UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

MULTIDRUG-RESISTANT BACTERIA IN HOSPITAL WASTEWATER OF THE KORLE BU TEACHING HOSPITAL

BY

DAISY SENA ADDAE-NUKU

(10803680)

THIS THESIS/DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MPHIL IN MEDICAL MICROBIOLOGY DEGREE.

JULY 2022

DECLARATION

I, Daisy Sena Addae-Nuku, hereby declare that the work presented in this thesis is the result of my own research carried out in the Department of Medical Microbiology Research Laboratory, School of Medicine and Dentistry, College of Health Sciences, Korle-Bu, under the supervision of Professor Eric Sampane-Donkor and Dr. Nicholas T. K. D. Dayie, and that all references cited here have been duly acknowledged.

Sign …………………. 12th July, 2022

Ms Daisy Sena Addae-Nuku

(Student)

Sign…………………..…… 12th July, 2022

Prof. Eric Sampane-Donkor

(Main Supervisor)

Sign…………………….. 12th July, 2022

Dr. Nicholas T. K. D. Dayie

(Co-Supervisor)

DEDICATION

I dedicate this work to God Almighty and to my lovely family, especially my mom, Madam Adwoa Pokua Amponsah, for her endless support.

ACKNOWLEDGEMENT

I deeply appreciate the efforts of the following individuals: my supervisors (Professor Eric Sampane-Donkor and Dr. Nicholas T. K. D. Dayie), Mr. Fleischer C. N. Kotey, and Ms. Mary-Magdalene Osei, all of the Department of Medical Microbiology, University of Ghana Medical School (UGMS), and all other members of the Department who contributed to the success of this research.

I am also grateful to my loving family for their support and encouragement over the years. A huge appreciation goes to my sister, Elikem Addae-Nuku, for her encouragement and the finances she expended in support of my postgraduate study. To my colleagues, friends, and all who played a role directly or indirectly to make this project a success also, I say "Thank you!"

TABLE OF CONTENTS

ABSTRACT

Background: Antimicrobial resistance (AMR) is one of the top ten (10) public health threats. It is, therefore, imperative to scale up efforts aimed at tackling the AMR menace. One approach could involve expanding the range of AMR surveillance targets to include hospital wastewater (HWW), a target that has largely been overlooked by researchers.

General Aim: The aim of this study was to evaluate the occurrence of multidrug-resistant bacteria in hospital wastewater of the Korle Bu Teaching Hospital (KBTH).

Methodology: This was a longitudinal study involving a total of 288 hospital wastewater samples consecutively collected across twelve weeks from the pool of wastewater emanating from two critical care units of the Korle Bu Teaching hospital, that is, the Child Health Unit and the Maternity Unit on Mondays and Thursdays, each week. The samples were immediately transported on ice to the laboratory and cultured for bacteria, which were identified using the Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) technique and subjected to antimicrobial susceptibility testing via the Kirby-Bauer method.

Results: In total, 294 bacteria of 23 different types, all being Gram-negative, were isolated from the 288 samples. The predominant ones were *Escherichia coli* (30.6%, n = 90), *Klebsiella pneumoniae* (11.2%, n = 33), *Citrobacter freundii* (10.9%, n = 32), *Alcaligenes faecalis* (5.8%, n =17), and *Pseudomonas mendocina* (5.4%, n = 16). *Escherichia coli* was the only organism whose proportion significantly differed between the two units studied [Maternity Unit = 23.8%, n = 37; Child Health Unit = 38.0%, n = 53, p = 0.02], and it persisted for weeks 1 to 6 in the wastewater from the Maternity Unit and Weeks 6 to11 in the wastewater from the Child Health Unit, while occurring intermittently during the other periods. The prevalence of multidrug resistance among the isolates was 55.4% (*n* = 163) [Maternity Unit = 53.4%, *n* = 87; Child Health Unit = 46.6%, *n* = 76, *p* = 0.22]. Moreover, the prevalence of extended-spectrum beta lactamase (ESBL) producers was 15.6% (*n* = 46) [Maternity Unit = 18.4%, *n* = 26; Child Health Unit = 13.1%, *n* = 20, *p* = 0.21]. *E. coli* accounted for the most ESBL-producing organisms (28.9%, *n* =26).

Conclusion: The wastewater generated by the Maternity and Child Health Units of KBTH harboured a wide range of multidrug resistant bacteria, with a good proportion of these being ESBL producers, and the predominant and persistent one being *Escherichia coli*. The study thus identifies the wastewater of KBTH as an important source of infection transmission, and underscores the significance of appropriate treatment of wastewater of the hospital and other clinical and related settings prior to its discharge.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AR……………………………………………………………………….………...Antibiotic residues

ARB……………………………………………………………...Antibiotic-resistant bacteria

ARG ……………..……………………………...…………………...Antibiotic resistance genes

BHIB………………..………………………………………………...…Brain heart infusion broth

CFU………………………………………………………………….…..Colony forming unit

CLSI………………………………………………...…Clinical and Laboratory Standards Institute

EC-MUG……………...………………….......*Escherichia coli* 4- methylumbelliferyl-β-glucuronide

ESBLEc ……………………………...……Extended-Spectrum Beta-Lactamase *Escherichia coli*

GWCL…………………..…………………...…………………………Ghana Water Company Limited

HPLC…………………………………………………High Performance Liquid Chromatography

HRT……………………...……………………………………...……… Hydraulic Retention Time

HWW………………..……………………………...………………………Hospital wastewater

HWWTP…………………………………………………...…Hospital wastewater treatment plant

KATH……………………………………………...…….........Komfo Anokye Teaching Hospital

KBTH……………………………………………………………....Korle Bu Teaching Hospital

LCMS/MS……………………Liquid Chromatography Mass Spectrometry/ Mass Spectrometry

LoD…………………………………………………………………………...…Limit of Detection

LTB…………………………………………………………………....Lauryl Tryptose Broth

MIA……………………………..……..…………………………..….Microbial Inhibition Assay

MPN…………………………………………….……………………Most Probable Number

MWWTP……..……………………...………………....Municipal WasteWater Treatment Plant

OPD……………………………………………...…………………Out Patient Department

SRT…………………………………………………………………..Solid Retention Time

TGYA……………………………………………………Tryptone Glucose Yeast Extract Agar

WHO…………..……………………………………………...…World Health Organisation

WWTP………………………………………………………….Wastewater Treatment Plant

WSP……………………………………………………………......Waste Stabilization Pond

CHAPTER ONE

1.0 INTRODUCTION

1.1Background

Hospital wastewater is any water generated from cleaning laboratories and other facilities within hospitals. It is a major source of environmental pollutants, as it contains pathogens and other potential contaminants of the aquatic environment (Prado *et al*., 2010; Gheethi *et al*., 2018). Moreover, as it is often discarded without prior treatment, it has a high potential as a source of human and animal infection, particularly, with those of multidrug-resistant pathogens (Wang *et al*., 2018a). Properly disposing liquid clinical waste is becoming a herculean task in the 21st century due to an increase in contagious and deadly diseases treated in healthcare settings and a variety of dangerous pathogens encountered. The act of discharging clinical liquid waste in the sanitary sewer into the aquatic environment and on bare soil has become normal, especially in developing countries (Fianko *et al*., 2016). Chemical and liquid wastes from hospital laboratories are dumped into public sewers without treatment, exposing a section of the public to infections and other healthcare hazards during floods due to blocked drains. Many developed countries have, however, developed strict guidelines to deal with the collection, segregation, storage, transportation, and disposal practices of hospital wastewater. Drug-resistant microbes have, over the years, spread and multiplied as a result of the unsuitable and unprofessional use of antibiotics in healthcare and community settings (Azanu *et al*., 2018).

The fact that many microbes are becoming increasingly resistant to antibiotics is a huge concern in the health industry given its contribution to the rise in clinical deaths, which could otherwise have been treated (Azanu *et al*., 2018). Antimicrobial resistance is prevalent in both developed and developing countries. Ghana, for instance, is dealing with multidrug-resistant pathogens like methicillin resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Enterobacteriaceae*, which are dangerous to human health (Donkor *et al*., 2019; Appiah *et al*., 2020; Donkor & Kotey, 2020; Dayie *et al*., 2021; Egyir *et al*., 2021). According to Jury *et al*. (2010) and Colque *et al*. (2019), antibiotic resistant strains are able to multiply in the gastrointestinal tract of humans and animals as a result of exposure to high concentrations of bacteria and sub-lethal doses of antibiotics.

1.2 Problem statement

The World Health Organization (WHO) has stated antimicrobial resistance as one of the top ten public health threats facing humanity (WHO, 2020). The cost of antimicrobial resistance to the economy is significant, spanning across prolonged illness and longer hospital stays, the need for more high-priced medicines, thereby, causing financial challenges for those impacted, disability, and death (Ledingham *et al*., 2019). Presently, not less than 700,000 lives are lost each year due to drug-resistant diseases (WHO, 2019). By 2030, AMR could force up to 24 million people into extreme poverty, and by 2050, it could result in the loss of 10 million lives each year (WHO, 2020). It is therefore imperative to scale up efforts aimed at effectively tackling the AMR menace. One approach could involve expanding the range of AMR surveillance targets, such as expanding to include hospital wastewater, which is a significant source of multidrug-resistant pathogens (Husain *et al*., 2020).

1.3 Justification

The high public health threat posed by AMR demands that efforts aimed at curbing the menace are done holistically. One approach could involve expanding the range of AMR surveillance targets, such as expanding to include hospital wastewater, which is a significant source of multidrug-resistant pathogens (Husain *et al*., 2020). That notwithstanding, the majority of AMR surveillance efforts have focused on human and animal populations (Egyir *et al*., 2016; Donkor *et al*., 2019; Ekli *et al*., 2019; Adzitey *et al*., 2020; Dayie *et al*., 2021a; Dayie *et al*., 2021b), with little attention paid to the environment. In this context, the environment refers to ambient air, soil, water, and other locations that are neither on nor within the bodies of humans and animals, as conceptualized by Huijbers *et al*. (2019). Elsewhere, AMR surveillance in the environment has spanned a variety of targets, including hospital wastewater, domestic wastewater, surface water, and sewage (Caplin *et al*., 2008; Korzeniewska *et al*., 2013; Zhang *et al*., 2013; Blaak *et al*., 2015; Kwak *et al.*, 2015; Li *et al*., 2015; Daoud *et al*., 2017; Buelow *et al*., 2018; Müller *et al*., 2018; Aarestrup *et al*., 2020). The little attention paid to the environment presents a major setback to having robust AMR surveillance data, a key critical to unlocking the AMR conundrum, and may explain some of the bottlenecks regarding the effective management of the AMR menace. As filling the identified knowledge gaps is important, this study aimed to evaluate the occurrence of multidrug-resistant bacteria in wastewater of the Korle Bu Teaching Hospital (KBTH). Specifically, the prevalence of different bacteria present in the hospital wastewater, the spatio-temporal distribution of the predominant wastewater-contained bacteria, and the prevalence of antimicrobial resistance and multidrug-resistant organisms, especially, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* among the hospital wastewater isolates, were investigated. Such insights, particularly, the distribution of HWW bacteria spatially and temporally, are limited in sub-Saharan Africa (Moges *et al*.,2014; Fekadu *et al*.,2015; Anssour *et al*., 2016; Asfaw *et al*., 2017; Tesfaye *et al*., 2019).

