MAKERERE UNIVERSITY

OCCURRENCE OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE IN WASTEWATER AND RECEIVING WATER BODIES, UGANDA

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DECLARATION

I **KATENDE GEORGE,** hereby do declare, that this is my own work and it has not been submitted to any organization or Institution. The work of others has been cited and referenced.

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DEDICATION

I dedicate this work to my beloved family, parents, relatives and friends for their love and support.

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LIST OF ABBREVIATIONS

WHO	World Health Organization
E. coli	*Escherichia coli*
K. pneumoniae	*Klebsiella pneumoniae*
Spp	Species
WWTPs	Wastewater Treatment Plants
AMR	Antimicrobial resistance
CFU/ml	Colony Forming units per milliliter
mg/l	Milligrams per liter
µm	Micrometer
Mm	Millimeter
µg	Microgram
UTI	Urinary Tract Infections
ATCC	American Type Culture Collection
CLSI	Clinical Laboratory Standards Institute
PCR	Polymerase Chain Reaction
NEMA	National Environment Management Authority
ESBL	Extended Spectrum Beta-lactamase

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ABSTRACT

Introduction: Antimicrobial resistance is taking center-stage in increasing morbidity and mortality especially in Low- and middle-income countries like Uganda. WHO drew a roadmap to increase antimicrobial resistance surveillance using the One-Health approach so as to attain information about this silent pandemic in an all-encompassing manner. This is because resistance determinants circulate within humans, animals and the environment. The environment acts as a reservoir for antibiotic resistance genes, these can be transferred through mechanisms such as conjugation, transformation and transduction to many other organisms hence encouraging antimicrobial resistance spread. However, information about the antimicrobial resistance state of the environment in Uganda is still lacking. Resistance to third generation cephalosporins is a key priority as they are among the most effective and safest antimicrobials. Therefore, this study set out to determine the presence of ESBL producing Enterobacteriaceae and carriage of ESBL genes blaCTX-M, blaTEM and blaSHV in wastewater and receiving water bodies. **Methodology:** This was a longitudinal study carried out between October 2022 and April 2023, from, Mbarara and Gulu city. Wastewater samples were collected from wastewater treatment plants (WWTPs) of Kizungu, Katete and Kakoba of Mbarara City, Gulu City wastewater treatment plant, hospital effluent of Mbarara Regional Referral Hospital and Gulu Regional Referral Hospital. Water samples of receiving waterbodies were collected from river Rwizi and Pece stream upstream and downstream treatment plants. Water samples were also picked from R. Aswa that does not receive wastewater directly from any treatment plant. Samples were on ChromESBL agar plates. All pink and blue colonies were subjected to VITEK automated identification system to confirm ESBL positive *E. coli* and *K. pneumoniae.* DNA was extracted from all positive isolates using the ZymoBiomics Kit and conventional PCR was done. **Results:** From Mbarara study site, 6 out of 7 wastewater samples grew *E. coli* and 1 *K. pneumoniae* isolate. All the 4 samples from R. Rwizi upstream the WWTP grew *E. coli* and 1 of these samples had both *E. coli* and *K. pneumoniae* isolated. For the 11 river water samples downstream WWTP, only 9 samples had *E. coli* and 3 these had both *E. coli* and *K. pneumoniae*. For samples from Gulu study site, 3 of the 5 wastewater samples grew *K. pneumoniae* and no *E. coli*. Both 2 stream water samples upstream WWTP had *K. pneumoniae* while only one of the 2 water samples downstream WWTP had *K. pneumoniae* and no *E. coli*. All the 4 samples from R. Aswa had no *E. coli* or *K. pneumoniae* isolated. All the 30 isolates (20 *E. coli* and 10 *K. pneumoniae*) carried the blaCTX-M gene while 22 of these also carried the blaTem gene and only 8 carried the blaSHV gene. **Conclusion:** This study found out that wastewater and receiving waterbodies contain antibiotic resistant bacteria in comparison to waterbodies that don't receive wastewater directly. **Recommendation:** The study recommends improved wastewater treatment that aims at disinfection before discharge. It also recommends further research on genomic relatedness of isolates to track the source in relation to clinical isolates.

CHAPTER ONE

1.0 Introduction

1.1 Background

Antimicrobial resistance (AMR) is a major public threat. Predictor models estimate that about 4.95 million people died globally in 2019 due to drug-resistant infections, with Africa having the highest mortality rate at 27.3 fatalities per 100,000 inhabitants (Murray et al., 2022). In Uganda, a report by Uganda National Institute of Public Health indicates a rise of resistance against commonly used antibiotics such as cephalosporins to about 70% (UNIPH, *bulletin March.2023*). However, with no intervention the situation is expected to get worse. WHO estimates that 10 million fatalities will occur globally each year due to drug resistant infections by 2050 (WHO, 2014).

International health institutions have set out to increase antimicrobial resistance surveillance so as measure the progress of the current interventions. Using the One Health approach, most surveillance data has been gathered from the human and animal samples, however, the environmental sector has been the least studied especially in Low- and Middle-Income Countries (LMICs) such as Uganda. Nevertheless, growing evidence indicates that the environment acts as a reservoir for antibiotic resistant bacteria and genes. For example, wastewater from homes, farms, industries and hospitals collects antibiotic residues and multi-drug resistant bacteria with hospital effluent reported to have concentrations up to 10 times than in other wastewater sources (Ngigi et al., 2019).

Currently, the few studies about wastewater contamination in Uganda have reported about the physico-chemical pollution of receiving waters. These have quantified parameters such as total solid suspension, heavy metals, dissolved oxygen, and electrical conductivity, (Atwebembeire et al., 2018). One study that quantified microbial contamination found out thermotolerant coliforms at 2.9×10^5 colony-forming units (CFU)/100ml and mean *Escherichia coli* of 9.9×10^4 CFU/100ml from water samples contaminated by wastewater at Nakivubo wetland area, Kampala (Fuhrimann et al., 2015). However, this study neither reported about phenotypic antimicrobial resistance patterns nor genomic characteristics of the isolated bacteria.

In ideal conditions, all wastewater should be channeled into treatment plants that neutralize antibiotic resistant bacteria and genes before being discharged into the environment. However, the treatment is not entirely effective. The excessive antibiotic residues and surviving antibiotic-resistant bacteria are channeled into receiving water bodies, where they may persist in the environment. The antibiotic resistant bacteria with virulence factors then contribute to disease

causation in human and animal life that communally utilize the contaminated water (Redha et al., 2022).

Beta-lactam drugs are commonly the drugs of choice against gram negative bacilli due to their affordability, safety and efficacy (Biondi, 2014). However, increasing resistance against these drugs is a major public health concern. Gram-negative bacilli produce beta-lactamases; enzymes that inactivate the antibiotic by digesting the beta-lactam ring. The genes encoding the beta-lactamases are majorly plasmid mediated hence can be spread to other bacteria of the same or different species within the environment (Millan, 2018).

In South Africa, diarrheagenic *E. coli* bearing the *daaE (*diffusely adherent *E. coli)* gene was isolated from wastewater effluent discharged into Kat and Brack rivers. it carried the erm gene responsible for erythromycin resistance and plasmid mediated mcr-1 gene that encodes colistin resistance (Igwaran et al., 2018). In the United States, the Enterobacteriaceae isolated were carbapenemase producing (Mathys et al., 2019a). In Uganda, extended spectrum beta-lactamases have been the most prevalently reported (Kateregga et al., 2015). These enzymes inactivate penicillin, monobactams and cephalosporins. Resistance to these drugs leaves no affordable treatment options.

Bacteria isolated from wastewater have been reported to be multidrug resistant (Akther et al., 2018), (Mukherjee et al., 2021). This is because of the sub-inhibitory concentrations of antibiotic residues in wastewater that keep exerting antibiotic pressure on these bacteria (Buriánková et al., 2021). *E. coli* and *K. pneumoniae*, are important indicator organisms for presence of pathogens in water and are commonly isolated from wastewater (Triggiano et al., 2020). They are able to share antibiotic resistance genes to potential pathogens. Additionally, those that possess virulence factors are able to cause disease to humans and animals (Rodríguez et al, 2017). These being discharged into river water that is used communally, poses a threat to people that utilize the water for irrigation, food preparation and other activities. Therefore, the goal of this study is to determine the occurrence of ESBL producing Enterobacteriaceae and the genetic determinants isolated from wastewater and its receiving waterbodies.

