonly used antibiotic in treating bacterial infections**

In a study conducted in Saudi Arabia, the antibiotics evaluated included imipenem, morepenem, ciprofloxacin and amikacin. *A. baumannii* isolates were highly resistant to amoxicillin/ clavulanic acid, ciprofloxacin, piperacillin, ceftazidime, trimethoprim/ sulfamethazole, gentamicin and amikacin. Data from northern region demonstrated 7.1% of isolates were resistant to most commonly used antibiotics (Ibrahim, 2019).

In a study conducted by (Hüsnü Baykal, 2022), the findings revealed the sensitivity rates for amikacin, tobramycin, gentamicin, imipenem, piperacillin and ciprofloxacin to 96.1%, 96.0%, 88.2%, 86.0%, 65.3% and 53.9% respectively.

*S. aureus* exhibited resistance to beta-lactam antibiotics. However much it was sensitive to ceftriaxone (73.3%), cefixime (66.7%) but highly resistant cefuroxime and ceftazidime (95.6%). Other resistant profile included amoxicillin/ clavulanic acid, gentamycin, cloxacillin and ciprofloxacin (Musa Y. Tula, 2016).

There was a high MRSA carriage in East Africa among patients and health care workers. In Kenya, the trend of MRSA susceptibility declined between 2014 to 2016, however much the MRSA were susceptible to tigecycline, tecicoplanin and vancomycin. They however recorded high resistance to gentamycin, erythromycin, levofloxacin and tetracycline. In Uganda, there was a high prevalence of carbapenem and third generation cephalosporins in patients in Mulago National referral hospital. The MRSA was fuelled by the pre-exposure to ampicillin and cotrimoxazole. High prevalence of third generation cephalosporin cefotaxime was found

among pregnant mothers with urinary tract infection at Muhimbili National Hospital in Tanzania. It further identified high resistance to MRSA isolates to kanamycin, gentamicin, ciprofloxacin and trimethoprim-sulphamethaxole. (Bugwesa Z. Katale, 2020).

## CHAPTER THREE

### MATERIALS AND METHODS

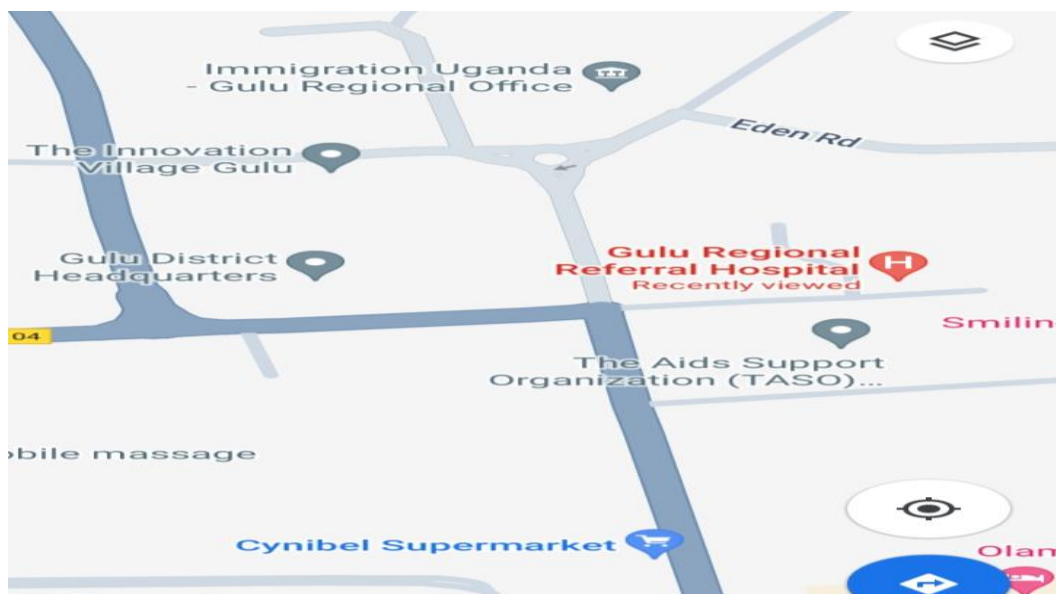
#### 3.1 Study Design

This was a cross-sectional study conducted between February, 2024 to April, 2024. It employed prospective method. The factors that were ascertained were; common pathogenic bacterial isolates, pattern of the commonly used antibiotics, and risk factors on a structured questionnaire. The cultured microorganisms were isolated, identified and drug susceptibility test performed to identify the susceptibility pattern. The findings were recorded later on analysed on STATA version 23.

#### 3.2 Study area

The study was conducted at Gulu Regional Referral Hospital. GRRH is located in Gulu district, approximately 340km north of the Uganda's capital city Kampala. GRRH is approximately 500 meters away from Gulu district headquarters from the east. The hospital serves and receives patients from all over the northern region being a referral hospital, which includes districts such as Amuru, Pader, Kitgum, Omoro amongst others.

Figure 1: A map showing the location of Gulu Regional Referral Hospital



### **3.3 Study variables**

#### **3.3.1 Dependant variable**

Antimicrobial resistance

#### **3.3.2 Independent variable**

Socio-demographic factors, risk factors

### **3.4 Study population**

The study comprised of out-patients receiving health care at GRRH between February 2024 to April 2024.

### **3.5 Sample size determination**

The sample size was determined using modified Kish and Leslie formula (Kish and Leslie 1965)

$$N = Z^2 pq / d^2$$

Where; N= Sample size

Z= Level of confidence at 95% (1.96)

d= maximum error 5% (0.05)

q= proportion of AMR (1 – p)

There was a paucity of data labelling the prevalence of AMR in Uganda, therefore, a prevalence from Saudi Arabia was be adapted basing on the basis of urban set at, hence 7.1% and considering as a recent data (Mutasim E, 2019)

Therefore, p = 0.071

$$N = 1.96^2 \times 0.071 \times (1-0.071) / 0.05^2$$

N= 101.3552; Therefore, a sample of 101 was drawn.

### **3.6 Inclusion and exclusion criteria**

#### **3.6.1 Inclusion criteria**

Only out-patients who presented at Gulu Regional Referral Hospital between February 2024 to April 2024 with a diagnostic need for culture and sensitivity and consented to the study.

#### **3.6.2 Exclusion criteria**

All in-patients and out-patients who presented at the time outside February 2024 to April 2024 at Gulu Regional Referral Hospital, those with no diagnostic need for culture and sensitivity and those that did not consent to the study.