1.4 General aim

The aim of the study was to evaluate the occurrence of multidrug-resistant bacteria in hospital wastewater of the Korle Bu Teaching Hospital.

1.5 Specific Objectives

The specific objectives of the study were:

1. To determine the prevalence and types of bacteria present in the hospital wastewater.
2. To determine the spatio-temporal distribution of the predominant hospital wastewater-contained bacteria.
3. To determine the prevalence of multidrug-resistant organisms, especially, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* among the hospital wastewater isolates.
4. To determine the antibiogram of the bacteria isolated from the hospital wastewater.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Hospital Wastewater

Any volume of water whose quality has been affected by human activities is referred to as wastewater (Fekadu *et al*., 2015). Hospitals generate lots of wastewater, which could be hazardous or non-hazardous, and pose serious health problems to individuals in the community (Ali *et al*., 2017; Husain *et al*., 2020). Hazardous, infectious or clinical wastewater, comprises liquid wastes from hospital laboratories, wards, surgical theatres, pharmacies, mortuaries, healthcare workers, patients and visitors (Fianko *et al*., 2016). Hospital wastewater is also a changing system in which pharmaceutical conjugates and biological alteration products are formed, with some of these products being pharmaceuticals themselves (Kovalova *et al*., 2012). Thus, hospital wastewater is very dangerous, as it contains drug residues, pathogenic microbes, chemicals, cultures from laboratory, blood, cultures of infectious agents, discarded vaccine, chemical toxins, biological products (infectious viruses and bacteria), unused and excreted non-metabolized pharmaceuticals and organohalogen compounds such as the halogenated organic compounds absorbable on activated carbon (Fianko *et al*., 2016). The makeup or composition of hospital wastewater discharged differs between countries, and this can be associated to the size of the hospital, proportion of in and out patients, practices, and the kind of services rendered at the various hospitals. The waste aggregation options available, proportion and use of reusable items, wealth of user and finally the growth of the country, also contributes to the makeup of hospital wastewater discharge (Aththanyaka *et al*., 2014). Although it has been stated by Fianko *et al*. (2016) that hospital wastewater discharge is dependent on the size of the hospital, it was estimated in a study by Aththanyaka *et al*. (2014) that the consumption of water was 400 to 1200 liters/day/bed for developed countries and 500 liters/bed/day for developing countries. Fianko *et al*. (2016) further estimated from a study conducted in Sunyani on the clinical management of three healthcare facilities, that the highest liquid waste was generated from the wards, that is, 7,817 liters/day, of which 199 liters and 7,618 liters were pathological and infectious wastes respectfully, followed by the surgical theatre which generated a total of 1,620 liters of which 560 liters were pathological waste and 1,060 liters were infectious waste, followed by the laboratory which generated 192.5 liters of which 162 liters were pathological wastes and 30.5 liters were infectious wastes. This was closely followed by 987 liters generated from the mortuaries of which 412 liters were pathological wastes and 575 liters were infectious wastes. Also, 45.5 liters and 19 liters of infectious wastes were generated from the pharmacy and clinics respectfully.

Due to the maximized use of antibiotics in hospitals, hospital wastewater contains more antibiotic residues and antibiotic-resistant bacteria than domestic wastewater (Fekadu *et al*., 2015).

2.2 Composition of Hospital Wastewater

Bacteria, antibiotic residues and antibiotic-resistance genes detected in untreated hospital wastewater are also sourced from human excreta and urine (Maheshwari *et al*., 2016). Itzel *et al*. (2018), in a study, noted that wastewater treatment plants (WWTPs) are not designed for the specific removal of micro pollutants. These pollutants are, however, disposed into receiving water surfaces in ng/L to µ/L range. Hospital wastewater can have several genotoxic, immunotoxic, cytotoxic and endocrine disrupting effects on these aquatic organisms. The abundance of *Escherichia coli* in the hospital environment, as stated by Wang *et al*. (2018), is characteristic of the environmental risk posed by humans, most especially patients on antibiotics, as *Escherichia* *coli* is dominant in the intestinal tracts of humans. The pH values of hospital wastewater has been found to be slightly acidic after satisfactory removal of BOD_5 (biochemical oxygen demand over 5 days), COD (chemical oxygen demand over 5 days), and $N - NH_3$(ammonium), this is an indication of the presence of high concentration of chemicals in hospital wastewater (Prado *et al*., 2010).

2.2.1 Microorganisms

Bacteria may be present in hospital wastewater, ground water, and municipal water. *Escherichia coli* is present in untreated hospital wastewater and in ground water, but absent in municipal water (Diwan *et al*., 2010; Wang *et al*., 2018). Bacteria akin to the genera *Aeromonas, Citrobacter, comamonas, Pseudochrobactrum, Morganella* and other opportunistic pathogens such as *Escherichia, Enterococcus, Acinetobacter, Proteus, Streptococcus, Pseudomonas Myroides*, et cetera, are prevalent in untreated hospital wastewater (Wang *et al*., 2018). Drug resistant bacteria, such as *Klebsiella* spp., are frequently isolated from untreated hospital wastewater, followed by *Escherichia coli*, *Pseudomonas* spp., *Citrobacter* spp., *Staphylococcus aureus* and *Shigella* spp., (Moges *et al*., 2014).

Strongyloides stercoralis, which was prevalent, followed by hookworms and *Opisthorchis viverini* were detected in treated hospital wastewater samples evaluated by Assanasen (2014) in their study of microbial and heavy metal contamination in treated hospital wastewater in Thailand.

Human adenoviruses (HAdV), rotaviruses A (RV-A), hepatitis A viruses (HAV) and noroviruses (NoV) Genogroup I and II have been found in hospital wastewater treatment plants (Prado *et al*., 2010). NoVs Genogroup II are responsible for acute gastroenteritis diseases due to NoVs. Noroviruses are excreted in faeces by infected patients and are eventually deposited into the acquatic environment via sewage or night soil systems (Kishida *et al*., 2012). NoVs are not completely eliminated in conventional treatment system and also, they persist for long periods, with temperature and solar radiation being a factor once they are discharged into the recipient water. NoVs are mostly detected in areas where fecal contamination exist in water environments (Kishida *et al* ., 2012). In a study of river basins used by some communities in drinking and cooking, it was observed that the presence of viral contamination was directly linked to the cases of acute gastroenteritis diseases in patients. The presence of viruses in the river basins was also due to the discharge of water through the wastewater treatment systems into the river basins, because NoVs are shed in faeces of infected patients. The detection of viruses in river water has become public health concern as most rivers receive effluents from wastewater treatment plant upstream, and supply drinking water through treatment plants downstream (Kishida *et al*., 2012). A study in Tunisia on three different neighbouring clinics by Ibrahim *et al*. (2018), reported the presence of high human adenoviruses (HAdVs) molecular detection frequency in 65 (64%) samples out of 102 hospital wastewater samples used. The fact that human adenoviruses have been recorded at high rates in treated hospital wastewater is a prerequisite for necessary steps to be taken for wastewater treatment surveillance (Ibrahim *et al*., 2018).

2.2.2 Antibiotic Resistance and Antibiotic Resistant Bacteria

The ability of bacteria, through genetic mutation or by obtaining antibiotic resistance genes (ARGs) to repel the effects of an antibiotic which they were initially susceptible to is termed as antibiotic resistance (Amarasiri *et al*., 2020). Resistance to antibiotics stems mainly from antibiotic misuse as a result of off label prescriptions, potential interactions with other drugs, non-completion of treatment, inadequate protocol implementation, mal-administration, partial treatment, lack of follow ups by health physicians, occurrence of error due to a disorder in the frequency between

IV and oral formulation of an antibiotic (Garcia-Vello *et al*., 2020). The reasons for these are due to the absence of a well- structured antibiotic stewardship programme (ASP), and also because of improper antibiotic management among health workers (Garcia-Vello *et al*., 2020). Another substantial reason for the development of antibiotic resistance is simply because, in Ghana, for instance, there are about 80% of lower healthcare facilities than hospitals (Ahiabu *et al*., 2016). These lower healthcare facilities generally prescribe higher numbers of antibiotics as compared to the hospitals. The National Health Insurance Scheme (NHIS), also plays an important role in the prescription practices of antibiotics, considering the difference in insurance coverage among outpatient compared with the general population of Ghana. Most health facilities are profit based, also, because NHIS has standardized reimbursement rates, it influences the prescription of antibiotics which again, are also viewed by both health professionals and the public as potent medicines (Ahiabu *et al*., 2016). Moreover, in the treatment of common infections like candida, sore throat and common flu, antibiotics play a key role. Afriyie (2014), however, suggested that the generic use of antibiotics has resulted in vast cases of antibiotic resistance. Although ARBs were detected in three hospitals that were sampled for a study, higher loads of the ARBS were found in the clinical isolation wards. In the same way, hospital wastewater discharged from wards with patients recovering from severe infection tends to contain higher levels of ARBs and this only plays a major role in the spread and emergence of ARBs, that is, if wastewater is not properly treated in the downstream environment, making this one of the major reasons why hospital wastewater should be sufficiently treated in wastewater treatment plant before its final release into the aquatic environment (Le *et al*., 2016). Without prior treatment of wastewater before its discharge into the treatment plants, effluents that contain large amounts of antimicrobials always exert huge pressure on ARBs (Hocquet *et al*., 2016). In the wastewater microbiome, disinfectants and heavy metals with antimicrobial activity may favour the perseverance of ARB. The selective pressure which is exerted on antimicrobials favours and promotes interspecies and intraspecies horizontal gene transfer of resistance genes (Hocquet *et al*., 2016).