1.2 Statement of the problem

Waterbodies get contaminated with effluent from wastewater treatment plants and other anthropogenic activities that discharge virulent antibiotic resistant bacteria and genes. In Uganda, antimicrobial resistance surveillance has majorly focused on clinical sampling and is yet to adequately address one of the potential sources of antimicrobial resistance; environmental sampling (Kivumbi & Standley, 2021). The published literature reports about physico-chemical pollutants of rivers barely informing about microbial pollution (Atwebembeire et al., 2018). Water samples from

Nakivubo wetland that receives effluent from Bugoloobi-Nakivubo wastewater treatment plant contained *Escherichia coli* and *Salmonella* species (Fuhrimann et al., 2015). However, the isolates were not tested for carriage of antimicrobial resistance and virulence determinants. Therefore, this study is aimed to investigate the presence of ESBL producing *E. coli* and *K. pneumoniae* in water samples of River Rwizi and River Aswa in reference to WWTPs. Results of the study will provide antimicrobial resistance surveillance information that will guide policy on reducing spread of antimicrobial resistance determinants.

1.3 Objectives

1.3.1 General Objective

To determine the occurrence of Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae and the genetic determinants carried by these organisms in wastewater and their receiving water bodies of Uganda

1.3.2 Specific objectives

Objectives:

1. To determine the presence of ESBL producing Enterobacteriaceae in wastewater treatment plants and their receiving water bodies

2. To determine carriage of ESBL encoding genes, (blaCTX-M, blaTEM variants, blaSHV variants) by ESBL positive *E. coli* and *K. pneumoniae* isolates

1.3.3 Research Questions

1. What is the prevalence of ESBL producing Enterobacteriaceae isolated from wastewater in comparison to receiving water bodies

2. Do the ESBL positive isolates carry the commonest ESBL encoding genes (blaTEM, blaSHV, blaCTX-M) isolated before in the Ugandan setting?

1.4 Significance and Justification of the study

This study provides information about the antibiotic resistance patterns and carriage of antibiotic resistance genes by Enterobacteriaceae from wastewater and receiving water bodies in Uganda. The results of this study promote the first and fourth strategic objectives of the Antimicrobial Resistance National Action Plan (2018-2023) by increasing awareness and surveillance data about antimicrobial resistance in Uganda. The study results also contribute to the WHO integrated global surveillance data on ESBL producing *E. coli* using a "One Health" approach.

1.5 Conceptual framework

Wastewater collects antibiotic resistant bacteria, antibiotic residues and antibiotic resistance genes from municipal cities and hospitals. Although wastewater treatment plants play a big role in neutralizing the waste, excessive pollutants end up through the effluent into receiving water bodies. This endangers aquatic life, animals and people that utilize the water for various activities. In Uganda, information regarding antimicrobial resistance surveillance through the "One Health" approach is still lacking especially with the environmental sector. Therefore, this study intervenes to give research-based information regarding occurrence of antibiotic resistant bacteria and genes from wastewater sources and their receiving water bodies in Uganda, as illustrated in Figure 1. The results of this study are able to guide policy change aimed at reducing antimicrobial resistance.

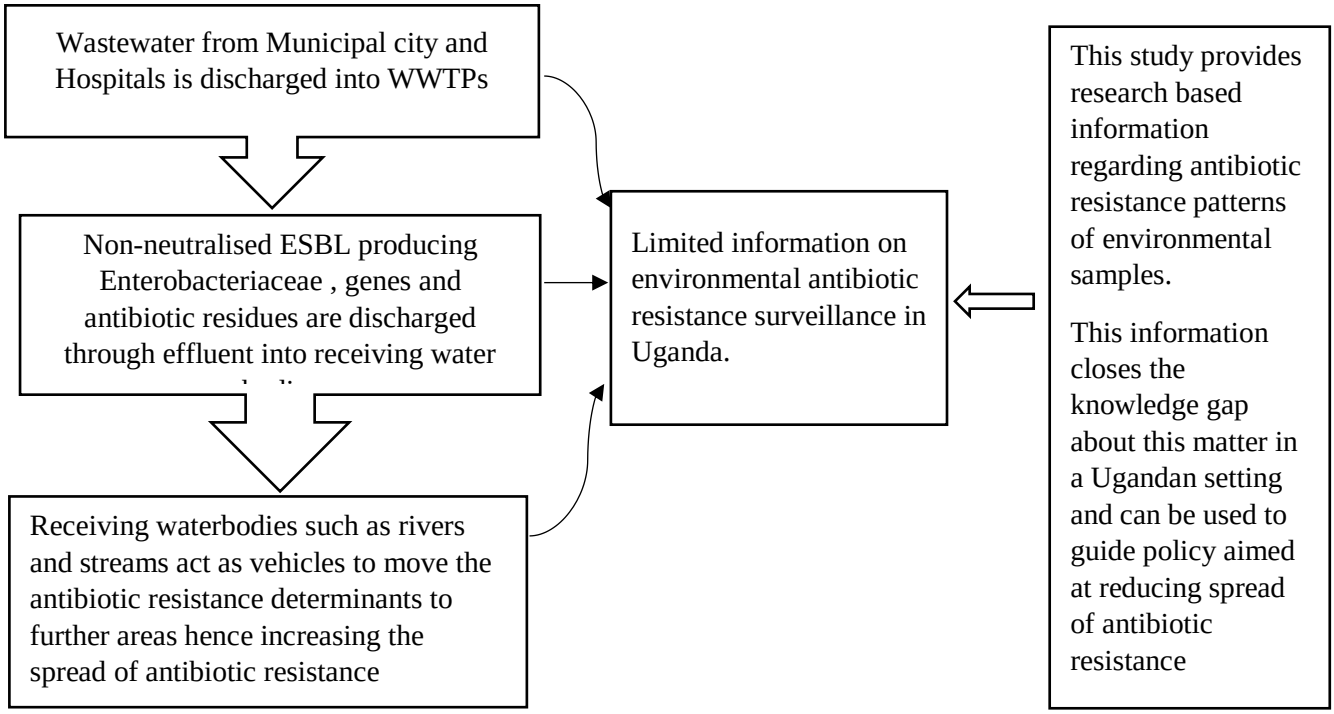


Figure 1: Conceptual framework indicating factors related to spread of ESBL producing Enterobacteriaceae from receiving waterbodies and the intervention of the study

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Wastewater and bacterial composition

Wastewater is a term used to describe water from any combination of industrial, domestic, commercial, agricultural activities, surface runoff/stormwater, and any sewer inflow or sewer infiltration. All these have loads of both clinically significant and insignificant bacteria from animal and human excreta and the environment. Predominant phyla of microorganisms in wastewater have been reported as *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* with an average abundance of $52.2 \pm 4.4\%$, $37.8 \pm 4.7\%$, $4.9 \pm 1.9\%$, and $2.2 \pm 0.2\%$, respectively (Numberger et al., 2019). Wastewater run-off to water bodies like rivers and lakes causes microbial and physico-chemical pollution. This pollution is minimized by national or municipal sewer lines that channel most of the wastewater to treatment plants before reaching receiving water bodies. The treatment plants then neutralize most of the chemical, biological and physical substances that may endanger the ecosystem (Aghalari et al., 2020).

2.2 Wastewater treatment

This is the process of converting wastewater into a less toxic form that can be discharged back into the environment. Various parameters are assessed before the effluent is discharged into the environment. These must meet required set standard values to minimize pollution to receiving environmental sites like water bodies. In Uganda, these standard maximum allowable limits are set and regulated by the National Environment Management Authority. The maximum allowable limits for effluent discharged into water or land are as follows; Biochemical Oxygen Demand (50 mg/l), Chemical Oxygen Demand (70 mg/l), Total metal concentration (10 mg/l), Total coliforms 400 CFU/100ml, Urea (1 mg/l) (NEE, 2020). The process of wastewater treatment involves a combination of various steps that are physical, chemical, and biological. These processes can be categorized into primary, secondary and tertiary as described below (Demirbas et al., 2017).