### **3.7 Sampling technique and procedure**

The study employed nonprobability convenience sampling technique due to related advantages that ascertains a lot of information that could otherwise describe the impact of the finding on the population and its ability of being time and cost effective. Biasness that arose due to the sampling technique was reduced by sampling on different days and time and diversifying data collection by recruiting as many respondents as possible.

All patients who presented for diagnostic need of culture and sensitivity at GRRH were identified according to the inclusion/ exclusion criteria protocol. Consent was sought and upon consenting, they were enrolled in the study, completed a structured questionnaire and samples collected aseptically for C/S.

### **3.8 Data collection tools and methods**

#### **3.8.1 Data collection tools**

- Pretested questionnaire
- Stationaries (Pens, papers, books)
- Computer (Laptop)
- Culture plates and biochemical tubes

- Antimicrobial discs

### **3.8.2 Data collection method**

#### **3.8.2.1 Ascertaining the associated risk factors**

The demographics and associated socio-economic and environmental factors were captured in a structured questionnaire. The questionnaire was made into two sections; section A that ascertained the socio-demographic factors and section B that ascertained the associated risk factors such as comorbidities, Hospital effects and antibiotics misuse.

#### **3.8.2.2 Identifying the common pathogenic bacteria**

The obtained samples were cultured on MacConkey agar and Chocolate agar for a period of 18 to 24 hours at 37°C. Bacterial isolates were identified based on the colony morphological appearance, gram stain and biochemical tests. The used biochemicals were Simmons Citrate agar, Indole and motility agar, Triple sugar ion agar, coagulase and catalase test. The results were recorded and sensitivity set.

#### **3.8.2.3 Determining the pattern of commonly used antibiotics**

The overnight colonies were emulsified in sterile physiological saline and a suspension made in correlation to 0.5 McFarland as per the CLSI standard. An inoculum by surface spreading was done on mueller hinton agar (MHA) for none fastidious organisms and Blood agar for fastidious organisms. The measure of zone of inhibition diameter was measured after 18 to 24 hours as per the CLSI standard and results reported as per table 1.

The antibiotic discs used included Ciprofloxacin 25 µg (CIP), Amoxicillin- clavulanic acid 30µg (AMC), Nalidixic acid 30 µg (NA), Ampicillin 10µg (AMP), Chloramphenicol 30µg (C), Gentamycin 10µg (CN), Imipenem 10µg (IPM), Tetracycline 30µg (TE), Cefuroxime 30µg (CXM), Sulfamethaxole-Trimethoprim 25µg (SXT), Amikacin 30µg (AK), Ceftriaxone 30µg

(CRO), Vancomycin 30µg (VA), Nitrofurantoin 300µg (F), Azithromycin 15µg (AZM), Doxycycline 30µg (DO), Penicillin 10µg (P), Erythromycin 15µg (E) and Streptomycin 10µg (S). These drugs were selected for this study because they are the most commonly used in the treatment of bacterial infections.

Table 1: A table showing how to interpret zone of inhibition for the antibiotics (CLSI, 2020)

<b>Drug</b>	<b>Resistant</b>	<b>Intermediate</b>	<b>Susceptible</b>
Ciprofloxacin 25 µg (CIP)	≤20	21 to 30	≥ 31
Amoxicillin clavulanic acid 30µg (AMC)	≤13	14 to 17	≥18
Nalidixic acid 30 µg (NA)	≤13	14 to 18	≥19
Ampicillin 10µg (AMP)	≤13	14 to 16	≥17
Chloramphenicol 30µg (C)	≤12	13 to 16	≥17
Gentamycin 10µg (CN)	≤12	13 to 14	≥15
Imipenem 10µg (IPM)	≤19	20 to 22	≥23
Tetracycline 30µg (TE)	≤11	12 to 14	≥15
Cefuroxime 30µg (CXM)	≤14	15 to 22	≥23
Sulfamethaxole-Trimethoprim 25µg (SXT)	≤10	11 to 15	≥16
Amikacin 30µg (AK)	≤14	15 to 16	≥17
Ceftriaxone 30µg (CRO)	≤22	23 to 25	≥26
Vancomycin 30µg (VA)	≤14	15 to 16	≥17
Nitrofurantoin 300µg (F)	≤14	15 to 16	≥17
Azithromycin 15µg (AZM)	≤12	11 to 13	≥13
Doxycycline 30µg (DO)	≤10	14 to 17	≥14
Penicillin 10µg (P)	≤13	14 to 22	≥18
Erythromycin 15µg (E)	≤13	12 to 14	≥23
Streptomycin 10µg (S)	≤11		≥15

The obtained data was captured in a Microsoft Excel worksheet that was later on analysed.

### **3.8.3 Data analysis and presentation**

The primary data was recorded in the lab results register. The data was then entered in Microsoft Excel office package and counter verified with the primary data for any transcriptional error. It was finally analysed on STATA version 23.

The findings are presented in form of tables, charts and graphs.

### **3.9 Quality control**

The integrity of the data was maintained by working along experienced technologists/ technicians in setting up culture and sensitivity. The data captured in the Microsoft Excel was counter verified with the primary source data before saving and analysis. Piloting was done on the questionnaire to ensure it captures all the relevant information with ease of use by the respondents. Filling the questionnaire was under the guidance of trained research assistants.

A copy of translated questionnaire was put in place for ease of clarity to the respondents who would find it familiar.

### **3.10 Ethical consideration**

An introductory letter from the Dean SBLs was presented to the Institutional Review Board, GRRH with a copy of the proposal that was used to sought permission to conduct the research within the institution. Upon approval, the research activity commenced.

There was strict adherence to privacy and confidentiality by using study assigned numbers as opposed to anything that identifies the patients such as names and only authorized personnel with access to the data by using a password locked computer where the data shall be store.

Only participants who consented or assent to the study were included.

### **3.11 Study limitation and mitigation measures**

Participants had the urge of financial compensation that subjected financial constraint, as it was not part of the budget. This was mitigated by explaining to the participants clearly during the time of recruitment (consent) so that they would not expect any financial compensation, hence avoiding conflicts during data collection.

There was a level illiteracy that made it harder for some participants to respond to the questionnaire. This was mitigated by explaining to the questionnaire and aiding in filling in their responses. Further, a Luo-translated version was made available for those with comfort in the Luo language.

### **3.12 Dissemination of findings**

Upon completion of data collection and analysis, the study findings were presented to GRRH research board and a copy submitted to Makerere University, School of Biosecurity Biotechnical and Laboratory Sciences, department of research.