Lee *et al*. (2016), in a study in Ghana about occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital, came to a conclusion that parts of Ghana prescribed more antibiotics in their hospitals and pharmacies than other countries such as Iran. Lee *et al*. (2016) further concluded that even though the concentration of specific antibiotics may be high in hospital wastewaters, this does not necessarily mean high resistance of bacteria to that antibiotic. A contributing factor to antibiotic resistance, especially among *Enterobacteriaceae*, is as a result of the use of antibiotics in animals in the retail food chain (Carlet *et al*., 2011). More so, *Escherichia coli* and *Salmonella* strains which showed resistance to fluoroquinolones and third generation cephalosporins in a study by Carlet *et al*. (2011) have been associated with meat and poultry products. Prudent use of antibiotics minimizes or lowers the risk of serious clinical problems, such as cost of retreatment, drug resistance, and super infections associated with antibiotic abuse and misuse (Carlet *et al*., 2011).

Staphylococcus aureus, a Gram positive bacterial pathogen responsible for a wide range of skin infections, pneumonia and septicaemia, in a research conducted by Moges *et al*. (2014) was observed to be 100% resistant to ampicillin, an isolate of *Staphylococcus aureu*s also showed resistance to 11 antimicrobials, which included methicillin, making it multidrug resistance but was however, susceptible to vancomycin. All *Staphylococcus* isolates were reported by Sakkas *et al*. (2019) to be resistant to penicillin and oxacillin when the cefoxitin disc was used for analysis. Also, amongst six methicillin resistant *Staphylococcus aureus* (MRSA) strains, one was found to be resistant to vancomycin. Dayie *et al*. (2021), in a study in Accra, on nasopharyngeal carriage and antimicrobial susceptibility profile of *Staphylococcus aureus* among children under five years in Accra, also confirmed reports of *Staphylococcus aureus* resistance to penicillin (98%), amoxiclav (20%), tetracycline (18%) and ampicillin (100%). The reason for the resistance of *Staphylococcus aureus* to β-lactam antibiotics like penicillin as given by Dayie *et al*. (2021) is because of its accessibility, that is, they are over the counter drugs and easily accessible, relatively cheaper than other antibiotics and lastly because they have been on the market for a very long time, hence reasons for their great misuse. Similarly, amoxiclav resistance by *Staphylococcus aureus* is as a result of amoxiclav being used in Ghana as a frontline presumptively prescribed antibiotics (Dayie *et al*., 2021).

Pseudomonas aeruginosa, is a nosocomial infection of the urinary and respiratory tract, usually common in the intensive care units (Breathnach *et al*., 2012). 15% of nosocomial infections are attributed to this pathogen (Luczkiewicz *et al*., 2015). *Pseudomonas aeruginosa*, unlike *Escherichia coli* is not a commensal bacterium, where the frequency of carriage among inpatients is high but comparatively low in *Pseudomonas aeruginosa*. There are many locations within the hospital water network where this bacterium can be located. *Pseudomonas aeruginosa* can be located at distal, better oxygenated parts of the water distribution system like the sinks, U bends, taps and the toilets (Hocquet *et al*., 2016).Refraction or resistance of *Pseudomonas aeruginosa* to many classes of antibiotics is through chromosomal mutations or horizontal gene transfer (Hocquet *et al*., 2016). Isolates of *Pseudomonas aeruginosa* (100%), *Klebsiella pneumoniae* (95.8%) and *Escherichia coli* (83.3%) were analyzed and found to be resistant to three or more classes of antibiotics. The treatment of *Pseudomonas aeruginosa* infections may be complicated because of its intrinsic resistance to many classes of antibiotics and its ability to acquire resistance to many effective antibiotics as well (Hocquet *et al*., 2016). Some isolates of *Klebsiella* *pneumoniae* were resistant to carbapenems while some of those resistant to carbapenems were also resistant to tigecycline (Moges *et al*., 2014). In one study, Tesfaye *et al*. (2019) observed that *Citrobacter, Enterobacter*, and *Escherichia coli* were multidrug-resistant and more common in isolates obtained from hospital wastewater than from other sources of wastewater. Also, other opportunistic human pathogens such as members of the genera *Myroides, Enterococcus, Proteus, Pseudomonas,* and *Streptococcus* were found to be prevalent in the multiple antibiotic resistant bacteria communities (MARB) in the hospital wastewaters (Wang *et al*., 2018).

Even though the number of ARBS in hospital wastewater is minimized after treatment with sewage processing techniques, various resistance factors are still present in greater proportions in hospital wastewaters than in surface waters. Also, wastewater treatment plants have been reported to provide the appropriate environment for the growth and propagation of ARBs (Wang *et al*., 2018). The widespread development of drug-resistance among pathogens has become one of the most serious challenges worldwide (Asfaw, 2019).

2.2.3 ESBL-producing Enterobacteriaceae

A study conducted by Hocquet *et al*. (2016) on antibiotic resistance in hospital wastewater systems, have shown high frequency of human intestinal carriage ESBLEc in both hospital and community settings. The *Escherichia coli* load in the hospital effluents was 3.8% - 39% higher than in urban effluents but within the same range of $\backsim 10^5 - 10^6$cfu/mL as in community effluents. This occurrence can be attributed to the high carriage rates of ESBLEc among in-patients in hospitals than in communities. The presence of large amounts of antibiotics and disinfectants in hospital wastewater is a contributing factor to the high survival rate of ESBLEc in the hospital wastewater network (Hocquet *et al*., 2016). On the contrary, *Escherichia coli* concentration was slightly greater in urban wastewaters than in hospitals, this is because of the high dilution rate in hospital wastewater resulting from the high daily water consumption per bed (Hocquet *et al*., 2016).

Escherichia coli in a study by Diwan *et al.*(2010) and Moges *et al*. (2014), showed resistance to ciprofloxacin, ampicillin and amikacin, whereas they showed sensitivity to amoxicillin and other antibiotics that were tested. Some Gram negative isolates including *Citrobacter* spp and *Enterobacter* spp were reported to be 100% resistant to ampicillin in a study by Moges *et al*. (2014). The above stated Gram negative isolates were also resistant to cotrimoxazole (38%), tetracycline (37%), naldixic acid (36%), cephalotin (49%) and cefotaxime (33%) but showed least resistance to ciprofloxacin (12%) (Diwan *et al*., 2010). In a study by Moges *et al*. (2014) as mentioned earlier, *Enterobacter* spp showed resistance to 12 antibiotics, *Klebsiell*a also showed resistance to 11 antibiotics, and *Pseudomonas* spp showed refractory to 10 antibiotics. Some Gram positive bacteria also exhibited multiple drug resistance to frequently used antibiotics in a study site at Gondar, Ethiopia (Moges *et al*., 2014).

2.2.4 Antibiotics and Antibiotic Residues

Antibiotics are incorporated in medicines and widely used by humans and animals to treat and prevent diseases (Jury *et al*., 2010). They are naturally occurring or synthetic chemical compounds with antimicrobial activities (Jury *et al*., 2010; Tesfaye *et al*., 2019). Antibiotics were one of the most prescribed drugs followed by analgesics in a health facility survey conducted in the Eastern region of Ghana (Ahiabu *et al*., 2016). In Ghana, some of the most prescribed antibiotics are amoxicillin, metronidazole, flucloxacillin, amoxicillin/clavulanic acid, ciprofloxacin, erythromycin, chloramphenicol and co-trimoxazole (Ahiabu *et al*., 2016; Azanu *et al*., 2018). The high prevalence and concentration of antibiotic residues detected in the hospital wastewater suggests that hospital wastewater is a major source of antibiotic contamination and a greater cause for public health concern (Petrovich *et al*., 2020). Pharmaceuticals like antibiotics (ciprofloxacin, erythromycin, tetracycline et cetera) are also known as emerging organic contaminants and are mostly endocrine disruptors or carcinogenic and this is usually based on the level of concentration detected (Bhattacharya, 2017). Wang *et al*. (2018) in a study ascertained that the types of antibiotic residues detected in untreated hospital wastewater samples also reflect the various antibiotics prescribed to patients. A research by Wang *et al*. (2018) on the occurrence and diversity of antibiotic resistance in untreated hospital wastewater revealed the presence of antibiotic residues, which included tetracyclines, sulfonamides, macrolides, quinolones, cephalexin, lincomycin and trimethoprim in hospital wastewater samples collected from three tertiary public hospitals in Xinxiang City, central China, in January 2016 and July 2016. Also, ciprofloxacin and sulfamethoxazole were detected in a study by Paulus *et al.* (2020) at concentrations of 2706 ng/L, 3752 ng/L and 367ng/L, 269ng/L respectively in two different hospitals. These concentrations were several orders of magnitude higher than in community wastewater samples (Paulus *et al*., 2020). However, erythromycin was not detected in both HWWTPs influent and effluent samples used by Al Qarni *et al*. (2016) in a study on investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. Among these, eleven antibiotic residues were detected in all samples analyzed (Al Qarni *et al*., 2016). The occurrence of quinolones, tetracyclines and cephalexin antibiotic residues were more prevalent than those of macrolides and sulfonamides in all wastewater samples. Municipal water and ground water were free from antibiotic residues as reported by (Diwan *et al*., 2010). Also, ceftriaxone, one of the most-prescribed antibiotics and other antibiotics with the β-lactam ring have never been detected in hospital effluents because of the easy degradation of the β-lactam ring, its high metabolic rate and the processes of decarboxylation (Diwan *et al*., 2010). Six (6) antibiotics residues out of eight (8) antibiotics used for a study by Tamhankar *et al*. (2013) in a teaching hospital wastewater samples were recorded. The antibiotics detected were the sulfonamides (sulfamethoxazole), imidazoles (metronidazole), and fluoroquinolones (ciprofloxacin, levofloxacin, ofloxacin, norfloxacin). Cephalosporins (ceftriaxone and cefeperazone) on the other hand were not detected in hospital wastewater samples used for the studies. High concentrations of antibiotics which included ciprofloxacin, clarithromycin and sulfamethoxazole were found in hospital wastewater influent in a study conducted in Saudi Arabia by Al Qarni *et al*. (2016). Similarly, high amounts of ciprofloxacin (11,352 to 15,733 ng/L) and erythromycin (7944 to 10613 ng/L) and low concentration of tetracyclines were found in hospital wastewater samples collected from KATH by Azanu *et al*. (2018) in a research on occurrence and risk assessment of antibiotics in water and lettuce in Ghana. It is worth noting, that in Azanu *et al*.'s (2018) research, the maximum concentration of ciprofloxacin that was detected in hospital wastewater and river samples analyzed were 15µg/L and 1.2µg/L respectively, this is respectively 150 and 2 times greater than the proposed concentration of 0.1µ/L purported to promote antimicrobial resistance. Generally, detecting high level of antibiotics in raw hospital wastewater is expected due to the high consumption of antibiotics in the hospitals. Even though concentrations of antibiotic residues in hospital wastewater varies across hospitals, similar dimensions of concentrations of ciprofloxacin, sulfamethoxazole and metronidazole have been recorded (Paulus *et al*., 2020). However, in Ghana, very little information on the actual use of antibiotics by humans is available due to the weak systems for the regulations of medicines. Obtaining data on the quantities of antibiotics sold are very difficult to come by. Tamhankar *et al*. (2013) and Diwan *et al*. (2010), however, did not record the presence of norfloxacin and sulfamethoxazole in hospital wastewater sample collected from a non-teaching hospital in India. Similarly, Tamhankar *et al*. (2013), recorded no antibiotic residues from incoming water samples for hospitals.