2.2.1 Primary treatment process

The first stage is physical and involves the removal of large and long items from the influent that would damage equipment, pumps, and valves downstream of the treatment plant. The influent then flows to the grit chamber where grit is removed. The grit might impede the flow of influent and also cause damage to plant equipment downstream. The next step is the primary clarifier. Solids termed sludge sink to the bottom of the tank and are then pumped into a sludge digester. The sludge is dried and hauled away. The sludge can be applied as an agricultural fertilizer.

2.2.2 Secondary treatment process

The next stage is biological and involves aeration. Air is pumped into the aeration tank to promote the conversion of ammonia to nitrate and also provide oxygen for bacteria to multiply. The bacteria then remove oxygen molecules from the nitrate molecules and the nitrogen is given off as a gas. At this stage, the Biochemical Oxygen Demand is the indicator for the amount of organic material present in the influent and is used to determine the effectiveness of the organic material breakdown. However, there are a number of other tests employed to ensure organic material breakdown such as measuring pH, temperature, Dissolved Oxygen, total suspended solids, hydraulic retention time, solids retention time, and mixed liquor suspended solids.

The wastewater then flows to the secondary clarifier. This allows any remaining organic sediment to settle out of the treated water flow. This organic sediment contains any small solids and is usually termed activated sludge since it contains mostly active bacteria. Part of this activated sludge is returned to the aeration tank to increase bacterial concentration, hence accelerating the organic material.

2.2.3 Tertiary treatment process

The next stage is chlorination. Chlorine is added to the flow to kill any bacteria and other microorganisms in the contact chamber. This ensures that higher than. specified concentrations of bacteria are not released into the environment. Other than chlorination, modern treatment plants employ ozone and UV disinfection (Zheng et al., 2017). The final stage of this process is dichlorination aimed to remove chlorine that was earlier deployed to kill microorganisms. This is done since chlorine is harmful to aquatic life in effluent-receiving water bodies. A compound called Sodium bisulfite is added to the wastewater to remove the chlorine ions.

The wastewater flow is then tested for various parameters before being discharged into the environment. These include pH levels, ammonia, phosphates, dissolved oxygen, and residual chlorine levels. If all the values from the water analysis conform to the standards, then the water is discharged as effluent to the immediate water channels such as streams, rivers, swamps, or lakes.

2.3 Bacterial composition of wastewater treatment plant effluent

Despite the various chemical treatments, wastewater contains significant concentration of microbes. These include bacteria, fungi, and viruses. Studies performed using metagenomic analyses have reported microbial communities from wastewater treatment plants and hospital effluent. *Proteobacteria, Bacteroides, Actinobacteria, Firmicutes, Tenericutes and Verrucomicrobia* phyla have been reported as the dominating the microbiomes in wastewater treatment plant effluent (Do et al., 2019). Although these bacteria are initially at lower concentrations in the effluent than in the influent of the treatment plant, the microorganisms multiply with time in the receiving water bodies,

given favorable conditions. Most of these bacteria pose a threat to humans and animals that utilize the contaminated water for they could possess virulent, factors and antibiotic resistance genes, therefore can be clinically significant.

2.3.1 *Escherichia coli*

E. coli is a gram-negative non-sporulating facultative anaerobe first identified in 1885 by a German bacteriologist, Theodor Escherich. It is species of the genus *Escherichia,* commonly found in the lower intestine of warm-blooded organisms. It is flagellated with cells that are typically rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter with a cell volume of 0.6–0.7 μm^3 (Tenaillon et al., 2010).

Biochemically, *E. coli* ferments glucose, xylose, trehalose, arabinose, mannitol, and lactose. However, some strains of *E. coli* when grown on MacConkey media have varying lactose fermenting power, some being late/slow lactose fermenters. It is positive for nitrate reduction, catalase, and indole tests, although negative for citrate, oxidase, and urease tests. It also does not produce hydrogen sulfide (Procop et al., 2017).

Phylogenetically, *Escherichia coli* is grouped into groups A, B1, B2, C, D, E, F, and clade I. Groups A and B1 have been reported to contain commensal strains while groups B2 and D are reported to contain pathogenic strains. Surprisingly, some studies have reported pathogenic strains in group B2 and D to be more susceptible to antibiotics as compared to the commensal strains in groups A and B1 (Chakraborty et al., 2015). However, report from phylogenetic analysis of uropathogenic *Escherichia coli* from Mulago hospital indicated that strains in Group B2 were highly multidrug resistant (Katongole et al., 2019).

2.3.1.1 Pathogenicity and virulence of *Escherichia coli*

Escherichia coli is a commensal organism in the gastrointestinal tract. However, the virulent strains of *E. coli* can cause various infections, depending on the pathotype. These infections are grouped into gastrointestinal and extra intestinal infections. The gastrointestinal infections include the enteropathogenic (EPEC), entero-toxigenic (ETEC), entero-invasive (EIEC), entero-hemorrhagic (EHEC) and the entero-aggregative (EaggEC) (Palaniappan et al., 2006). The extraintestinal infections include Urinary Tract Infections (UTIs), meningitis, septicemia and wound infections.

Escherichia coli can possess various virulence factors to aid causation of disease. These include adhesins such as Type 1, Dr, P, S and F1C fimbriae for attachment on host cells; invasins such as Ibe ABC for cell invasion into host tissues. Iron acquisition factors such as aerobactin, iron repressible protein, salmochelin, ChuA, Hma and SitABC. Toxins such as Serin protease autotransporter that degrades mucins, proteolytic toxins (Secreted auto-transporter and vacuolating

auto-transporter toxin) that aid cell vacuolization, hemolysin A that aids cell lysis, Cytotoxic necrotizing factor for cell necrosis, and Cytolethal distending toxin (Sarowska et al., 2019). These factors can be encoded chromosomally on pathogenic islands or extra-chromosomally on plasmids, transposons and other mobile genetic elements.

2.3.2 *Klebsiella pneumoniae*

This is also another bacterial species in the *Enterobacteriaceae* family that was first described by a German microbiologist, Carl Friedlander in 1882. It is a non-motile, oxidase-negative, Gram-negative rod with a thick polysaccharide-based capsule. The cells are generally between 0.3 to 1.5µm in width and 0.5 to 5.0 µm in length (Lawlor et al., 2005). *Klebsiella pneumoniae* bacteria do not have special growth requirements and can grow on ordinary laboratory media, unlike other members of the *Enterobacteriaceae* family. They appear as mucoid lactose fermenters on MacConkey agar. The species are facultative anaerobes and their ideal growth temperature is 35-37ºC at pH of 7.2. In nature, they are ubiquitous; commonly inhabiting environmental places like surface water, sewage, soil and mucosal surfaces of mammals such as horses (Struve et al., 2004).

2.3.2.1 Pathogenicity and virulence of *Klebsiella pneumoniae*

In humans and animals, *Klebsiella pneumoniae* is both a commensal and an opportunistic pathogen. It is prevalent in normal microbiota of the intestinal tract, colon, mouth and skin where it initially does not cause disease. However, it can progress into pathogenicity causing infections especially in immune-compromised individuals and those that are exposed to regular administration of broad-spectrum antibiotics that disrupt normal flora in the body (Rafii et al., 2008). Infections caused by *K. pneumoniae* include pneumonia, bloodstream infections, wound infections, meningitis and UTIs. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks, hence accounting for a significant proportion of hospital-acquired infections (Peltier et al., 2019).

Klebsiella pneumoniae possess a collection of virulence factors utilized for its pathogenicity. These include capsules, endotoxins, iron-scavenging systems, siderophores and adhesions. The capsule is a very important virulence factor for it covers the bacterial surface. It promotes inflammation, sepsis and it is responsible for resistance against host phagocytosis and antimicrobial peptides and peptones. Capsular molecular typing has identified about 78 capsular types (Pan et al., 2013). Types K1, K2, K3, K5, K20, K54, and K57 are the most studied and some studies have reported Capsular type K5 and K20 with the highest occurrence in isolates from Uganda (Ssekatawa et al., 2021).