## CHAPTER FOUR

### RESULTS

#### 4.1 Sociodemographic factors of the patients

A total of 101 patients who presented with bacterial growth were recruited in the study comprising of 55 (54.46%) females and 46 (45.54%) males. The majority of the patients were in the age group of 15 to 24 (40.59%), followed by 25 to 34 (22.77%),  $\geq 65$  (12.87%), 45 to 54 (9.09%), 35 to 44 (8.92%),  $\leq 14$  (3.96%) and the least being 55 to 64 (0.99%). The mean age was 33.62 ( $\pm 19.13$ ). Secondary level of education was the most predominant level at 52 (51.49%), followed by primary at 32 (31.68%) and tertiary at 15 (14.85%) with the least being 2 (1.98%), who never had any formal education. The majority of the population were peasant farmers at 40 (39.60%), followed by students 29 (28.71%), market vendors at 26 (25.74%) and teachers who were 4 (3.96%) and the least being 2 (1.98%) children who had no formal occupation type. The family size that was most represented was the range of 5-7 (51.49%) followed by  $\leq 4$  at 33 (32.67%) and the least being  $\geq 8$  at 16 (15.84%)

*Table 2: Sociodemographic of the patients who had bacterial infection*

Variable	Frequency (n=101) (Percentage %)
<b>Sex</b>	
Female	55 (44.6)
Male	46 (55.4)
<b>Age group (years)</b>	
$\leq 14$	4 (3.96)
15-24	41 (40.59)
25-34	23 (22.77)
35-44	9 (8.92)

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45-54	10 (9.90)
55-64	1 (0.99)
≥65	13 (12.87)
<b>Education level</b>	
Primary	32 (31.68)
Secondary	52 (51.49)
Tertiary	15 (14.85)
None	2 (1.98)
<b>Occupation</b>	
Peasant farmer	40 (39.60)
Market Vendor	26 (25.74)
Student	29 (28.71)
Teacher	4 (3.96)
None	2 (1.98)
<b>Family size</b>	
≤4	33 (32.67)
5-7	52 (51.49)
≥8	16 (15.84)

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## 4.2 Associated risk factors

Out of the 101 patients that were recruited in the study, 95 (94.06%) never had diabetes with only 6 (5.94%) who had Diabetes, none had lung cancer nor bronchitis, 86 (94.06%) were HIV negative, 6(5.94%) were HIV positive and 9 (8.91%) did not know their HIV status. 89 (88.12%) never had the history of ICU hospitalization with 12 (11.88%) who had history of ICU hospitalization, 86 (85.15%) never had a history of prolonged hospitalization with 15 (8.91%) who recorded history of prolonged hospitalization. 92 (91.09%) never had a history of invasive procedures with only 9 (8.91%) who had a history of having received an invasive procedure. 54 (53.47%) were purchasing drugs over the counter and 47 (46.53%) mentioned they were purchasing drugs over the counter. 53 (52.48%) did not practiced self-medication with 48 (47.52%) who practiced self-medication. 78 (77.23%) did not know antibiotics were used in the food/ animal production they consume, 17 (16.83%) were not using antibiotics in food or animal production and 6 (5.94%) were using antibiotics in food or animal production. 52 (51.49%) were not using antibiotics to treat common cold and flu whereas 49 (48.51%) did.

*Table 3: Associated risk factors to AMR among the patients who had a bacterial isolate*

Variable	Frequency (n=101)	Percent (%)
<b>History of Diabetes</b>		
No	95	94.06
Yes	6	5.94
<b>Lung Cancer</b>		
No	101	100.00
Yes	00	0.00
<b>Bronchitis</b>		
No	101	100.00

Yes	00	0.00
<b>HIV Status</b>		
Negative	86	94.06
Positive	6	5.94
Unknown	9	8.91
<b>ICU Hospitalization</b>		
No	89	88.12
Yes	12	11.88
<b>Prolonged Hospitalization</b>		
No	86	85.15
Yes	15	14.85
<b>Invasive procedures</b>		
No	92	91.09
Yes	9	8.91
<b>Purchasing drugs over the counter</b>		
Yes	54	53.47
No	47	46.53
<b>Self-medication</b>		
No	53	52.48
Yes	48	47.52
<b>Antibiotics use in food/ animal production</b>		

Unknown	78	77.23
No	17	16.83
Yes	6	5.94

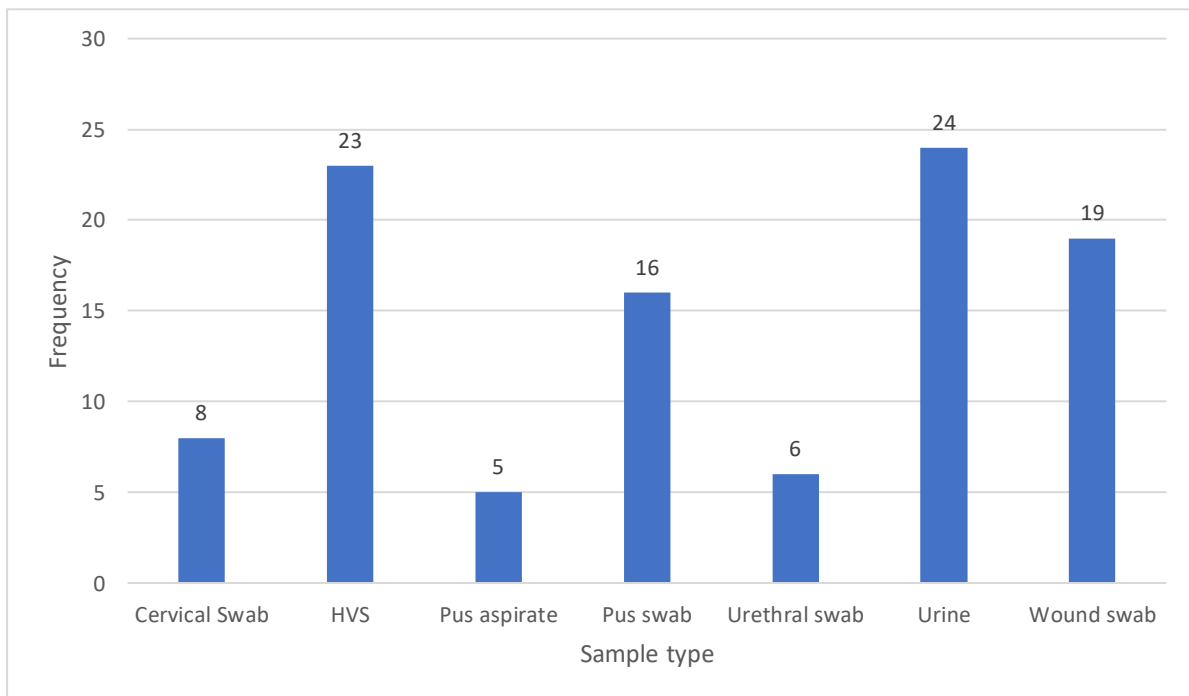
**Antibiotics to treatment of cold and flu**