The efficient removal of antibiotics from wastewater treatment plants varies depending on the treatment method adopted. Ciprofloxacin, for instance, in a published work by Al Qarni *et al*. (2016) was efficiently removed using the activated sludge treatment method, whereas the efficient removal of sulfamethoxazole was lower using the activated sludge treatment plant in temperate regions. It is to be noted, however, that removal efficiency is relatively higher during summer than in winter (Al Qarni *et al*., 2016). Overall, the removal of antibiotics in HWWTPs in a study was efficient during the hotter tropical climate seasons of Saudi Arabia. The higher temperatures of >26˚C which is available all year round in Saudi Arabia contributed to the removal efficiency of these compounds (Al Qarni *et al*., 2016). The reason is that tropical conditions may lead to an increase in microbial activity during the active sludge process, leading to an increase in the biodegradation kinetics. This process has been supported by the theory that microorganisms living in reactors reach their optimal activities usually at temperature rates within the range of 25˚C to 35˚C (Al Qarni *et al*., 2016). Nevertheless, the availability of sunlight which is important for photo degradation also plays a major role in the efficient removal of pharmaceutical compounds in hospital wastewater. However, the removal of pharmaceutical products from effluents does not signify absence or reduced level of toxicity (Al Qarni *et al*., 2016).

Rivers, streams and other aquatic bodies are at the receiving end of partially treated or untreated hospital wastewater and this explains the reason for the occurrence of antibiotics in the rivers and streams (Azanu *et al*., 2018). A study in Kumasi by Azanu *et al*. (2018) indicated that all water bodies were significantly contaminated with antibiotics and as such the concentration of antibiotics recorded in rivers were usually high with ∽3μg/L, while the concentration in irrigation water were comparatively low with ∽0.2µg/L. These concentrations were influenced by the wastewater discharge from surrounding hospitals. Sulfamethoxazole (2861 ng/L) recorded the highest concentration in river samples analyzed by Azanu *et al*. (2018), followed by ciprofloxacin and erythromycin. Amoxicillin was least represented with a maximum concentration of 2.7 ng/L. Also, the concentrations of antibiotics detected in the upstream samples collected before entry of WSP effluents was lower than antibiotics concentrations detected and recorded in the midstream and downstream river samples (Azanu *et al*., 2018). Overall, for wastewater used for irrigation purposes, occurrence of antibiotics detected in a study suggested that the concentrations of antibiotics in water used for the irrigation of vegetables were lower than the concentration of antibiotics detected in river which ranged from <LOD to 146 ng/L for ciprofloxacin. However, differences in concentrations of tetracycline and cefuroxime residues detected in a river sample compared to its concentration in the irrigation water sample was statistically significant, with tetracycline ($p=0.04$) and cefuroxime ($p=0.01$). Differences in concentrations were, however, not significant for ciprofloxacin, metronidazole, erythromycin, trimethoprim, sulfamethoxazole, amoxicillin, ampicillin, oxytetracycline, chlortetracycline and doxycycline (Azanu *et al*., 2018). Again, out of 12 antibiotics that were used in a study in the Ashanti region of Ghana, five (erythromycin, ciprofloxacin, cefuroxime, sulfamethoxazole, metronidazole) were detected in market samples, trimethoprim and ampicillin together with these five antibiotics were also detected in farm samples (Azanu *et al*., 2018).

Antimicrobial resistance is gradually built by the selective pressure that is exerted onto the wastewater micro flora as a result of the chemical residues present in the hospital wastewater. Hospital wastewater, in a study by Paulus *et al*. (2020), was found to contain more antibiotic resistance-genes and antibiotic residues as compared to wastewater samples from the communities and urban wastewater treatment plants. In a study conducted in Netherlands, it was reported that hospital wastewater samples exhibited .04 to1.8 fold higher, relative to antibiotic resistance-genes concentration than community wastewater samples. However, no ARGs were detected in significantly higher concentrations in community wastewater samples. Comparatively, ARGs levels per milliliter in hospital wastewater samples and community wastewater samples differed significantly with 0.8 and 2.3 fold increase in the former (Paulus *et al*., 2020). The higher concentrations of ARGs detected in hospital wastewater implies higher occurrences of antibiotic resistance which can result in multidrug-resistant bacteria. Moges *et al.*(2014) further reported that seasons do not have effect on the occurrence of bacteria in untreated hospital wastewater. Similarly, hospital wastewater samples collected 100 meters away from hospitals were reported to have had higher numbers of antibiotics and higher antibiotic residual levels than samples collected in close proximity to the hospital (Diwan *et al*., 2010). In fact, Azanu *et al*. (2018) concluded in a study that 94% of antibiotics detected in water bodies were from hospital effluents and the remaining 6% were from WSPs. Diwan *et al.* (2010) in another study discovered a link between antibiotics prescribed to in-patients and the antibiotics detected in the hospital effluents. Gupta (2017) indicated in a study on sources, effects and control of water pollution, that contamination of an aquatic environment affects places that are hundreds or even thousands of miles away. This correlation is as a result of factors such as the metabolism of antibiotics, water flow rate, time of sample collection, temperature etc. On the contrary, Wang *et al*.(2018) in a study, reported that the antibiotic residues that were detected in the hospital wastewater samples used for survey on the occurrence and diversity of antibiotic resistance in untreated hospital wastewater did not positively correspond with the abundance of the various ARGs and ARBs, indicating that these ARBs were already present in the human excreta, especially intestinal excreta. It is important to note that the types and mass load of the various antibiotic residues found in hospital wastewater is dependent on the geographical location and the type of healthcare facility as well (Khan *et al.*, 2020).

2.3 Management of Hospital Wastewater

Managing hospital wastewater is a serious issue in many parts of the world, as mismanagement can result in health, safety and environmental risk to inhabitants. Most developing countries like Asia, Africa and the Middle East have legislature governing the disposal of wastes, of which hospital wastewater is inclusive, however, the implementation of these regulations are short coming. Rachel e*t al*. (2012) confirmed in a study that hospital staff responsible for wastewater management are very few and marginalized. Sadly, these category of staff are undertrained, resulting in alarmingly low level of knowledge and awareness regarding effective management of hospital wastewater. Moreover, most of these categorized staff are not immunized and they do not wear protective clothing when handling these wastes, as such, they are mostly infected through inhalation and dermal exposures (Rachel *et al*., 2012). Some individuals who are exposed to wastewater include: people residing near spray irrigation sites and spray irrigators (Rachel e*t al*., 2012). Most scavengers on sites where some of these wastewater are dumped are also not immunized, exposing them to adverse health risks (Rachel *et al*., 2012).

Hospital wastewater requires additional efforts to ensure safe storage, conveyance and treatment (Aththanyaka *et al*., 2014; Fianko *et al*., 2016; Ibrahim *et al*., 2018; Husain *et al*., 2020). Developing countries require special attention in the management of hospital wastewater due increased urbanization, lack of resources and the relatively large population. Most developing countries do not have the expertise and technology that are necessary in the effective management of hospital wastewater, hence, steps to be taken to reduce the adverse effects of such wastes include: waste reduction, minimization and source segregation (Ali *et al*., 2017)

2.4 Treatment of hospital wastewater

Treatment employed by the various hospitals affect the quality of water discharged into water bodies which are eventually used for cooking, drinking and irrigation (Diwan *et al*., 2010; Beattie *et al*., 2020). Bhattacharya (2017) explained that contaminated drinking water can be detected by scrutinizing or examining the odor, taste, color and turbidity of the water. However, most contaminated water cannot be easily detected unless tested in the laboratory. Efficient treatment of hospital wastewater is vital to the health of individuals because dissemination of poorly treated hospital wastewater into the aquatic environment and its consumption has adverse effects on inhabitants, this, is usually dependent on the individual's vulnerability and immune strength as explained by Bhattacharya (2017). The EPA (2015), however, did not find the correlation between long-term exposure to low levels of pharmaceuticals in drinking water and adverse health implications. A similar study conducted by Beattie *et al*. (2020) on survivor of microbial population in post chlorinated wastewater, identified the presence of multiple microbial taxa in treated and disinfected effluents, these included potential pathogenic species such as *Escherichia coli*, and β-lactam resistant microbial communities. Similarly, findings by Lien *et al.* (2017) in a study conducted on antibiotic resistance and antibiotic-resistance genes in *Escherichia coli* isolates from the hospital wastewater in Vietnam, reported that even after treatment, antibiotic resistant bacteria and antibiotic-resistance genes were found in hospital wastewater. Fekadu *et al*. (2015), also revealed in a study that hospital effluents that are released into recipient water bodies contain antibiotic-resistant bacteria and eventually pose public health threat.