2.4 How Bacteria develop resistance

The emergence of drug resistance in the clinical environment has been a constant threat since the beginning of the antibiotic era. For example, penicillin-resistant strains of *Staphylococcus aureus* were already isolated in 1944, just 2 years after the introduction of this antibiotic on the market (Bhattacharjee, 2016). The same trend has been observed for most of other manufactured antibiotics. Antibiotic resistance mechanisms have probably evolved from genes present in organisms producing antibiotics (Davies & Davies, 2010). The ultimate source of many of these genes is almost certainly the *actinomycetes* that make the antibiotics and therefore need self-protective mechanisms to avoid suicide (Cundliffe et al., 2010a). The *Actinomycetes* are known to produce almost two thirds of natural antimicrobial drug compounds such as Streptomycin, actinomycin, streptothricin among others (Takahashi et al., 2018).

In avoidance of suicide, expression of genes encoding antibiotic biosynthesis and those encoding resistance to produced antibiotics needs to occur in a timely manner. Hence the two processes either occur concomitantly or the resistance genes can be induced by their cognate antibiotics or by intermediate molecules from their biosynthetic pathways. This is possible since the genes can occur contiguously on chromosomal DNA or on plasmids (Cundliffe et al., 2010b).

2.5 Antimicrobial resistance transfer mechanisms

In bacteria, genetic information about antimicrobial resistance is encoded on genes located on the chromosome or extra-chromosomally on plasmids. The genetic information can be transferred from one bacterial cell to another through either vertical or horizontal transmission. within the same species or to other species (Lerminiaux et al., 2019). Horizontal gene transfer, however, potentiates at a greater extent, the spread of antibiotic resistance like a wild fire. Bacterial communities in wastewater are able to share a vast pool of antibiotic resistance genes since it collects antibiotic resistant bacteria and genes from various environments. Hospital wastewater has been shown to increase proportions of bacteria acquiring new antibiotic resistance genes (Hutinel et al., 2021).

There are three basic ways for the exchange of DNA between bacteria: Conjugation, transduction, and natural transformation. During conjugation, a piece of DNA/Plasmid from one bacterium is transferred to another via a temporary connection; a conjugative pilus. In transduction, the transfer of DNA occurs with aid of a bacteriophages and in transformation, DNA that is located outside the cell is fragmented and imported into the cell after which it replaces a piece of original DNA in the chromosome of host via recombination (Soucy et al., 2015).

2.6 Antibiotic resistance genes carried by *E. coli* and *K. pneumoniae*

Infections caused by *E. coli* and *K. pneumoniae* are commonly treated by variety of classes of antibiotics. Beta-lactams, quinolones, aminoglycosides, sulphonamides, phenicols, are the

commonest classes administered (Pontefract et al., 2020). However, bacteria have devised means to render these antibiotics in-effective. Some bacteria can possess genes that encode for any of the various mechanisms of resistance such as antibiotic-modifying enzymes, drug efflux permeases, bypass targets, and ribosome modification or mutation (Gomez et al., 2017).

Various studies have reported genes encoding antibiotic –modifying enzymes like beta-lactamases in bacteria isolated from samples in a Ugandan setting. The most reported are the extended spectrum beta-lactamase (ESBL) genes; CTX-M, TEM and SHV (Mbyemeire et al., 2021a). These enzymes confer resistance to penicillins, monobactams and third generation cephalosporins but not carbapenems. They are also inhibited by clavulanic acid, tazobactam and sulbactam (Ghafourian et al., 2015). Genes encoding broad spectrum beta-lactames; the carbapenemases, have also been studied in a Ugandan setting. Carbapenamases are able to inactivate carbapenem drugs that are options for infections caused by ESBL producing bacteria (El-Gamal et al., 2017). Genes commonly reported include blaIMP, blaVIM, blaNDM, blaKPC, blaOXA48, (Ssekatawa et al., 2021). Although these genes have been reported from clinical samples in Uganda, other studies elsewhere have reported Carbapenemases from wastewater samples (Mathys et al., 2019b). Genes conferring resistance to sulphonamides (Sul1, Sul2, and Sul3), fluoroquinolones (*aac(3)-Ib-cr, qnr)*, aminoglycosides(*aad*) phenicol (cat), and tetracycline tet(A), tet (B), tet(D), tet(E) and tet(G), have been reported from clinical samples (Decano et al., 2021). In wastewater, genes conferring sulphonamide and tetracycline resistance have been the most studied with findings indicating inefficient removal of these genes by wastewater treatment plants (Sabri et al., 2020).

2.7 *Escherichia coli* and *Klebsiella pneumoniae* isolated from river water

Genomic analyses of *E. coli* and *K. pneumoniae* isolated from river water is yet to be adequately studied in Uganda. There is still scarce information on the extent of threat that these bacteria might pose on the communities. However, for studies done elsewhere, one study that isolated *E. coli* from Yamato River, Japan found out that there was no significant difference in virulence gene content analysis between environmental and clinical strains. Some of the isolates belonged to clinically important clonal groups; ST95, ST127, ST14 and ST131 complexes and were multidrug resistant hence posing a major public health threat (Ryota et al., 2017). In another study done in Tunisia, *E. coli* and *K. pneumoniae* isolates from Rouriche river were ESBL producing and some were multidrug resistant. They also possessed Class 1 integrons and IncP/IncFIB plasmids, which are mobile genetic elements that could transmit antibiotic resistance genes to potential pathogens (Hassen et al., 2020a).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design and setting

This was a longitudinal study carried out between October 2022 and April 2023 from two study sites namely; River Rwizi catchment in Mbarara city; and River Aswa in Gulu city.

3.1.1 Mbarara City study site

Mbarara is a city in the south western region of Uganda. At this study site, wastewater samples were collected from lagoons of three wastewater treatment plants namely Katete, Kizungu and Kakoba WWTPs. Another wastewater sample was also collected from the effluent of Mbarara Regional Referral Hospital. Wastewater water samples are known to contain antibiotic resistant bacteria. Samples from influents and effluents of the lagoons were collected to determine presence of ESBL producing Enterobacteriaceae. River water samples were picked along River Rwizi upstream and downstream of Katete WWTP. R. Rwizi is the second longest river in Uganda. The river has its source from Buhweju hills and runs through Buhweju, Bushenyi, Sheema, Ntungamo, Isingiro, Mbarara, and other south-western districts. It pours its waters into Lake Victoria after transecting through lakes Mburo, Kachera, Nakivale and Kijanebalola (Atwebembeire et al., 2018). The river receives the effluent of wastewater lagoons that contains virulent and antibiotic resistant bacteria that might endanger lives of people utilizing the river water. Since the river also flows through many communities, it essentially acts as a vehicle to spread antibiotic resistant determinants to further areas.

3.1.2 Gulu City study site

Wastewater samples were collected from lagoons of the city WWTP to assess the presence of ESBL positive Gram negative Enterobacteriaceae. Another wastewater sample was collected from the Gulu Regional Referral Hospital effluent. The city WWTP discharges its effluent into the nearby Pece stream. Water from this stream is used to irrigate gardens of vegetables and cereals planted along it. Water samples were collected upstream and downstream the discharge point from the stream. River water samples were collected along River Aswa at points acting as drinking points for cattle from nearby farms. At these points, fecal matter from the cattle washes into the river and these might be the points of transmission of antibiotic resistant bacteria in a One Health perspective. The River drains most of the northern plateau of Uganda before it joins the White Nile River in South Sudan.

3.1.3 Laboratory sample analysis

Samples were analyzed from the Microbiology and Molecular Biology laboratories at the College of Natural sciences, Department of Zoology, Entomology and Fisheries Sciences, Makerere University. Laboratory analysis was qualitative and targeted presence of two ESBL positive

organisms from the Enterobacteriaceae family; that is *E. coli* and *K. pneumoniae*. The two coliforms are reliably used as indicator organisms for fecal contamination of water sources and they possess capability to transfer antibiotic resistance genes to other species. Possession of the ESBL genotype gives these organisms the capability to confer resistance to critically important antibiotics (WHO, 2021).