No	52	51.49
Yes	49	48.51

**4.3 The common pathogenic bacteria isolated**

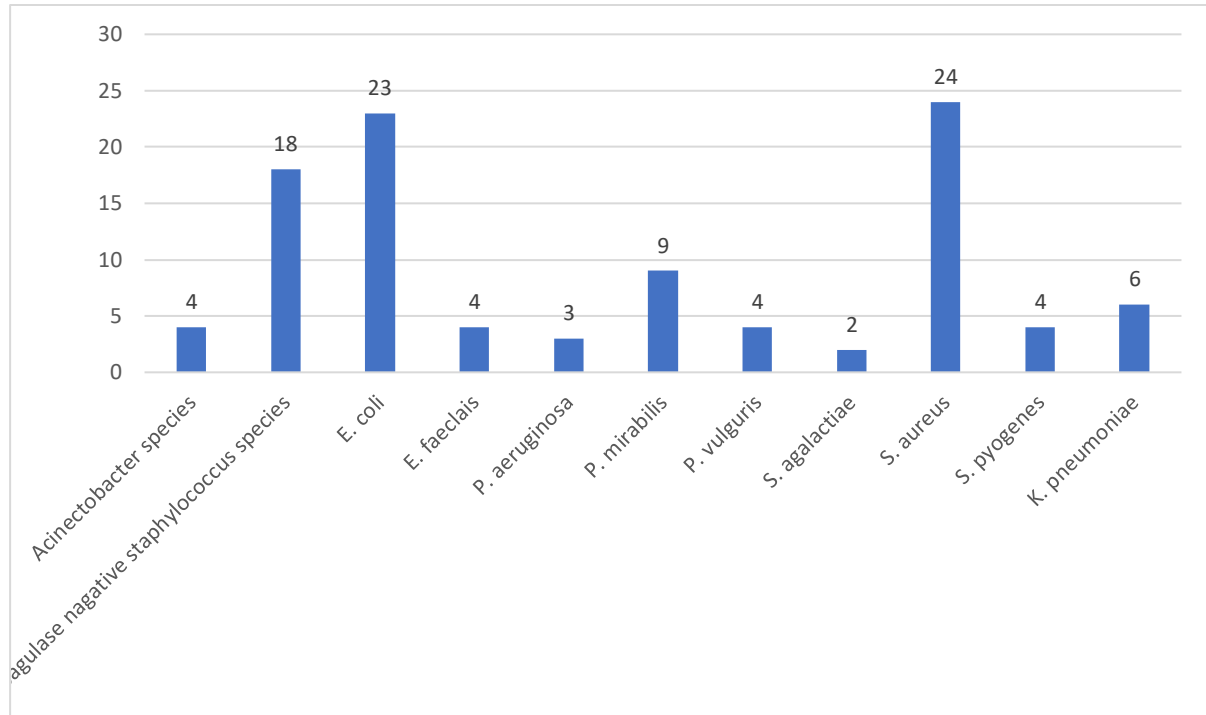
There were 7 different types of samples that were received. Urine was the most predominant received type of sample 24 (23.76%), followed by HVS 23 (22.77%), wound swab 19 (18.81%), pus swab 16 (15.84%), cervical swab 8 (7.92%), urethral swab 6 (5.94%) and the least were pus aspirate 5 (4.95%)

*Figure 2: Showing the various samples that were obtained from the patients for analysis*



There were 11 bacteria species isolated. *Staphylococcus aureus* was the most predominant bacteria at 24 (23.76%), followed by *E. coli* 23 (23.77%), Coagulase negative staphylococcus *aureus* 18 (17.82%), *P. mirabilis* 9 (8.91), followed by *K. pneumoniae* 6 (5.94%). *Acinetobacter* species, *E. faecalis*, *P. vulgaris* and *S. pyogenes* were all 4 (3.96%) respectively, *P. aeruginosa* (2.97%). The least was *S. agalactiae* 2 (1.98%)

Figure 3: Showing the isolated bacteria from the patients



#### 4.4 Antibiotic resistance profile of the isolates to the commonly used drugs

The highest antibiotic resistance was recorded in Streptomycin at 100% followed by Nalidixic acid (NA) at 95.24%, Ampicillin (AMP) at 94.87%, Cefuroxime (CXM) at 83.72%. Relative resistance was observed with Ceftriaxone (CRO) at 79.25%, Ciprofloxacin (CIP) at 71.43%, Azithromycin (AZM) at 67.74% and Amoxicillin-clavulanic acid (AMC) 62.75%. An intermediate resistance was observed with Chloramphenicol (C) 50.68%, Vancomycin (VA) and Erythromycin (E) at 50%, Sulfamethaxole-Trimethoprim (SXT) at 47.72%, Tetracycline (TE) at 42.31%, Penicillin (P) at 42.22%, Gentamycin (CN) at 40.85% and Doxycycline (DO) 34.88%. The least resistance was observed with Amikacin (AK) and Imipenem (IPM) at 0.00% followed by Nitrofurantoin (F) at 10.53%

Table 4: Pattern of the commonly used antibiotics

Variables	Frequency (N)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
<b>Antibiotic discs</b>				
Ciprofloxacin (CIP)	91	65 (71.43)	18 (19.78)	8 (8.79)
Amoxicillin-clavulanic acid (AMC)	51	32 (62.75)	9 (17.65)	10 (19.61)
Nalidixic acid (NA)	42	40 (95.24)	0 (0.00)	2 (4.76)
Ampicillin (AMP)	39	37 (94.87)	0 (0.00)	2 (5.18)
Chloramphenicol (C)	73	37 (50.68)	0 (0.00)	36 (49.32)
Gentamycin (CN)	71	29 (40.85)	7 (9.86)	35 (49.29)
Imipenem (IPM)	40	0 (0.00)	6 (15.00)	34 (85.00)
Tetracycline (TE)	52	22 (42.31)	4 (7.69)	26 (50.00)
Cefuroxime (CXM)	43	36 (83.72)	4 (9.30)	3 (6.98)
Sulfamethaxole-Trimethoprim (SXT)	44	21 (47.72)	6 (13.64)	17 (38.64)
Amikacin (AK)	43	0 (0.00)	4 (9.31)	39 (90.69)
Ceftriaxone (CRO)	53	42 (79.25)	2 (3.77)	9 (16.98)
Vancomycin (VA)	4	2 (50.00)	0 (0.00)	2 (50.00)
Nitrofurantoin (F)	19	2 (10.53)	0 (0.00)	17 (89.47)
Azithromycin (AZM)	31	21 (67.74)	0 (0.00)	10 (32.26)
Doxycycline (DO)	43	15 (34.88)	4 (9.31)	24 (55.81)
Penicillin (P)	45	19 (42.22)	2 (4.45)	24 (53.33)
Erythromycin (E)	42	21 (50.00)	9 (21.43)	12 (28.57)
Streptomycin (S)	4	4 (100.00)	N/A	0 (0.00)