Disposal of clinical wastewater has been defined by Fianko *et al*. (2016) as the final placement of treated liquid waste on the land or into water bodies based on local rules and conditions. In most developing countries, hospital effluents do not undergo any treatment before disposal into the aquatic system (Khan *et al*., 2020). Besides, Fianko *et al*. (2016) also revealed in a study that the act of discharging untreated water down the sewer into water bodies and onto bare soil has been normalized in certain hospitals especially in developing countries. A typical example is the Accra Municipal Assembly (AMA) treatment plant, which initially diverted the sewage to the original pipe and further discharged the treated wastewater into the ocean. Now, with a faulty pipe, the wastewater is now discharged directly into the Korle Lagoon despite an ecological restoration project that is multi-million dollar (Murrel and Dreschel, 2011). Similarly, Azanu *et al*. (2018) has revealed in a study that wastewater from the Komfo Anokye Teaching Hospital (KATH), Kumasi, flows directly into surface water, serving as a great source of introduction of antibiotic residues and antibiotic resistant bacteria into the environment. Also, in the Sunyani municipality, treated clinical liquid wastewater is discharged into nearby rivers where it is used by inhabitants for drinking, cooking and irrigation purposes.

Lien *et al*. (2017) reported that the occurrence of antibiotic resistant bacteria is minimized in advanced wastewater treatment processes such as ozone, UV, and ultrafiltration. Even with such advanced treatment processes, resistant bacteria are not completely removed. Most hospital wastewater treatment plants used are inefficient in getting rid of the microorganisms and chemical residues, resulting in the transfer of these substances into the environment, an example is the high occurrence of antibiotic resistance and antibiotic-resistance genes detected in *Escherichia coli* isolates from hospital wastewater samples before treatment and after treatment (Lien *et al.*, 2017). Therefore, it has become necessary for hospitals to be provided with effective wastewater treatment plants with treatment processes that can completely get rid of antibiotic resistant bacteria (Lien *et al*., 2017; Asfaw, 2019). Analytical evaluation, periodic assessment and treatment of hospital wastewater that is released into the aquatic environment is necessary to limit or reduce its public health impact (Asfaw, 2019).

Treatment method is any technique used to change the chemical and physical composition of any waste (Fianko *et al*., 2016) . The need to protect the aquatic environment and provide potable water has led to the use of science-based solutions for hospital wastewater treatment methods that provide solution to the problem. It has therefore become necessary that the quality and quantity of hospital wastewater be given the necessary attention (Bhattacharya, 2017). Although the effective solution to be used is dependent on the nature and extent of contamination and infrastructure, that is, power, man power, presence of chemicals, and cost of production as well as acceptability of technology, these play essential roles in the effective treatment of wastewater (Bhattacharya, 2017).

In most instances, hospital wastewater is directly connected to the urban sewer system without pre-treatment. Municipal sewage systems, however, have not been designed to remove medical or pharmaceutical wastes (Al Qarni *et al*., 2016).

Sedimentation, distillation and disinfection are some of the common wastewater treatment and purification methods as stated by (Bhattacharya, 2017).

2.4.1 Sedimentation

According to Fianko *et al*.(2016), many teaching hospitals in developing countries still use conventional treatment methods and waste stabilization ponds in the treatment of their clinical wastes. For example, a regional hospital in Sunyani uses on-site conventional treatment method, where the sedimentation tank processes are in three stages, that is, primary, secondary, and tertiary. Here, the grits and particulate matter, biological-floc, sludge, odour and chemical-floc are removed by pumping chlorine through a piping system into the sedimentation tank to disinfect the liquid of any bacteria present, afterwards it is left for a day or more, then it is finally dislodged into a tunnel connected to a stream (Fianko *et al* (2016). One of the main disadvantages of conventional treatment plant is the fact that effluent still contains large number of pathogens even after treatment, whereas the waste stabilization pond uses the suns energy for its operation and treatment by selecting appropriate organic loadings, retention periods and pond depth to promote the maximum growth of organic material needed for treatment, thus making it easier and more effective especially in the removal of total faecal coliform bacteria (Fianko *et al*., 2016). Fianko *et al*. (2016) in a microbial analysis that was made on a conventionally treated liquid waste revealed the total coliform and the faecal coliform to be 18 and 16 respectively, this however, does not meet the standard total and faecal coliform count of the WHO and the GWCL, which are 0 and 0 respectively. This is an implication of the presence of bacteria in treated effluents which is far above the standards of WHO and GWCL (Fianko *et al*., 2016). Results from Azanu *et al*. (2018) revealed that WSPs significantly minimises antibiotic loads to about 96% indicating that sewage sludge is the main reservoir of antibiotics as a result of their effective sorption properties.

2.4.2 Distillation

Distillation is the most common separation technique used in the treatment of water. Heat is applied in the separation technique to separate the mixed constituents of the wastewater. Separation is achieved based on the variance of the boiling points of the constituents, where water is separated from inorganic substances such as lead, calcium, magnesium etc (Bhattacharya, 2017). Distilled water is safe, however, it is not considered healthy for consumption purposes as it does not contain nutrient minerals (Bhattacharya, 2017). Treating water by the distillation method removes a wide range of contaminants which includes heavy metals, toxic chemicals, bacteria, viruses, parasites etc., also, treatment by this method does not require additional disinfection processes, and does not rely on physical barriers like filters. However, treatment by distillation requires careful maintenance to ascertain purity, and also, treatment of wastewater by this method makes use of huge amount of energy in terms of cooling and heating (Veenstra *et al*., 1997).

2.4.3 Disinfection

Disinfection method of treating wastewater is categorised into physical and chemical methods. The physical method involves using ultra violet radiation and the chemical method involves using chlorine (Bhattacharya, 2017). With the ultra violet radiation, the water to be treated is passed through germicidal ultraviolet light that is configured inside a low pressure lamp. The genetic components of the microbes are destroyed as the contaminants pass through the ultra violet purifier and are exposed to the ultra violet light (Kataki *et al*., 2021). Advantages of this method include its ability to destroy and inactivate pathogenic microbes, degrade some organic contaminants and, it also has no effect on minerals in water. This method, however, cannot be achieved without electricity (Bhattacharya, 2017) .

Chlorine together with its compounds chloramine or chlorine oxide are used in the disinfection of wastewater (Kataki *et al*., 2021). Chlorine acts against bacteria or protozoa that form cysts. One disadvantage of using chlorine is that it reacts with natural organic compounds in the water to form harmful chemical by-products such as trihalomethanes and haloacetic acids which have been shown to cause cancer (Bhattacharya, 2017).

Treating and disinfecting drinking water to reduce water-borne diseases is one of the major public health advances of the 20th century (EPA, 2015). No technology has been 100% effective in treating hospital effluents or contaminated water, so the concept of hybrid technology has been adopted to improve effective treatment of hospital effluents and contaminated water (Al Qarni *et al*., 2016). The biodegradation efficiency of some pharmaceutical compounds under anaerobic processes has fluctuated from no elimination to high elimination, an example is the high elimination rate that was observed in some antibiotics like sulfamethoxazole and natural oestrogens, and the no elimination rate that was observed in carbamazepine in a published article by Al Qarni *et al.* (2016). In using the biological treatment, it has been found that the level of removal efficiency is dependent on the physicochemical properties of the compounds, the type of wastewater treatment technology, the hydraulic retention time (HRT), the solids retention time (SRT) and the climatic conditions which include rainfall, dilution, level of sunlight and temperature (Al Qarni *et al*., 2016). The variations are mostly as a result of these parameters and other physicochemical properties of compounds that have effects on microbial activity and growth, thereby causing changes in the quality of effluents. Moreover, the temperature variations of the biological treatment processes can have effects on microbial activities and growth in the wastewater (Al Qarni *et al.,* 2016). Furthermore, the stability of temperature in the biological wastewater treatment process is an important factor in the overall removal of micro pollutants especially in arid and semi-arid areas like Saudi Arabia where the temperature is >25˚C (Al Qarni *et al*., 2016). This eventually leads to higher temperatures in wastewaters compared to both winter and summer conditions in temperate countries. Scarcity of rainfall and intense sunlight are also important factors in the removal efficiency of microbes in biological wastewater treatment processes (Al Qarni *et al*., 2016).

Among the treatment plants identified in a work by Murray and Drechsel (2011), about 30% were capable of treating influent but not capable of rendering it safe for public consumption and usage. Again, another observation was made by Murray and Drechsel (2011), that the problems with treatment plants were usually the result of institutional bottlenecks, missing aeration devices, failing electrical supply and broken pumps or motors. A further observation was made, that in facilities like the hospitals where the treatments plants were non-functioning, the wastewater generated was deliberately diverted into nearby streams or lagoons. Even though there are many treatment plants in Ghana, only a small percentage serves the population, for instance, 31 wastewater treatment plants (WWTPs) have been identified in Accra and have been shown to serve less than 10% of the population (Murray and Drechsel, 2011). The malfunctioning of most treatment plants in Ghana are not to be blamed solely on "inappropriate technologies", simply because in Ghana, very simple technologies like waste stabilization ponds (WSPs) and aeration tanks/ponds are used in the treatment of wastewater, but it is the reliance of most treatments plants on electricity that has contributed to its fragility as a result of irregular power supply in the country (Murray and Drechsel, 2011). A discussion with treatment plant operators further blamed the failure of these treatments plants on the cost of electricity and stealing of electrical power lines and its component (Murray and Drechsel, 2011).

In a study, Al Qarni *et al*. (2016) using the sand filtration treatment method, found it to have negligible effect on the removal of pharmaceutical products in wastewater. Chlorination, however, plays a relatively important role in the removal of some pharmaceutical compounds like ciprofloxacin and sulfamethoxazole, with the pH influencing the level of removal achieved (Al Qarni *et al*., 2016).

The hospital has a responsibility to protect the health needs of inhabitants in its environs because treated effluents which are disposed into nearby aquatic environments are used for irrigation purposes and are also, in most instances used by straying animals for drinking (Azanu *et al*., 2018). More so, inhabitants along the aquatic environments that are not connected to the GWCL pipeline depend on these streams for cooking and drinking water (Fianko *et al*., 2016). Another important factor to consider with inhabitants living around the sedimentation pool aside using the contaminated water to cook and drink is the fact that flies can go and sit on their drinking waters and food. Additionally, these wastewater may also permeate into the ground and eventually cause adverse health effects such as gastro enteric infections and diseases as a result of faecal coliform and *Escherichia coli*. Pathological, radioactive, chemical, infectious and pharmaceutical residues left untreated can escalate the outbreak of communicable diseases such as cholera, enteric illnesses, skin diseases and diarrhoea (Aththanyaka *et al*., 2014). Similarly, reports from hospitals and clinics within the Sunyani Municipal Assembly in a study by Fianko *et al*. (2016) suggested an increase in the cases of cholera, typhoid and diarrhoea due to water-borne related diseases. These effects emphasize the need for pre-treatment of hospital wastewater before its discharge, and an important reason to effectively train wastewater treatment operators and also ensure the functioning of these treatment plants.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Design

This was a longitudinal study.