3.2 Rationale of selecting R. Rwizi and R. Aswa

Three wastewater treatment plant lagoons in Mbarara City; Katete, Kizungu and Kakoba discharge their effluent into River Rwizi, increasing the extent of contamination of the river with antibiotic resistant bacteria such as coliforms like *E. coli* and *K. pneumoniae*. In comparison, River Aswa is less anthropogenically disturbed. It majorly flows through rural areas that are less populated and does not directly receive wastewater treatment plant effluent.

3.3 Sample size and sample collection technique

Samples from the rivers were collected at a safe river bank by dipping a sterile 500ml capacity bottles until it was full of water. The bottles were then tightly closed, labelled and preserved in a cold chain until the they reached the laboratory. A clean bucket was used to collect wastewater samples from drainages and the sample was poured into the sterile 500ml bottle

From Mbarara study site; Eleven (11) samples were downstream WWTP along R. Rwizi. Of these, 5 samples also acted as distance controls for they were far, about 30 km from the effluent discharge. Four (4) samples were collected upstream WWTP along R. Rwizi. Three (3) samples were WWTP influent and 3 samples were WWTP effluent. One (1) sample was collected from hospital effluent.

From Gulu study site, 4 samples were collected from R. Aswa, 2 wastewater samples from Gulu City WWTP and 2 wastewater samples from Gulu Regional referral hospital effluent

Table 1.0. Samples collected at Rivers Rwizi and Aswa catchments

Study site	Collection point	Number of samples
Mbarara	R. Rwizi water (Upstream WWTP)	4
	R. Rwizi water (Downstream WWTP)	11
	WWTP influent	3
	WWTP Effluent	3
	Hospital effluent	1
Gulu	WWTP influent	2
	WWTP effluent	2
	Hospital effluent	2
	Pece stream (upstream WWTP)	2
	Pece stream (downstream WWTP)	2

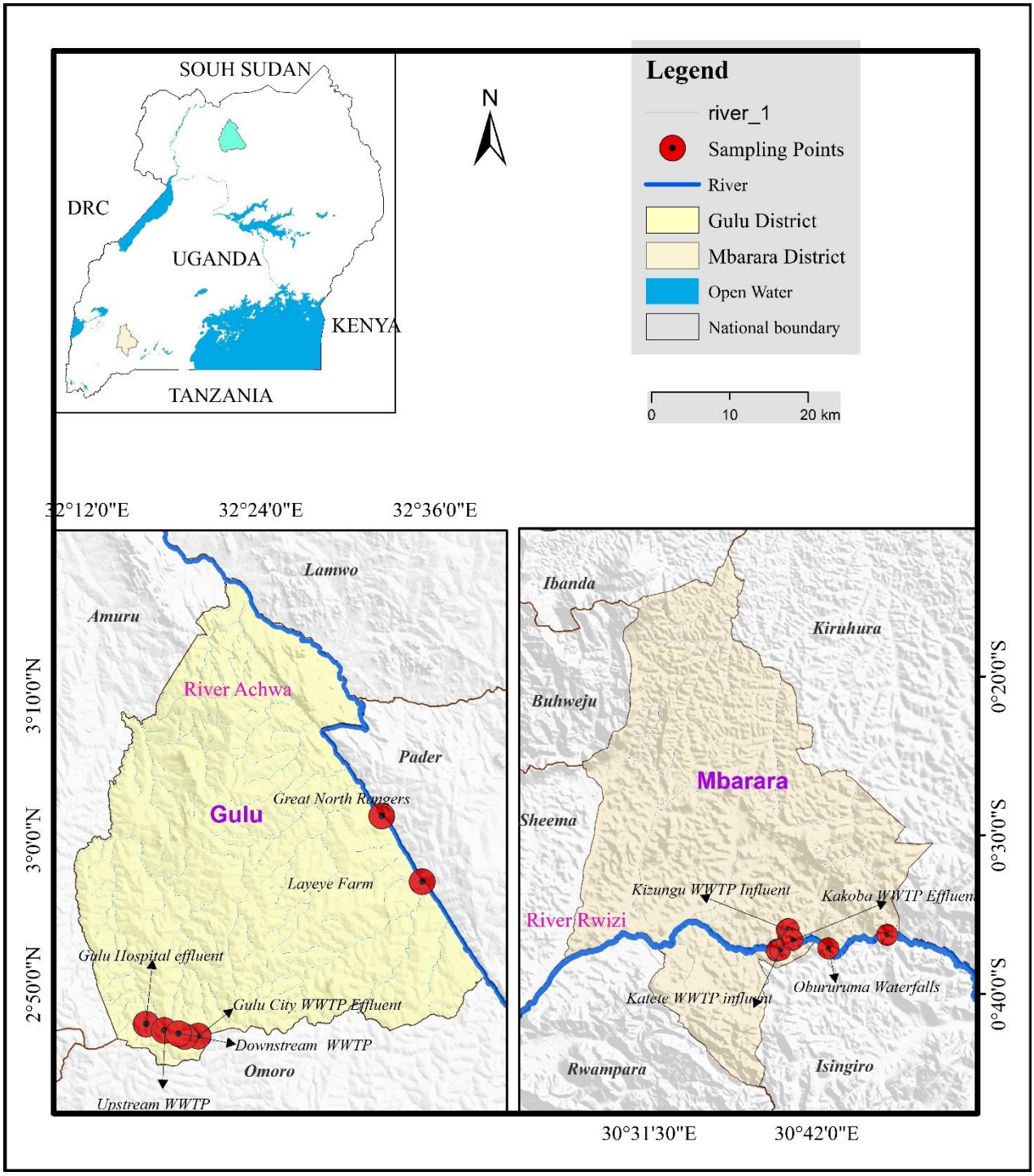


Figure 2: Showing sampling points at the study sites in Gulu and Mbarara

3.4 Laboratory analysis of samples

3.4.1 Sample processing for culture on ChromESBL agar

At the laboratory, 20- 200mls of the homogenized river water sample and wastewater were poured into a 500ml manifold cup. The sample was filtered using a vacuum pump through a 0.45µm gridded membrane filter. The filter was then aseptically removed from the manifold and incubated in 5mls of Buffered peptone water (BD, Germany) at 37°C for 24hrs. A graduated plastic loop was used to pick 10µl of the incubated broth and inoculated on ChromESBL agar plates ESBL (CHROMagar, France) prepared following manufacturer's instructions. The inoculated plates were then incubated 37°C for 24hrs for growth.

During plate reading, pink and blue colonies were preliminarily identified as *Escherichia coli* and *Klebsiella pneumoniae* respectively. The pure colonies were further subjected to VITEK 2 automated system (bioMerieux, France) at Kiruddu National Referral Hospital Microbiology laboratory for confirmatory identification. The Vitek ID GNB card was used according to the manufacturer's instructions. The card contains 47 colorimetric tests that measure utilization of carbon sources, enzymatic activities and resistance to inhibitory substances. A specific kinetic algorithm calculation and a modified optical system are employed by the system to give final identification within 2-10 hours (Renaud et al., 2005).

Quality Control: ATCC 700603 *Klebsiella pneumoniae* a known ESBL producer was used as a positive control and grew with blue colonies on ChromESBL agar. ATCC 35218 *Escherichia coli* was used as positive control and grew as pink colonies on ChromESBL agar. ATCC 25922 *Escherichia coli* was used as negative control and did not grow on ChromESBL agar since it is a known ESBL negative organism.