## CHAPTER FIVE

### DISCUSSION

Antibiotic resistance poses a profound threat to human health with associated increased mortality rates and the odds ratio of readmission for resistant infections is as high as 1.492 (49.2%) (Poudel, A. N, 2023). It has become empirical to treat bacterial infections without proper identification and susceptibility testing to guide the treatment due to the increase drug resistance. This study therefore analyzed 101 samples (100% respondent rate) while aiming at determining the antimicrobial resistance profile of isolates among out-patients receiving health care services at Gulu-Regional Referral Hospital, Gulu Uganda.

The study findings indicated a lack of significant correlation between antibiotic resistance and various risk factors such as diabetes, lung cancer, HIV positive status, history of ICU hospitalization, prolonged hospitalization, and history of invasive procedures. Surprisingly, this contradicts previous studies where different risk factors were identified. For example, Baykal H (2022) highlighted a higher risk in patients with a history of invasive procedures, Bugwesa Z. Katale (2020) associated resistance with comorbidities, and Mutesi E (2019) linked resistance with prolonged hospitalization. The study revealed an unclear link between antibiotic use in food and animal production and antimicrobial resistance. Most patients (77.23%) were unaware of antibiotic use in these sectors, while only 5.94% were directly aware. This conflicts with previous studies such as Chowdhury, S. (2021), FAO (2024), and Obiebe, T. (2023) which showed a strong association between antimicrobial resistance and the use of antibiotics in food production. On the other hand, a significant correlation was found between purchasing drugs over the counter and self-medication. This finding is consistent with earlier studies by Zeb, S. (2022) and Sachdev, C. (2022) which linked high resistance to self-medication, as well as Darko, E. (2020) who identified high resistance due to purchasing drugs over the counter. RFI (2022) and Nakakande, J. (2023) also reported a high level of resistance associated with purchasing drugs from over the counter and self-medication. The limited correlation between high antimicrobial resistance and common risk factors, as demonstrated in past research, may be attributed to the compelling evidence that animal-derived food contains substantial amounts of resistant bacteria and their resistance genes. Consumers are exposed to these bacteria through contact with or consumption of animal products (Bonnie M, 2011). Additionally, the prevalence of peasant farmers among the majority of the patients could greatly contribute to their heightened exposure to poultry faeces as seen in a study (Ruth A. A,

2020), that reported a high MARI with a mean distribution over 0.73. This finding suggests that poultry faeces serves as a significant carrier and transmitter of antibiotic-resistant genes to humans.

The highest recoveries of the isolates were from urine followed by swabs which is consistent to previous findings in a similar study in Nigeria where majority of the isolates were recovered in urine sample and followed by swabs (Tula M Y, 2016).

The isolated bacteria agree to the previously isolated resistant gene in East Africa as according to Bugwesa Z. K, (2020). The most commonly recovered bacterial species in this study were *S. aureus* and *E. coli*, followed by coagulase-negative staphylococci. This finding is consistent with other studies, such as the one conducted by Claujens Chastel, (2020) in Congo and a meta-analysis in LMIC by Nji, E. (2021), revealed a high recovery of *E. coli*. Similar study, Kayili, E. (2020) and Garoy, E. Y. (2019) revealed a higher association with *S. aureus* in Eritrea. Debora B. G (2023) revealed a high prevalence of coagulase-negative staphylococci. This high prevalence is likely due to the widespread presence of resistant species of *S. aureus* and *E. coli* as shown by Nji, E. (2021) and Kayili, E. (2020). *P. mirabilis*, *K. pneumoniae*, *Acinetobacter* spp., *E. faecalis*, *P. vulgaris*, *S. pyogenes*, and *P. aeruginosa* were recovered in moderate numbers. However, these findings are in contrast to previous studies, such as Açma, A. (2021), which showed a higher prevalence of *P. mirabilis*, *K. pneumoniae*, *Acinetobacter* spp. and *P. aeruginosa*. This discrepancy in the findings could be attributed to differences in the study populations, as the focus of this study was on outpatients, unlike studies conducted among inpatients. For example, a study conducted in western Uganda by Obakiro, S. B. (2021) on inpatients isolated mostly *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. The least isolated organism was *S. agalactiae*, which may be due to its susceptibility to most antibiotics. The high number of resistant isolates among outpatients could be attributed to the wide spread in the community in recent years, as opposed to the past when it was largely confined to hospital settings, as shown in a study conducted by Ak Narayan Poudel, (2023).

The study findings indicated a significant level of resistance to various antibiotic discs. Streptomycin, Nalidixic acid, Ampicillin, and Cefuroxime showed particularly high levels of resistance, which is consistent with earlier studies by Keikhaie, K. R (2018), J Ayatollahi (2013), and Alam, M. Z. (2013). The increased resistance may be attributed to the growing misuse of these antibiotics, as supported by previous study by Joseph A. A., (2017). Intermediate resistance was observed with Ceftriaxone, Ciprofloxacin, Azithromycin,

Amoxicillin clavulanic acid, Chloramphenicol, Vancomycin and Erythromycin, Sulfamethaxole-Trimethoprim, Tetracycline, Penicillin, Gentamycin and Doxycycline, aligning with findings from Berhe, D. F. (2021), Mahmudul Hassan, M. (2021), and Sokolović, D. (2023) who showed resistance to similar antibiotics. However, these results contrast with studies by Gharpure, R. (2021) and Assegu Fenta, D. (2020) which indicated better susceptibility to those drugs. The lowest level of resistance was seen with Amikacin and Imipenem, supporting previous findings by Naqid, I. A. (2020) showing complete susceptibility to these antibiotics. This could be attributed to strict control measures preventing their misuse and abuse as recommended by WHO (2023). However, these results contradict a study by Farhan S. M., (2021) indicating a high level of resistance to Amikacin and Imipenem, potentially due to the specific isolation of *P. aeruginosa* in their study, which was among the least isolated in this study.