3.2 Study Site

The study was conducted in the Greater Accra Region of Ghana. A referral hospital, Korle Bu Teaching Hospital was selected for this study because it is has a huge attendance and admission records. The Child Health Unit has an OPD attendance of 28,152 and 9,790 admissions and the Maternity Unit has a total OPD attendance of 83,001 and 14,070 admissions. It is also the premier health facility, serving an estimated population of 15 million people within southern Ghana. KBTH is equipped with 2000 beds and a central laboratory with several departments, including microbiology to which all microbiological specimens from patients are brought for processing. Hospital wastewater sampling was done at wastewater outlets of the two critical care units selected, Maternity and Child Health, where wastewater comes out of the wards after being contaminated with microorganisms (Diwan *et al.*, 2010). All the wastewater from the Maternity Unit exit through one channel into a manhole. This is similar for wastewater from the Child Health Unit. Samples were collected from sites where the wastewater from the two units do not merge. The wastewater outlets for these two units were traced and marked by a personnel from the plumbing department of the Korle Bu Teaching Hospital. As of the time of collection, there was no known treatment of wastewater from these two wards.

3.3 Sample Size Determination and Collection

Sterile bottles were used for the collection of the hospital wastewater samples. A total of 288 wastewater grab samples of 100 ml volumes each (144 from each Unit) were collected across 12 weeks, on two days, that is, Mondays and Thursdays of each week, twice each sampling day (morning and evening), three samples per sampling site at each sampling time, in the months October to December at ambient temperatures ranging from 24˚C to 31˚C. Hence 24 samples were collected each week from both Units. In the mornings, samples were collected from 7:00 a.m. and 8:00 a.m., and in the evenings, between 5:00 p.m. and 6:00 p.m. The samples were kept on ice and conveyed to the research laboratory (within 2 hours) of the Department of Medical Microbiology, University of Ghana Medical School, for microbiological analysis.

The minimum sample size was calculated as follows:

$$n = \frac{z^2 \text{ x } p\ (1-p)}{m^2}$$

Descriptions as follows:

n = Minimum sample size

z = Confidence level at 95% (standard value of 1.96)

m = Margin of error at 5% (standard value of 0.05)

p = Estimated Enterobacteriaceae prevalence = 5.3% or 0.0053 (Urase *et al*., 2020)

$$n = \frac{1.96^2 \times p\ (1-p)}{0.05^2} = 77.12$$

The minimum sampling size was calculated to be 77. However, the sample size was increased to 288. This was done to increase the statistical power of the study.

3.4 Laboratory Analysis

3.4.1 Isolation and Identification of Bacteria

The samples were cultured for bacteria by direct streaking of 0.5 ml on 5% sheep blood agar and chocolate agar, the resultant plates of which were incubated at 37 ºC in 5% CO_2, as well as mannitol salt agar and MacConkey agar, the resultant plates were aerobically incubated at 37 ºC. After 18–24 hours of incubation, each of the plates were examined for growth, and subcultures done until pure cultures were obtained. The pure bacterial cultures were subsequently identified using the Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) biotypher.

3.4.2 Antimicrobial Susceptibility Testing

Following the directives of the Clinical and Laboratory Standards Institute (CLSI, 2021), disc diffusion assay on Mueller-Hinton agar plates were used, the antimicrobials used were: amikacin (30 µg), ampicillin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefepime (30 µg), ciprofloxacin (10 µg), cotrimoxazole (trimethoprim/sulfamethoxazole) (1.25 µg/23.75 µg), imipenem (10 µg), ertapenem (10 µg), gentamicin (10 µg), and amoxicillin-clavulanic acid (25 µg). Using a nephelometer, a suspension similar in turbidity to that of 0.5% McFarland standard was achieved by emulsifying the test isolate in normal saline. A sterile cotton swab was dipped into the suspension and swabbed evenly across the entire surface of a Mueller Hinton agar, in three different dimensions. This was done to obtain a semi-confluent growth following incubation. The plates were incubated at 37˚C for 18-24 hours, after which the zones of inhibition around the antimicrobial discs were measured and interpreted according to the breakpoints of CLSI (2021). *Escherichia coli* ATCC 25922 was used as control strain. The CLSI guidelines were used for the interpretation of the bacterial isolates being resistant, susceptible or intermediate. A bacterial isolate was regarded as multidrug-resistant when they showed resistance to three or more classes of antimicrobial drugs.

3.4.3 Phenotypic screening of Extended Spectrum Beta Lactamase in *Enterobacteriaceae*

A standard inoculum (0.5 McFarland) of test isolate was streaked on Mueller Hinton agar and tested for ceftazidime (30 µg) and cefotaxime-clavulanic acid (30 µg/10µg) using the double disc synergy test (DDST), a disc of clavulanic acid was placed in the center of Mueller Hinton agar plates (90 mm) at 20 mm distance to ceftazidime and cefotaxime. ESBL production was detected by the appearance of keyhole effect due to the enhanced activity of ceftazidime and cefotaxime with clavulanic acid. *E.coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive control strains, respectively.

3.5 Ethical Consideration

Ethical approval was sought from the Institutional Review Board of KBTH. All data, both electronic and physical, were stored in a secure password-protected computer.

3.6 Data Analysis

Data were entered into and analyzed with Microsoft Excel, 2019, and Statistical Products and Services Solutions (SPSS), version 25. The data on the types of bacteria and their spatio-temporal distribution, as well as antimicrobial resistance data were summarized using descriptive statistics. Chi square tests were used to compare the two critical care units with regard to the proportions of the bacteria isolated from their wastewater effluents, at an alpha level of 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Prevalence and types of bacteria present in the hospital wastewater

The 288 samples collected from the wastewater pools of the two critical care units selected for this study, that is, Maternity and Child Health, yielded 294 bacteria, with all being Gram-negative. The Maternity Unit accounted for 48.0% (*n* = 141) of these, whereas the Child Health Unit accounted for 52.0% (*n* = 153).

The bacteria were of 23 different types, with the predominant ones being *Escherichia coli* (30.6%, *n* = 90), *Klebsiella pneumoniae* (11.2%, *n* = 33), *Citrobacter freundii* (10.9%, *n* = 32), *Alcaligenes faecalis* (5.8%, *n* = 17), and *Pseudomonas mendocina* (5.4%, *n* = 16). Besides *Escherichia coli* recording the highest prevalence (30.6%, *n* = 90), it was the only organism whose proportion significantly differed between the two units studied [Maternity Unit = 23.8%, *n* = 37; Child Health Unit = 38.0%, *n* = 53, *p* = 0.02]. The distribution of the organisms isolated from the wastewater samples is presented in Table 1.

Table 1: Prevalence of the bacteria in the hospital wastewater samples

Organism	Total	Maternity (*N* = 141)	Child Health (*N* = 153)	*p* value
*Escherichia coli***	90 (30.6%)	37 (41.1%)	53 (58.9%)	0.02
Klebsiella pneumoniae	33 (11.2%)	15 (45.5%)	18 (54.6%)	0.4
Citrobacter freundii	32 (10.9%)	15 (46.9%)	17 (53.1%)	0.6
Alcaligenes faecalis	17 (5.8%)	8 (47.0%)	9 (52.9%)	0.4
Pseudomonas mendocina	16 (5.4%)	12 (75.0%)	4 (25.0%)	–
Pseudomonas alcaligenes	10 (3.4%)	5 (50.0%)	5 (50.0%)	–
Acinetobacter towneri	10 (3.4%)	2 (20.0%)	8 (80.0%)	–
Acinetobacter kookii	9 (3.1%)	6 (66.7%)	3 (33.3%)	–
Acinetobacter junii	8 (2.7%)	5 (62.5%)	3 (37.5%)	–
Acinetobacter gerneri	8 (2.7%)	4 (50.0%)	4 (50.0%)	–
Enterobacter bugandensis	8 (2.7%)	4 (50.0%)	4 (50.0%)	–
Aeromonas hydrophila	8 (2.7%)	3 (37.5%)	5 (62.5%)	–
Aeromonas caviae	8 (2.7%)	5 (62.5%)	3 (37.5%)	–
Comamonas jiangduensis	6 (2.0%)	3 (50.0%)	3 (50.0%)	–
Acidovorax temperans	6 (2.0%)	2 (33.3%)	4 (66.7%)	–
Enterobacter cloacae	5 (1.7%)	3 (60.0%)	2 (40.0%)	–
Aeromonas veronii	5 (1.7%)	2 (40.0%)	3 (60.0%)	–
Pseudomonas extremorientalis	4 (1.3%)	4 (100.0%)	0 (0.0%)	–
Acinetobacter baumannii	3 (1.0%)	3 (100.0%)	0 (0.0%)	–
Comamonas testosteroni	3 (1.0%)	1 (33.3%)	2 (66.7%)	–
Pseudomonas koreensis	2 (0.7%)	2 (100.0%)	0 (0.0%)	–
Enterobacter aerogenes	2 (0.7%)	0 (0.0%)	2 (100.0%)	–
Rahnella aquatilis	1 (0.3%)	0 (0.0%)	1 (100.0%)	–

$p < 0.05$

4.2 Spatio-temporal distribution of the predominant bacteria isolated from the hospital wastewater samples across the twelve weeks of sampling

As observed in Figure 1, *Escherichia coli* was present in the wastewater samples collected from the Maternity Unit throughout Weeks 1 to 6 for the first days of sampling of those weeks. The organism, however, demonstrated an intermittent occurrence (Weeks 1 to 3, Weeks 5 and 6, Week 9, and Weeks 11 and 12) on the second days of sampling (Figure 1).

The other hospital wastewater-contained organisms that recorded a high prevalence – *Klebsiella pneumoniae*, *Citrobacter freundii*, *Alcaligenes faecalis*, and *Pseudomonas mendocina* – occurred sporadically during the sampling period. *Citrobacter freundii* was additionally present from Weeks 3 through to 6, and *Klebsiella pneumoniae*, Weeks 10 to 12, on the second days of sampling.