3.4.2 Molecular characterization

3.4.2.1 DNA extraction

DNA was extracted from ESBL positive *Escherichia coli* and *Klebsiella pneumoniae* isolates. The ZymoBiomics™ DNA Miniprep kit (Zymo Research, USA) was used following the manufacturer's instructions in the kit insert with pure bacterial cells as starting material. Briefly, the bacterial cells were added into bead lysis tubes and lysis solution added. After shaking at 300 revolutions per minute for 30 minutes, the tubes containing the lysate were centrifuged at 10,000g for 1 minute. The supernatant was loaded onto the Zymo-spin filter. After centrifugation at 8,000g for 1 minute, the filters were discarded and DNA binding buffer was added to the filtrate. The mixture was then transferred into a spin column and centrifuged. Wash buffer was added to the spin column and centrifuged at 10,000g for 1 minute and the flow through discarded. Dnase free water was added to the column matrix and incubated for 1 minute. The tubes were centrifuged at 10,000g for 1 minute

to elute the DNA and the eluted DNA was then filtered using HRC filter to remove any remaining contaminants and enzyme inhibitors. The filtered DNA was then suitable for PCR and other downstream applications.

To check quality of extracted DNA, the gel electrophoresis method was used. 5µl of DNA mixed with 1µl of loading dye was run on a 2% gel for 40 minutes at 130 volts, 121mA using a Power pack (Consort EV243, Belgium). The Gel was then visualized using a UV transilluminator (GeneFlash Syngene, Biocompare, USA). Attainment of thick bands implied good quality DNA.

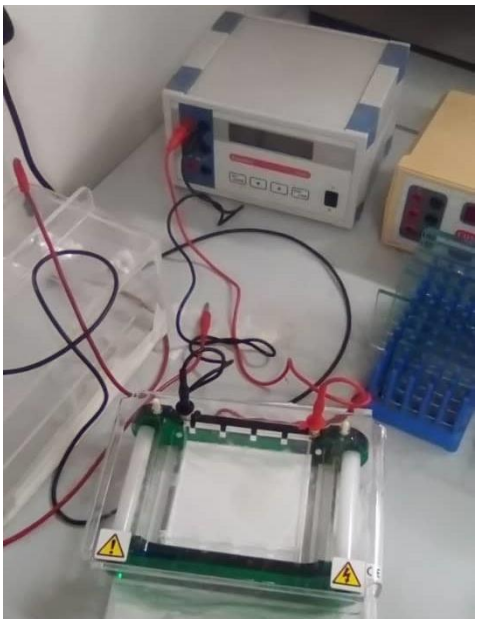


Figure 3a: Showing gel electrophoresis setup

Figure 3b: Showing the UV transilluminator

3.4.2.2 PCR

PCR was done to ascertain presence ESBL encoding genes carried by positive *E. coli and K. pneumoniae* DNA samples. The genes were amplified using MasterCycler® Nexus (Eppendorf, Germany). A 25 µl reaction containing 12.5 µl of quickload 2x Taq mastermix (Biolab, USA), 0.5 µl of each 10µM primer (forward and reverse), 10 µl of nuclease free water and 1.5 µl of DNA sample was prepared following laboratory protocols under ice. The amplification for the genes was done using PCR conditions shown in table 2.

Table 2. PCR conditions set for blaCTXM, blaTEM, and blaSHV

Gene	Primer Sequences	PCR conditions	Reference
blaCTXM	Fw: 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' Rev: 5'-TGGGTRAARTARGTSACCAGAAYCAGC GG-3'	Initial den.: 95°C for 5m Den.: 94°C for 30s Annealing: 56°C for 45s Ext.: 72°C for 45s Final Ext. 72°C for 10m	(Boyd et al., 2004)
blaTEM	Fw: 5'-TTTCGTGTCGCCCTTATTCC-3' Rev: 5'-ATCGTTGTCAGAAGTAAGTTGG-3'	Initial den.: 95°C for 5m Den.: 94°C for 30s Annealing: 53°C for 45s Ext.: 72°C for 45s Final Ext. 72°C for 10m	(Bali et al., 2010)
blaSHV	Fw: 5'-ATGCGTTATATTCGCCTGTG-3' Rev: 5'-AGCGTTGCCAGTGCTCGATC-3'	Initial den.: 95°C for 5m Den.: 94°C for 30s Annealing: 66°C for 45s Ext.: 72°C for 90s Final Ext. 72°C for 10m	(Rafiee et al., 2018)

The amplification was done within 35cycles. After the completion of the PCR run, the amplicons were subjected to the Gel electrophoresis method as described in section 3.4.2.1 above using a 100 bp as ladder marker.

Figure 4: Showing the thermocycler used for amplification of the genes

3.5 Data management and analysis

Data was entered into Microsoft excel version 2019, cleaned and sorted. Then presented in form of tables for all the objectives. Proportions of ESBL *K. pneumoniae* and *E. coli* were then estimated from the overall isolates identified. Quantum Geographical information software (QGIS) was used to display global positioning system points captured from the sample collection sites on the Maps of Gulu and Mbarara districts.

3.6 Ethical consideration

This study was approved by the Department of Immunology and Molecular biology, College of Health Sciences, Makerere University and the School of Biomedical Sciences Research Ethical Committee under REC NO.- SBS 2023-354. The parent study entitled "Dispersal of Antibiotic Resistance and antibiotics in Water ecosystems and Influence on livestock and aquatic wildlife", which supported this study obtained approval from the Uganda National Council of Science and Technology, School of Veterinary Medicine and Animal Resources Institutional Animal Care and Use Committee (SVAR IACUC), REF Number: SVAR_IACUC/104/2022, and City Councils in Mbarara and Gulu.

CHAPTER FOUR

4.0 RESULTS

4.1 Isolation of ESBL positive Enterobacteriaceae

This study sought out the two indicator organisms *E. coli* and *K. pneumoniae.* Twenty (20) out of the 36 (55.55%) samples had *E. coli* isolated, while only 10 (27.77%) samples had *K. pneumoniae*.

At the Mbarara City study site, all the four (4) river water samples upstream WWTP had *E. coli* isolated while one (1) sample had both *E. coli* and *K. pneumoniae* isolated. For the eleven (11) water samples downstream WWTP, 9 samples grew *E. coli* and 3 of these had both *K. pneumoniae* and *E. coli* isolates. For the wastewater samples, 2 out of 3 WWTP influent samples grew *E. coli* and *K. pneumoniae* while all the 3 WWTP effluent samples grew *E. coli* but no *K. pneumoniae.* The hospital effluent sample grew *E. coli* but no *K. pneumoniae.*

Table 3: Showing ESBL positive organisms isolated from wastewater and river water samples of R. Rwizi catchment area.

Organisms Isolated	**Hospital Effluent** (1 sample)	**WWTP Influent** (3 samples)	**WWTP effluent** (3 samples)	**R. Rwizi upstream WWTP** (4 samples)	**R. Rwizi downstream WWTP(<4km)** (6 samples)	**R. Rwizi downstream WWTP (about 30km)** (5 samples)
E. coli	*1*	*2*	*3*	*4*	*6*	*3*
K. pneumoniae	*0*	*1*	*0*	*1*	*2*	*1*
Enterobacter spp	*0*	*0*	*0*	*1*	*0*	*1*
Pantoea spp	*1*	*1*	*0*	*0*	*1*	*0*
Aeromonas spp	*0*	*0*	*0*	*0*	*2*	*2*
Sphingomonas spp	*0*	*1*	*2*	*0*	*1*	*1*

At Gulu study site, no *E. coli* or *K. pneumoniae* was isolated from the river water samples, from the two (2) WWTP influent samples only one (1) *K. pneumoniae* was isolated but no *E. coli* and the two (2) WWTP effluent samples grew one (1) *K. pneumoniae* and no *E. coli.*

Two (2) *K. pneumoniae* isolates were from the two (2) stream water samples that WWTP discharges into. Only one (1) of the two (2) hospital effluent samples had *K. pneumoniae* isolated and no *E. coli.*

Table 4: Showing ESBL positive organisms isolated from wastewater and river water samples of R. R.Aswa catchment area.