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 CONCLUSION

Out of the 101 patients, females 55 (54.46%) had a higher level of antibiotic resistance. The most affected age group was 15 to 24 (40.59%) with the mean age of 33.62 ( $\pm$ 19.13). Secondary level of education was the most predominant level at 52 (51.49%), higher antibiotic resistance was amongst peasant farmers at 40 (39.60%) than other occupation. Higher antibiotic resistance was amongst family size of 5-7 (51.49%). Urine was the most received type of sample 24 (23.76%), followed by HVS 23 (22.77%), wound swab 19. Purchasing the drugs over the counter and self-medication were the most direct associated risk factor in this study with no considerable association to other factors such as presence of diabetes, lung cancer, HIV positive status, history of ICU hospitalization and prolonged hospitalization and history of invasive procedure. *S. aureus* was the most predominant bacteria at 24 (23.76%), followed by *E. coli* at 23 (23.77%), Coagulase negative staphylococcus 18 (17.82%), with the least being *S. agalactiae* at 2 (1.98%). The highest antibiotic resistant was recorded in Streptomycin (S) at 100% followed by Nalidixic acid (NA) at 95.24%, Ampicillin (AMP) at 94.87%, Cefuroxime (CXM) at 83.72% and a complete susceptibility to Amikacin and Imipenem.

#### 6.2 RECOMMENDATIONS

Treatment of choice should be Amikacin and Imipenem due to less resistance meanwhile refraining from the use of antibiotics such as streptomycin, nalidixic acid, ampicillin and cefuroxime due to high resistance

Clinicians/ prescribers should base on laboratory guided prescriptions avoiding empirical treatment.

There is need for urgent sensitization of the population about self-medication and antibiotic misuse.

Since there is a widespread of resistant gene bacteria, the public should be sensitized to always to maintain high level of hygiene including on their wounds and other mucous membranes, approach hospital for timely diagnosis and intervention.

## REFERENCE

- Açma, A., Williams, A., Repetto, E., Cabral, S., Sunyoto, T., Woolley, S. C., & Mahama, G. (2021). Prevalence of MDR bacteria in an acute trauma hospital in Port-au-Prince, Haiti: a retrospective analysis from 2012 to 2018. *JAC-Antimicrobial Resistance*, 3(3). <https://doi.org/10.1093/jacamr/dlab140>
- Alam, M. Z., Aqil, F., Ahmad, I., & Ahmad, S. (2013). Incidence and transferability of antibiotic resistance in the enteric bacteria isolated from hospital wastewater. *Brazilian Journal of Microbiology*, 44(3), 799–806. <https://doi.org/10.1590/s1517-83822013000300021>
- Alekshun MN, L. S. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 1037–50. doi:doi: 10.1016/j.cell.2007.03.004
- Bugwesa Z. Katale, G. M. (2020). Genetic diversity and risk factors for the transmission of antimicrobial resistance across human, animals and environmental compartments in East Africa: a review. *Antimicrobial Resistance and Infection Control*, 1-20. Retrieved from <https://doi.org/10.1186/s13756-020-00786-7>
- Chowdhury, S., Ghosh, S., Aleem, M. A., Parveen, S., Islam, Md. A., Rashid, Md. M., Akhtar, Z., & Chowdhury, F. (2021). Antibiotic Usage and Resistance in Food Animal Production: What Have We Learned from Bangladesh? *Antibiotics*, 10(9), 1032. <https://doi.org/10.3390/antibiotics10091032>
- Claujens Chastel Mfoutou Mapanguy, Ayodele Adedoja, Leissie Graciela Vittickat Kecka, Jeannhey Christevy Vouvoungui, Etienne Nguimbi, Thirumalaisamy P. Velavan, Francine Ntoumi (22 September 2020). High prevalence of antibiotic-resistant *Escherichia coli* in Congolese students. (2021). *International Journal of Infectious Diseases*, 103, 119–123. <https://doi.org/10.1016/j.ijid.2020.09.1441>
- Dadgostar, P. (2019, Oct 21). Antimicrobial Resistance: Implications and. doi:DOI: 10.2147/IDR.S234610
- Darko, E., & Owusu-Ofori, A. (2020). Antimicrobial resistance and self-medication: A survey among first-year health students at a tertiary institution in Ghana. *International Journal of Infectious Diseases*, 101, 43. <https://doi.org/10.1016/j.ijid.2020.09.145>
- Debora Brito Goulart. (2023). Pathogenicity and Antimicrobial Resistance in Coagulase-Negative Staphylococci. *Journal of Biosciences and Medicines*, 11(05), 9–29. <https://doi.org/10.4236/jbm.2023.115002>

- FAO (2024). *Animal production / Antimicrobial Resistance / Food and Agriculture Organization of the United Nations*. (n.d.). [Www.fao.org](http://www.fao.org).  
<https://www.fao.org/antimicrobial-resistance/key-sectors/animal-production/en/>
- Farhan, S. M., Raafat, M., Abourehab, M. A. S., Abd El-Baky, R. M., Abdalla, S., EL-Gendy, A. O., & Azmy, A. F. (2021). Effect of Imipenem and Amikacin Combination against Multi-Drug Resistant *Pseudomonas aeruginosa*. *Antibiotics*, *10*(11), 1429. <https://doi.org/10.3390/antibiotics10111429>
- Garoy, E. Y., Gebreab, Y. B., Achila, O. O., Tekeste, D. G., Kesete, R., Ghirmay, R., Kiflay, R., & Tesfu, T. (2019). Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients—A Multicenter Study in Asmara, Eritrea. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2019*, 1–9. <https://doi.org/10.1155/2019/8321834>
- Hüsni Baykal, D. Ç. (2022, November 10). Clinical features, risk factors, and antimicrobial resistance of *pseudomonas putida* isolates. *Medicine*, *101*:48(e32145)., 1-6.
- Ibrahim, M. E. (2019). Prevalence of *Acinetobacter baumannii* in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance . *Clin Microbiol Antimicrob*, 1-12. Retrieved from <https://doi.org/10.1186/s12941-018-0301-x>
- IHME. (2023 , Feb 16). The burden of antimicrobial resistance (AMR) in Uganda. *Institute for Health Metrics and Evaluation*, 1-3.
- J Ayatollahi, Shahcheraghi, S.H., R Akhondi and SS Soluti (2013). Antibiotic Resistance Patterns of *Escherichia coli* Isolated from Children in Shahid Sadoughi Hospital of Yazd. *PubMed*.
- Kayili, E., & Sanlibaba, P. (2020). Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *International Journal of Food Properties*, *23*(1), 1441–1451. <https://doi.org/10.1080/10942912.2020.1814323>
- Keikhaie, K. R., Moshtaghi, F., Asadi, M. S., Nejad, S. S., & Bagheri, G. (2018). Antimicrobial Drug Resistance in *Escherichia coli* from Humans, and Identification of Carbapenemase-Producing *E. coli* in the City of Zabol, Iran. *International Journal of Infection*, *5*(3). <https://doi.org/10.5812/iji.62552>