Similar to the case of the wastewater samples collected from the Maternity Unit, in the wastewater collected from the Child Health Unit, *Escherichia coli* was observed to have persisted, particularly, during Weeks 1 to 4 and 6 to 11 of the first days of sampling and Weeks 3 to 7 and 9 and 10 of the second days of sampling (Figure 2). Similarly, the occurrence of each of the other organisms was intermittent, with *Klebsiella pneumoniae* additionally occurring repeatedly during Weeks 2 to 4 of the second days of sampling.

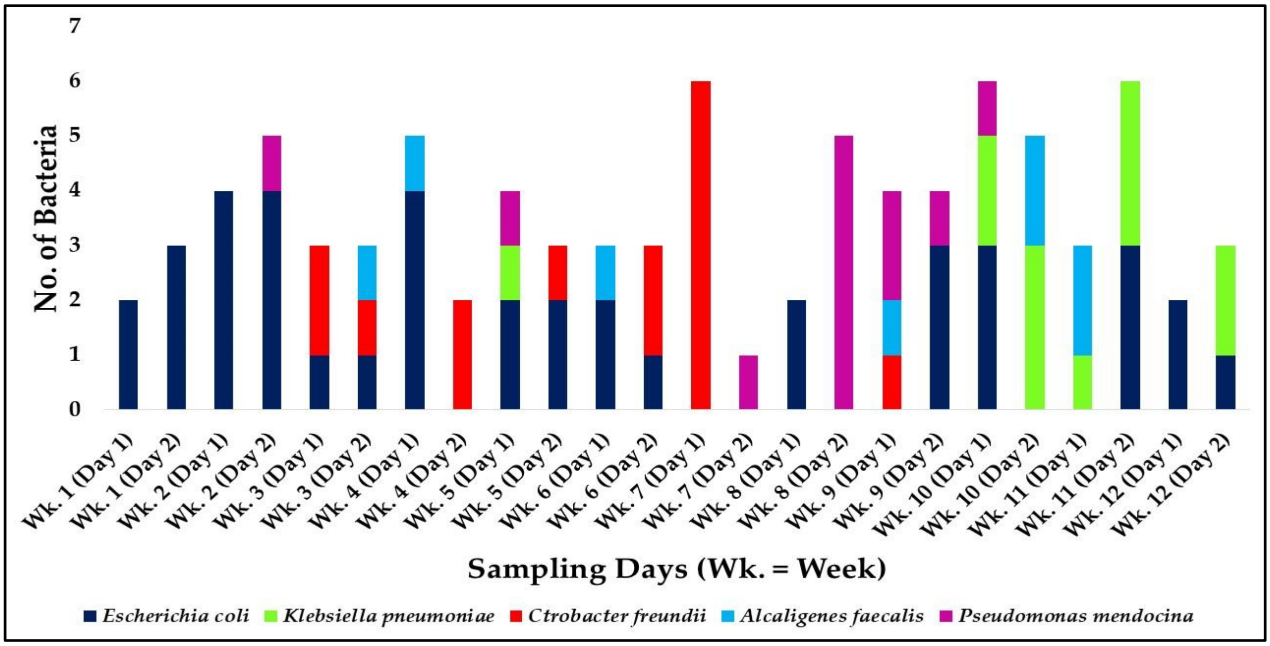

Figure 1: Spatio-temporal distribution of the predominant bacteria recovered from hospital wastewater collected from the Maternity Unit

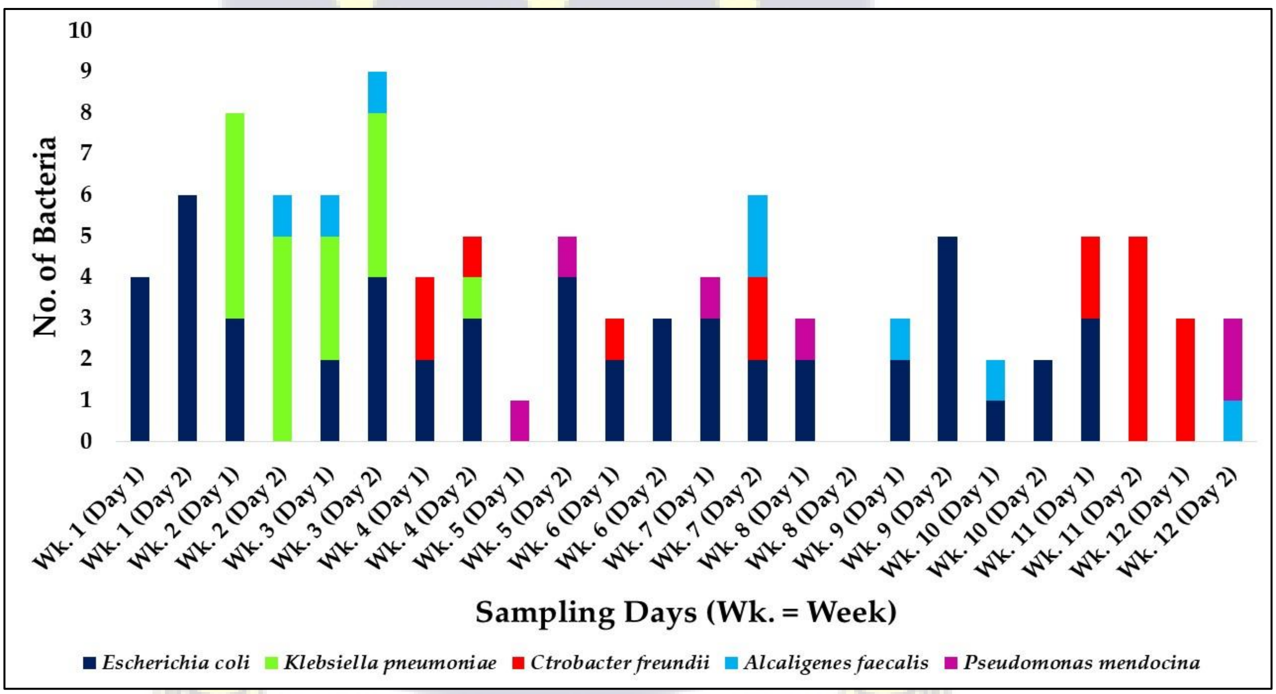

Figure 2: Spatio-temporal distribution of the predominant bacteria recovered from hospital wastewater collected from the Child Health Unit

4.3 Prevalence of multidrug resistance among the hospital wastewater isolates

The prevalence of multidrug resistance among the hospital wastewater isolates was 55.4% (*n* = 163) [Maternity Unit = 53.4%, *n* = 87; Child Health Unit = 46.6%, *n* = 76, *p* = 0.22]. Moreover, the prevalence of ESBL producers among the hospital wastewater isolates was 15.6% (*n* = 46) [Maternity Unit = 18.4%, *n* = 26; Child Health Unit = 13.1%, *n* = 20, *p* = 0.21]. *E. coli* accounted for the highest proportion of ESBL-producing organisms (28.9%, *n* = 26). The distribution of the predominant ESBL-producing bacteria is presented in Table 2.

Table 2: Distribution of the predominant ESBL-producing bacteria in the hospital wastewater samples

Organism	Total	Maternity	Child Health	*p* value
Escherichia coli (*n* = 90)	26 (28.9%)	11 (42.3%)	15 (57.7%)	0.27
Klebsiella pneumoniae (*n* = 33)	10 (30.3%)	10 (100.0%)	0 (0.0%)	–
Citrobacter freundii (*n* = 32)	6 (18.8%)	3 (50.0%)	3 (50.0%)	–
Enterobacter cloacae (*n* = 5)	2 (40.0%)	2 (100.0%)	0 (0.0%)	–
Enterobacter aerogenes (*n* = 2)	2 (100.0%)	0 (0.0%)	2 (100.0%)	–

4.4Antimicrobial resistance patterns of the hospital wastewater bacteria

All the *Escherichia coli* isolates recovered were resistant to cotrimoxazole and ampicillin, but not gentamicin, amikacin, imipenem, and ertapenem, and the resistance rates recorded for amoxiclav, cefuroxime, ceftriaxone, ceftazidime, and cefepime ranged between 24% and 30%. A similar trend was recorded for those of them that were ESBL-producing, except that the ESBL-producing ones were additionally wholly-resistant to amoxiclav, cefuroxime, ceftriaxone, ceftazidime, and cefepime. *Klebsiella pneumoniae* resistance rates for amoxiclav, cefuroxime, ceftazidime, and cefepime ranged between 10% and 67%. A similar trend was recorded for those of them that were ESBL-producing, except that the ESBL-producing ones were additionally wholly-resistant to gentamicin, amoxiclav, cefuroxime, ceftriaxone, and ceftazidime. Also, *Citrobacter freundii* resistance rates for cefuroxime, ceftazidime, and cefepime ranged between 20% and 30%. A similar trend was recorded for those of them that were ESBL-producing, except that the ESBL-producing ones showed full resistance to cotrimoxazole, gentamicin, ampicillin, amoxiclav, cefuroxime, ceftriaxone, ceftazidime, cefepime, and ciprofloxacin. In addition, all the *Alcaligenes faecalis* isolates recovered were resistant to ampicillin and amoxiclav, but showed no resistance to the other antibiotics tested. Moreover, all the *Pseudomonas mendocina* isolates were resistant to cotrimoxazole, ampicillin, ceftriaxone, ciprofloxacin, imipenem, and ertapenem. All the ESBL-producing *Enterobacter cloacae* showed full resistance to ampicillin, amoxiclav, cefuroxime, ceftriaxone, ceftazidime and cefepime, but they showed no resistance to cotrimoxazole, gentamicin, amikacin, ciprofloxacin, imipenem, and ertapenem. Likewise, all the ESBL-producing *Enterobacter aerogenes* showed full resistance to ampicillin, amoxiclav, cefuroxime, ceftriaxone and ceftazidime but they showed no resistance to cotrimoxazole, gentamicin, amikacin, cefepime, ciprofloxacin, imipenem and ertapenem. Details of the antimicrobial resistance patterns of the predominant bacteria are presented in Table 3.