Organisms Isolated	**Hospital Effluent** (2 sample)	**WWTP Influent** (2samples)	**WWTP effluent** (2 samples)	**Pece upstream WWTP** (2 samples)	**Pece downstream WWTP** (2 samples)	**R. Aswa** (4 samples)
Escherichia coli	1	0	0	0	0	0
Klebsiella pneumoniae	0	0	0	2	1	0
Enterobacter cloacae	1	1	1	0	0	0
Citrobacter freundii	0	0	1	0	0	0
Vibrio vulnificus	1	0	0	0	0	0
Sphingomonas paucimobilis	0	0	0	0	0	1
Brucella melitensis	0	1	0	0	0	0
Acinetobacter lwoffii	0	0	0	0	0	1
Pseudomonas fluorescens	0	0	0	0	1	0

4.2 Amplification results for ESBL genes (blaCTX-M, blaTem and blaSHV)

All the 20 ESBL positive *E. coli* and 10 ESBL *K. pneumoniae* isolates from all the samples carried the blaCTX-M gene. From R. Rwizi samples downstream WWTP, 5 out of the 9 *E. coli* isolates, carried the blaTEM gene while only 1 out of the 9 *E. coli* isolates carried the blaSHV gene. All the 3 *K. pneumoniae* carried the blaTEM gene and only 1 carried the SHV gene. From samples of R. Rwizi upstream WWTP, all the 4 *E. coli* carried both blaCTX-M and blaTEM gene while only 1 carried the all the three genes (blaCTX-M, TEM and SHV). The only *K. pneumoniae* isolated at this point carried both blaCTX-M and blaTEM but no blaSHV gene. The 2 *E. coli* isolates from WWTP influent carried both blaCTX-M and blaTEM but no blaSHV gene. The 1 *K. pneumoniae* isolate isolated at this point carried all the three genes (blaCTX-M, TEM, and SHV). For 3 *E. coli* isolated from WWTP effluent, only 2 carried both blaCTX-M and blaTEM while 1 carried only blaCTX-M gene. All the 3 *E. coli* from this point did not carry the SHV gene. The only *E. coli* isolate from the hospital effluent sample carried only blaCTX-M gene but no blaTEM or blaSHV.

For samples from Gulu study site, the *E. coli* isolate from the hospital effluent carried only the blaCTX-M gene. The *K. pneumoniae* isolate from the WWTP influent carried all the three genes (blaCTX-M, TEM, SHV). The *K. pneumoniae* isolate from the WWTP effluent carried both blaCTX-M and TEM but no SHV gene. Both the 2 *K. pneumoniae* from Pece stream water samples upstream WWTP carried all the three genes and the *K. Pneumoniae* from the water same downstream WWTP also carried all the three genes. These results are summarized in table 5

Table 5: Showing amplification results from *E. coli* and *K. pneumoniae* isolates

Site	Source	Organism	Number (n) of isolates	SHV gene	TEM gene	CTX-M gene
Mbarara		*E. coli*	9	1	5	9
	R. Rwizi Downstream WWTP	*K. pneumoniae*	3	1	3	3
		E. coli	4	1	3	4
	R.Rwizi Upstream WWTP	*K. pneumoniae*	1	0	1	1
	WWTP influent	*E. coli*	2	0	2	2
		K. pneumoniae	1	1	1	1
	WWTP effluent	*E. coli*	3	0	2	3
	Hospital Effluent	*E. coli*	1	0	0	1
Gulu	Hospital Effluent	*E. coli*	1	0	0	1
	WWTP influent	*K. pneumoniae*	1	1	1	1
	WWTP effluent	*K. pneumoniae*	1	0	1	1
	Pece Upstream WWTP	*K. pneumoniae*	2	2	2	2
	Pece Downstream WWTP	*K. pneumoniae*	1	1	1	1
Total(N)			30	8	22	30

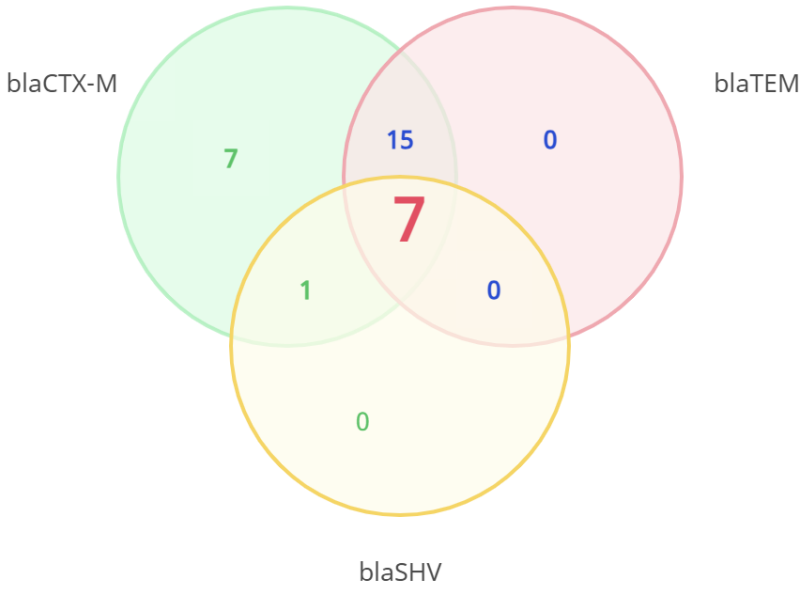
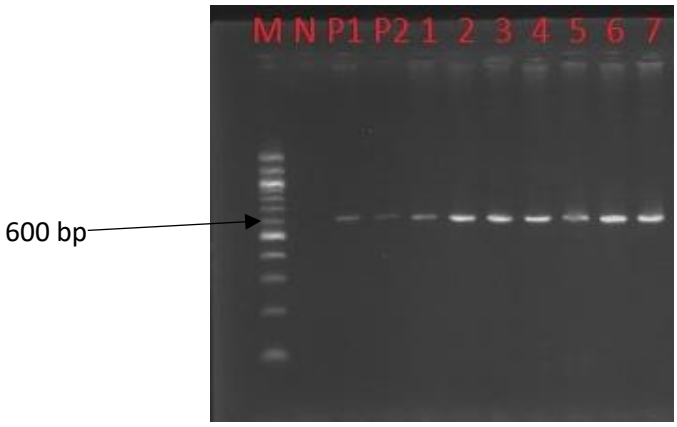


Figure 5: A venndiagram showing an overlap of presence of detected ESBL genes



Key: From left to right,
M-marker (100bp ladder),
N-negative control,
P1-Positive Control *E. coli*,
P2 –Positive Control *K. pneumoniae*,
wells 1-7 positive water samples at 600bp

Figure 6: Showing a representative gel image of blaCTX-M.

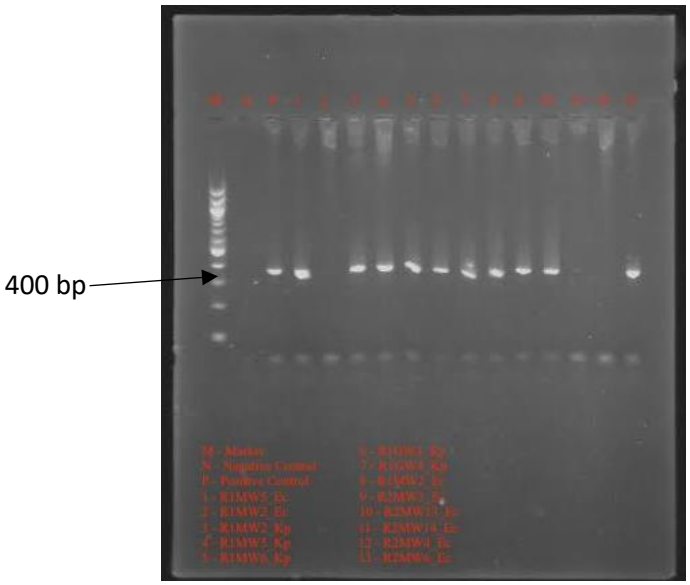
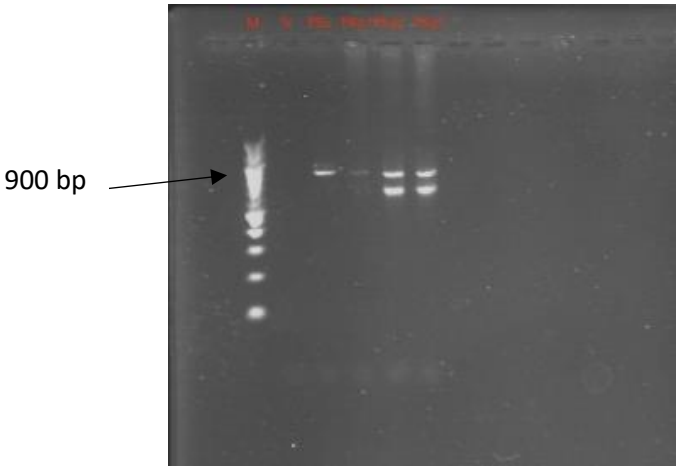


Figure 7: A representative gel image showing positive bands for the blaTEM gene at 400bp size for water isolates



Key: From left to right,
M-marker (100bp ladder),
N- negative control,
P1-Positive Control *E. coli*,
P2 –Positive Control *K. pneumoniae*,

Figure 8: A representative gel image showing positive water samples and negative samples for the blaSHV gene at the 900bp mark.