- Levy, B. M. (2011, Oct). Food Animals and Antimicrobials: Impacts on Human Health. *Clinical microbiology reviews*, Vol. 24, No. 4. doi:doi:10.1128/CMR.00002-1
- Markus Huemer, S. M. (2020, November 2). Antibiotic resistance and persistence— Implications for human health and treatment perspectives. *Review, Reports (2020) 21: e51034*, 1-24. doi:10.15252/embr.202051034
- MOH. (2022). *Antimicrobial Resistance National Action Plan 2018-2023*.
- Musa Y. Tula, A. V. (2016). Antimicrobial susceptibility pattern and plasmid-mediated antibacterial resistance in Staphylococcus aureus and coagulase-negative staphylococci. *Highland Med Res J(16(2):)*, 80-86.
- Negar Narimisa, Razavi, S., & Faramarz Masjedian Jazi. (2024). Prevalence of antibiotic resistance in Salmonella Typhimurium isolates originating from Iran: a systematic review and meta-analysis. *Frontiers in Veterinary Science, 11*. <https://doi.org/10.3389/fvets.2024.1388790>
- Naghavi, M. (2022, Feb 22). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Vol 399*, pp. 630-655. Retrieved from [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Nji, E., Kazibwe, J., Hambridge, T., Joko, C. A., Larbi, A. A., Dampthey, L. A. O., Nkansa-Gyamfi, N. A., Stålsby Lundborg, C., & Lien, L. T. Q. (2021). High prevalence of antibiotic resistance in commensal Escherichia coli from healthy human sources in community settings. *Scientific Reports, 11(1)*, 3372. <https://doi.org/10.1038/s41598-021-82693-4>
- Nakakande, J., Taremwa, I. M., Nanyingi, M., & Mugambe, S. (2023). The Utility of Internet-Enabled Antibiotic Self-Medication and Its Associated Factors Among Patients Attending Private Clinics in Kawempe Division in Kampala Capital City, Uganda: Community-Based Cross-Sectional Study. *Drug, Healthcare and Patient Safety, 15*, 85–91. <https://doi.org/10.2147/DHPS.S405072>
- Naqid, I. A., Balatay, A. A., Hussein, N. R., Saeed, K. A., Ahmed, H. A., & Yousif, S. H. (2020). Antibiotic Susceptibility Pattern of Escherichia coli Isolated from Various Clinical Samples in Duhok City, Kurdistan Region of Iraq. *International Journal of Infection, 7(3)*. <https://doi.org/10.5812/iji.103740>

- Obakiro, S. B., Kiyimba, K., Paasi, G., Napyo, A., Anthierens, S., Waako, P., Royen, P. V., Iramiot, J. S., Goossens, H., & Kostyanev, T. (2021). Prevalence of antibiotic-resistant bacteria among patients in two tertiary hospitals in Eastern Uganda. *Journal of Global Antimicrobial Resistance*, 25, 82–86. <https://doi.org/10.1016/j.jgar.2021.02.021>
- Obiebe, T., Williams Omotola Tanimowo, Kayode Olayinka Afolabi, Iqbal Kabir Jahid, & Rine Christopher Reuben. (2023). Antimicrobial use and resistance in food animal production: food safety and associated concerns in Sub-Saharan Africa. *International Microbiology*. <https://doi.org/10.1007/s10123-023-00462-x>
- Osundiya OO, O. R. (2013, September). Multiple antibiotic resistance (MAR) indices in Pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital. *African journal of clinical and experimental microbiology*, Vol14 No.3, 164-168. Retrieved from <http://www.ajol.info/journals/ajcem>
- Poudel, A. N., Zhu, S., Cooper, N., Little, P., Tarrant, C., Hickman, M., & Yao, G. (2023). The economic burden of antibiotic resistance: A systematic review and meta-analysis. *PLOS ONE*, 18(5), e0285170. <https://doi.org/10.1371/journal.pone.0285170>
- Puca, V., Marulli, R. Z., Grande, R., Vitale, I., Niro, A., Molinaro, G., Prezioso, S., Muraro, R., & Di Giovanni, P. (2021). Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. *Antibiotics*, 10(10), 1162. <https://doi.org/10.3390/antibiotics10101162>
- Qi Chen, D. L. (2021, May 21). Risk factors for antibiotic resistance development in healthcare settings in China: a systematic review. *Epidemiology and Infection*, 1–10. Retrieved from <https://doi.org/10.1017/S0950268821001254>
- Raminder Sandhu, S. D. (2016). Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of Acinetobacter species among inpatients. *Indian J Microbiol*, 299-304.
- RFI (2022). *Silent epidemic as self-medicating Ugandans cause antibiotic resistance*. (2022, April 17). RFI. <https://www.rfi.fr/en/africa/20220417-silent-epidemic-self-medicating-ugandans-cause-dangerous-antibiotic-resistance>
- Rosini R, N. S. (2020, June). Vaccines Against Antimicrobial Resistance. *Front. Immunol.*, 1-9. doi:doi: 10.3389/fimmu.2020.01048

Sachdev, C., Anjankar, A., & Agrawal, J. (2022). Self-Medication With Antibiotics: An Element Increasing Resistance. *Cureus, 14*(10). <https://doi.org/10.7759/cureus.30844>

WHO. (2021). Antimicrobial resistance in the African WHO region: a systematic literature review. 1-22.

Zeb, S., Mushtaq, M., Ahmad, M., Saleem, W., Rabaan, A. A., Naqvi, B. S. Z., Garout, M., Aljeldah, M., Al Shammari, B. R., Al Faraj, N. J., Al-Zaki, N. A., Al Marshood, M. J., Al Saffar, T. Y., Alsultan, K. A., Al-Ahmed, S. H., Alestad, J. H., Naveed, M., & Ahmed, N. (2022). Self-Medication as an Important Risk Factor for Antibiotic Resistance: A Multi-Institutional Survey among Students. *Antibiotics, 11*(7), 842. <https://doi.org/10.3390/antibiotics11070842>

## APPENDICIES

### **Appendix I: Consent form**

I Akena Stephen, a third-year student pursuing a Bachelor of Biomedical Laboratory Technology at Makerere University conducting a study entitled “Antimicrobial resistance profile of pathogenic isolates among out-patients receiving health care services at Gulu Regional Referral Hospital, Gulu – Uganda.”

### **Purpose**

The study is intended to assess the Antimicrobial resistance profile of isolates from patients receiving health care services at Gulu Regional Referral Hospital, Gulu district and the information obtained from the study will help to in addressing the burden of antibiotic resistance as one of the world latest health threats and providing possible mitigation measures.

### **Expectations**

Upon consenting to the study, your sample will be obtained aseptically once depending on the requisition by the clinicians. The samples will be analysed and a copy of the results conveyed to you within 72 hours (3 days).

### **Remuneration**

There will be no direct financial benefit at any moment.

However, you will be briefed on Antimicrobial resistance knowledge, spread, comorbidities and possible mitigation measures that should help improve your health hand the society.

### **Confidentiality and privacy**

All information concerning you shall be kept with utmost confidentiality and only be used for this research. Only authorised personnel will have access to your data.

Study number, not names shall be used to capture the information at all processes. Should you feel any of these obligations are breached or needs clarification, do not hesitate to contact the principle investigator by phone on 0776481344 or by Email: [khenakenacredentials@gmail.com](mailto:khenakenacredentials@gmail.com).

**Right to refuse or withdraw**

Your participation in the study is entirely voluntary and you are free to accept to take part or refuse.

**Consent**

I have read (been explained) and understood the consent form and voluntarily consent to be part of the study.

Sign/Thumbprint: .....

Date: .....

**Appendix II: Study Questionnaire**

Study number: .....

This questionnaire seeks to obtain the associated factors to antibiotic resistance. I urge you to answer it with most level of honesty for data accuracy.

**Section A (Socio-demographic factors)**

**Fill in by answering besides the question in this section.**

Sex: .....

Age: .....

Occupation: .....

Family size: .....

Current education level: .....

Annual income estimate: .....

Area of residence: .....

**Section B (Associated risk factors)**

**Put Y for Yes and N for N besides the question as your answer in this section.**

**i) Comorbidities**

Do you have any of the following disease condition(s)?

i. Diabetes mellitus:

ii. Lung cancer:

iii. Bronchiectasis:

HIV

**ii) Hospital effects**

Are you or have ever had?

i. Been hospitalized in ICU

ii. Prolonged hospitalization

iii. Invasive procedures e.g., surgery

**iii) Antibiotics misuse**

i. Been buying drugs from over the counter

ii. Practicing self-medication

iii. Use of antibiotics in food and animal production

iv. Treat common cold and every flu case with antibiotics

### Appendix III: Approval at GRRH

Hospital Director's 256- (0)774408225

REC Administration :256 (0) 772060564

E-mail: [guluhospital@gmail.com](mailto:guluhospital@gmail.com)

Website: <https://guluhospital.net>

In any correspondence on this matter quote:

**REC/2024/01/08**



THE REPUBLIC OF UGANDA

MINISTRY OF HEALTH

GULU REGIONAL REFERRAL HOSPITAL

P. O. Box 160,

Gulu, Uganda.

**Date: 29<sup>th</sup> / JAN / 2024**

### RESEARCH AND ETHICS COMMITTEE

AKENA STEPHEN

REG NO: 21/U/19609/EVE

MAKERERE UNIVERSITY

#### **RE: ANTIMICROBIAL RESISTANCE PROFILE OF PATHOGENIC ISOLATES AMONG PATIENTS RECEIVING HEALTH CARE SERVICES AT GULU REGIONAL REFERRAL HOSPITAL.**

This is to inform you that the Gulu Regional Referral Hospital, Research and Ethics Committee (GRRHREC) has approved your study documents for the above referenced research study. Note that this approval expires after one year; the current approval is therefore valid until Date: **29th/JAN/2025**. Continued approval is conditional upon your compliance with the following requirements:

1. No other consent forms, questionnaire and /or advertisement documents should be used.
2. Consent forms must be voluntarily signed by study subjects prior to commencement of data collection. Queries raised regarding consent too should be addressed.
3. Study bias aversion strategies should be employed as discussed to improve quality and reliability of findings.
4. All the protocol amendments and changes to other research documents must be re submitted to the COVAB-REC for approval and made known to the Gulu RRH REC.
5. Significant changes to the current protocol and all anticipated problems that may involve risks to study subjects or patient information or that may affect integrity of the research must be promptly reported to COVAB-REC and Gulu RRH REC.
6. Specification of support required from GRRH and inputs from the researcher (Research Assistants, Stationary etc.) to the Department.
7. Renewal of the study approval is upon feedback from the continuing research and will be done within 2 months prior to the expiry of the current protocol approval date.

You will be required to share the study findings with the GRRHREC and the responsible Departments to support our efforts to improve quality of patient care.

Yours faithfully

  
.....  
Dr Baifa Arwinyo  
OBO, Chair GRRHREC  
For Hospital Director-GRRH

**Appendix IV: Photos**



**A**



**B**

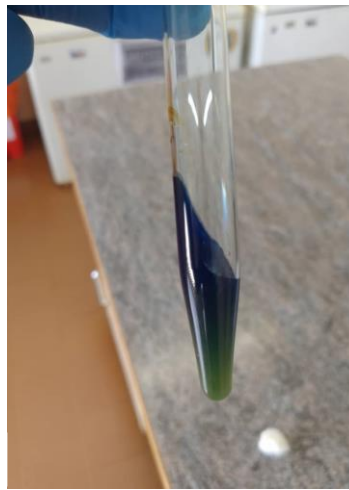


**C**

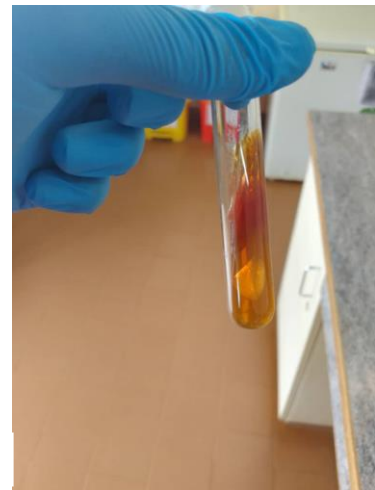
**A: MIC on a MHA B: A growth on chocolate agar C: A growth on MaCkonkey agar**



**A**



**B**



**C**

**A: An indole and Motility test B: A positive Citrate test C: A TSI agar**