CHAPTER FIVE

5.0 DISCUSSION

5.1 DISCUSSION

This section provides a detailed explanation of the results of the study.

Out of the 30 indicator organisms isolated from all the samples, 20 (66.66%) were *E. coli* 10 (33.33%). These findings are similar to those from wastewater and receiving water bodies in Ethiopia where frequency of *E. coli* isolation was higher than *K. pneumoniae. E. coli* and *K. pneumoniae* are both gut commensals and hence used as indicators for fecal contamination of the environment. However, findings from (Auban et al., 1998) indicate a positive correlation between frequencies of the two indicator organisms and water temperature; *K. pneumoniae* predominating at high water temperatures while *E. coli* showing predominance at cold periods. This could be the reason for the findings in this study since sample collection was done mostly during rainy periods. On addition to the *E. coli* and *K. pneumoniae* isolates, organisms such as *Sphingomonas paucimobilis, Aeromonas hydrophillae, Pantoe species* and *Brucella melintensis* were isolated as well. These organisms are commonly isolated from the environment; however, some reports indicate isolation of these organisms in clinical samples causing multidrug resistant infections such as septicemia especially in immunocompromised individuals (Agyepong et al., 2018).

Hospital effluent samples from Mbarara Regional Referral Hospital and Gulu Regional Referral Hospital had ESBL producing *E. coli* isolated. These findings are expected since hospital effluent is known to have higher concentration of antibiotic residues that influence acquisition of antibiotic resistance determinants. Similar findings were attained from effluent of Mulago National Referral hospital (Bagaya et al., 2023) and also from two healthcare facilities in Nigeria (Adekanmbi et al., 2019). Two (2) out of the three (3) WWTP influent samples from Mbarara City study site had ESBL positive *E. coli and K. pneumoniae* isolated while one (1) of the two (2) WWTP influent samples from Gulu City study site had ESBL positive *K. pneumoniae* isolated. WWTP influent definitely contains fecal material from the municipal cities and hospital effluent, however, presence of fecal coliforms that are resistant to common and affordable antibiotics is a threat to public health. This is because these ESBL positive organisms with possession of virulence factors, are able to cause drug resistant infections or transfer resistance determinants to other virulent organisms, even of different species. These results give a picture of the level of cephalosporin resistance in these communities. In comparison with the results from effluent samples, all the three (3) WWTP effluent samples from Mbarara City study site had ESBL positive *E. coli* isolated while one of the two (2) WWTP effluent

samples from Gulu City study site had ESBL positive *K. pneumoniae* isolated. The WWTP lagoons concentrate a lot of antibiotic residues and antibiotic resistant bacteria that share resistance markers to other bacteria. Since there is no disinfection stage at this point such as chlorination, antibiotic resistant bacteria are discharged into receiving bodies close to the lagoons. Consequently, samples from the receiving water bodies had ESBL positive *E. coli* and *K. pneumoniae* isolated. Nine (9) of the eleven (11) samples collected from R. Rwizi downstream WWTP discharge had *E. coli* and *K. pneumoniae* isolated. Additionally, Samples collected downstream of Pece stream that receives effluent from Gulu City WWTP also had ESBL positive *K. pneumoniae.* These results are comparable to a study done in Tunisia that also isolated ESBL positive *E. coli* and *K. pneumoniae* from Rouriche river (Hassen et al., 2020). The results of this study indicate that the effluent from WWTP indeed contain antibiotic resistant bacteria and these are discharged directly into these water bodies. Therefore, it is one source of the presence of resistant bacteria in receiving waterbodies. Interestingly, this study found out that even water samples from river Rwizi and Pece stream collected upstream the WWTP effluent discharge point had ESBL positive *E. coli* and *K. pneumoniae* isolated. This indicates that regardless of the WWTP discharge, water bodies receive fecal contamination from other various sources such as wastewater from farm fertilizers and wastewater that is directly channeled to the river before treatment. However, in this study this finding is attributed to urban settlements along the receiving waterbodies, since there was no ESBL positive *E. coli or K. pneumoniae* isolated from R. Aswa that does not flow through the city and also does not receive wastewater treatment plant effluent.

The thirty (30) isolates from this study were further subjected to conventional PCR to amplify ESBL encoding genes. All the isolates carried the blaCTX-M gene (100%), and of these, 73.3% also carried the blaTEM gene, while 26.7% carried the blaSHV gene. These results agree with findings of a study done on clinical isolates from Mbarara Regional Referral Hospital, Southwestern Uganda that had expression of the blaCTX-M gene highest in comparison to blaTEM and blaSHV genes (Moses et al., 2014). The blaCTX-M gene has been the most prevalent Class A ESBL gene reported worldwide. First reported in Munich, Germany, it has been responsible for breakdown of cephalosporins more preferably Cefotaxime and Ceftriaxone in comparison to Ceftazidime. Its ability to be plasmid mediated has fueled its intercontinental spread. On addition to the overuse of cefotaxime/ceftriaxone in Uganda (Manirakiza et al., 2019.), it is not surprising that this study found high prevalence of blaCTX-M gene. This over use is linked to selection pressure that increases the expression levels of the gene. However, a study done in Iraq found out blaTEM as the most prevalent gene among ESBL positive *E. coli* and *K. pneumoniae* isolates in comparison to blaSHV and blaCTX-M (Pishtiwan et al., 2019). The blaSHV gene was the least prevalent with only 8 positive isolates out of the total 30 isolates tested. Six (6) of the 8 blaSHV positive isolates were *K. pneumoniae* while only 2 out of the

8 were *E. coli.* Similar findings are observed in a study on clinical isolates from Mbarara Regional Referral Hospital (blaSHV 34%, blaTEM 47%, blaCTX-M 70%) (Moses et al., 2014b). However, results from a study in the same region reported highest prevalence of blaSHV (42%) from isolates at Kampala International University Teaching Hospital in comparison to blaCTX-M (22.4%) and blaTEM (27.3%) (Mbyemeire et al., 2021b). Although the blaSHV gene ancestor has been reported to be a chromosomal species-specific penicillinase, first isolated from a fecal *K. pneumoniae* isolates of neonates; transferable subtypes of the gene have since been reported even in other species such as *E. coli* (Liakopoulos et al., 2016). Plasmid mediation is the commonest mode of transfer of these genes reported worldwide and therefore organisms can transfer resistance genes to each other at environment, human and animal interface.CHAPTER SIX

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study found out that wastewater and receiving waterbodies contain antibiotic resistant bacteria in comparison to waterbodies that don't receive wastewater directly. These bacteria also carried resistance genes that have been found in clinical settings and therefore wastewater sources contribute to the spread of antimicrobial resistance in communities.

6.2 Recommendation

According to the findings, this study recommends improved wastewater treatment that aims at disinfection before discharge. It also recommends further research on genomic relatedness of isolates to track the source in relation to clinical isolates.

6.3 Study Limitations

This study did not determine the presence of other ESBL genes such as Oxa, VEB, PER, GES, TLA, and BES. However, the three ESBL genes studied, CTXM, TEM and SHV are the commonest genes reported in Uganda.

Some sampling sites were not accessible on the second round of sampling, so number of sampling times were not uniform for some sources.

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