Table 3. Antimicrobial resistance patterns of the wastewater bacteria

Antibiotics / Organisms	Rates of Resistance (%)											
	COT	GEN	AMK	AMP	AMC	CFU	CFTX	CFTZ	CFP	CIP	IMI	ERT
Escherichia coli **(*n* = 90)**	100.0	0.0	0.0	100.0	26.7	28.9	28.9	28.9	28.9	5.6	0.0	0.0
ESBL *E. coli* **(n=26)**	100.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	19.0	0.0	0.0
Klebsiella pneumoniae **(*n* = 33)**	45.5	0.0	0.0	100.0	4.5	30.3	100.0	30.3	21.2	12.1	0.0	0.0
ESBL *K. pneumoniae* **(n=10)**	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	70.0	40.0	0.0	0.0
Citrobacter freundii **(*n* = 32)**	100.0	96.9	0.0	100.0	100.0	12.5	100.0	20.0	25.3	100.0	0.0	0.0
ESBL *C. freundii* **(n=6)**	100.0	100.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
Alcaligenes faecalis **(*n* = 17)**	0.0	0.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas mendocina **(*n* = 16)**	100.0	0.0	0.0	100.0	17.6	0.0	100.0	0.0	0.0	100.0	100.0	100.0
ESBL *E. cloacae* **(n=2)**	0.0	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0
ESBL *E. aerogenes* **(n=2)**	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0

COT = Cotrimoxazole; GEN = Gentamicin; AMK = Amikacin; AMP = Ampicillin; AMC = Amoxiclav; CFU = Cefuroxime; CFTX = Ceftriaxone; CFTZ = Ceftazidime; CFP = Cefepime; CIP = Ciproflloxacin; IMI = Imipenem; ERT = Ertapenem

CHAPTER FIVE

5.0 DISCUSSION

This study evaluated the occurrence of multidrug-resistant bacteria in hospital wastewater of the Korle Bu Teaching Hospital. One aspect of this study investigated the prevalence and types of bacteria isolated from the Korle Bu Teaching Hospital wastewater. The bacterial organisms present in the hospital wastewater sampled from both the Maternity and the Child Health Units comprised 23 different species of Gram negatives, with the predominant ones being *Escherichia coli*, *Klebsiella pneumoniae, Citrobacter freundii*, *Alcaligenes faecalis*, and *Pseudomonas mendocina*.

The range of bacteria recovered from the wastewater emanating from the two KBTH units could be a reflection of the bacterial infections managed at the units during the study period, and compares well with many of the hospital wastewater evaluation studies conducted previously. For instance, Daoud *et al*.(2017) who conducted their study in a hospital in Lebanon, reported the range of organisms they found to be *E. coli, Enterobacter cloacae, Klebsiella pneumoniae*, *Klebsiella oxytoca,* and *Serratia odorifera*. Lee *et al*. (2016) also reported *Acinetobacter junii*, *Comamonas testosteroni*, *Enterobacter* spp., and *Pseudomonas* spp., as the organisms they recovered from the wastewater of a hospital in Singapore. Similarly, Anssour *et al*. (2016) recovered *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Escherichia vulneris*, and *Citrobacter koseri/farmer* from the wastewater of an Algerian hospital. Likewise, Moges *et al*. (2014) also recovered *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella ozaenae, Pseudomonas* spp., *E.coli, Shigella* spp., *Citrobacter* spp., *S. aureus*, *Enterobacter* spp., *Provendencia* spp., *Edwardsiella* spp., *Serratia* spp., and *Morganella* spp., from the wastewater of Gondar University Hospital in Ethiopia. The findings of these studies, along with those of the current study, confirm that individuals who rely on water bodies contaminated with hospital wastewater for domestic use, without appropriate prior treatment, put themselves at risk of a wide range of microbial infections.

The subtle disparities between the range of organisms reported in the current study and those of the other studies highlighted could be accounted for by differences in the bacterial identification methods employed. Most of these other studies used manual methods of bacterial identification, compared to the MALDI-TOF technique used in the current study which is more specific, and hence would identify a wider range of organisms, following their isolation (Croxatto *et al*., 2012). Also plausible is the potential role that differences in the content and brands of agar used may have played in the growth of the target bacteria (Stecchini *et al*., 2001; Donkor *et al*., 2007; Costa *et al*., 2021).

Moreover, in the current study, five bacterial species dominated in both units, with *Escherichia coli* being the frequently isolated organism, at a prevalence of 30.6%, followed by *Klebsiella pneumoniae* (11.2%), *Citrobacter freudii* (10.9%), *Alcaligenes faecalis* (5.8%) and *Pseudomonas mendocina* (5.4%). Similar to these findings, Daoud *et al*. (2017) in a study on Lebanese hospital wastewater, isolated *Escherichia coli* as the predominant organism accounting for 56.3%, Anssour *et al*.(2016), likewise recovered *Escherichia coli* as the predominant bacteria, with a prevalence of 85%, *Klebsiella pneumoniae* (6.7%), and *Citrobacter freundii* (3.3%) from hospital wastewater in Algeria. Again, Sakkas *et al*. (2019), also recorded *Escherichia coli* and *Klebsiella pneumoniae* as the predominant bacteria with a prevalence of 34.1% each, followed by *Pseudomonas* spp. (12.8%). Contrarily, Moges *et al*. (2014) recorded *Klebsiella pneumoniae* as the frequently isolated organism accounting for 23.5%, followed by *Pseudomonas* spp., (16.8%), *Escherichia* *coli* (11.5%) and *Citrobacter* spp., (11.5%). Asfaw *et al*.(2017) in a study on antibiotic resistant bacteria in a North Ethiopia hospital wastewater, also isolated *Klebsiella pneumoniae*, *Escherichia coli*, and *Citrobacter freundii*, with prevalence of 17.9%, 12.7% and 6.7%, respectively.

One other key objective of this study was to determine the spatio-temporal distribution of the hospital wastewater-contained bacteria. This study is one of the few in sub-Saharan Africa to go beyond determining the prevalence of different bacteria in hospital wastewater to use a design that allows for gaining insights into the dynamics in bacterial composition of hospital wastewater effluents overtime. As observed, *Escherichia coli* persisted for several of the weeks in the wastewater from the two critical care units. While it is difficult to explain the intermittent absence of *E. coli*, it is conceivable that the wastewater system may host environmental *E. coli* contaminants that may not have originated from the critical care units studied. Accordingly, changes in environmental conditions within the wastewater system may have played a role in the *E. coli* transiency. As *E.coli* is a known indicator of faecal contamination, its persistence during those periods could also have been influenced by shedding of the organism by patients and hospital staff alike into the lavatory wastewater of the two KBTH units during those periods (Croxen *et al*., 2013). Possibly, too, it could reflect a prevalence of *E. coli*-associated diarrhoea, *E. coli* UTI, some other *E. coli* infections like neonatal meningitis, cholangitis, or pneumonia, or a mixture thereof, during those weeks at the units studied. In like manner, the sporadic occurrence of the other organisms could be a reflection of sporadic cases of infection involving those bacteria at the units during the specified periods. The absence of a distinction between facultative and obligate pathogenic *E. coli*, however, minimizes the conclusiveness of this infection hypothesis. Future studies on microbiological evaluation of hospital wastewater could include an investigation of cases managed at the units of the hospital from which the effluents emanate to add to the robustness of the data generated.

This study additionally investigated the prevalence of multidrug-resistance among hospital wastewater isolates that were recovered. The prevalence of multidrug resistance among the hospital wastewater isolates was 55.4%, and that of ESBL producers was 15.6%, with the organisms generally demonstrating high resistance rates. The high antibiotic resistance rates, including multidrug resistance, is consistent with the high rates reported by various studies among clinical isolates emanating from the study hospital (Newman *et al*., 2011; Labi *et al*., 2014; Obeng-Nkrumah *et al*., 2015; Opintan *et al*., 2015; Donkor *et al*., 2018; Anafo *et al*., 2021; Anafo *et al*., 2021) Several studies have also reported high prevalence of multidrug resistant bacteria in hospital wastewater. For example, Voigt *et al*. (2020) found between 85.7% and 96.4% of hospital wastewater they sampled from different sites to contain ESBL *E. coli*, *Citrobacter* spp., *Enterobacter* spp., and *Klebsiella* spp. That in the current study, organisms with the identified resistance traits were recovered from the wastewater effluent of a leading referral hospital in the country is worrying, as the wastewater effluents could potentially end up in water bodies used in community settings. Previous studies have demonstrated a high potential for transmission of multidrug resistant organisms from such contaminated water bodies to humans, such as those involving IMI2-producing *Enterobacterales* and KPC3-producing *K. pneumoniae* in south of France and Frankfurt, Germany, respectively (Heudorf *et al*., 2018; Laurens *et al*., 2018).Consequently, the pool of hospital wastewater evaluated in this study, and by extension, those generated in the hospital, could serve as a reservoir for dissemination of drug-resistant infections. In fact, previous studies in the country have demonstrated that fomites, as well as cockroaches, are important in harbouring and disseminating multidrug resistant organisms and drug resistance determinants (Donkor *et al*., 2018; Donkor *et al*., 2019; Obeng-Nkrumah *et al*., 2019).

The public health concerns raised by the findings of the current study may be accentuated by the fact that besides potentially providing a snapshot of the nature of the wastewater generated by other hospitals in the country, the hospital studied is a major referral healthcare facility, and by virtue of this status, may have more robust waste management strategies than would lower-tier hospitals in the country. It is also possible that the findings of the current study may be a reflection of the complexity of cases handled at the hospital, and not necessarily be a general reflection of the wastewater of the entire healthcare system of the country. To clarify this hypothesis and adequately quantify the aforementioned public health threat, it would be necessary to replicate this study at other hospitals in the country which belong to other tiers of healthcare classification. As an additional public health measure to safeguard antimicrobials, it is important that hospitals in the country routinely monitor the microbiological safety of their wastewater effluents and disinfect them. These monitorings could encompass evaluation of prevalence and types of antibiotic residues. Moreover, as noted earlier, the high rates of resistance recorded highlight the need to intensify antimicrobial stewardship efforts to help tackle the antimicrobial resistance menace efficiently.

CHAPTER SIX

6.0 CONCLUSION, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusion

The main conclusions of the study are as follows:

The wastewater generated by the Maternity and Child Health Units of KBTH harboured a wide range of multidrug resistant bacteria, with a good proportion of these being ESBL producers, and the predominant and persistent one being *E. coli*. The study thus identifies the wastewater of KBTH as an important source of multidrug resistant organisms, and underscores the significance of appropriate treatment of wastewater of the hospital and other clinical and related settings prior to its discharge.

Findings from this study, particularly, the high prevalence of multidrug resistance, suggest that studies of this nature should be done routinely and expanded to other hospitals in the country. Extensive research is to be conducted to identify efficient and cost-effective ways to remove antibiotic resistant organisms from hospital effluents. Again, hospitals should be built to include waste treatment plants, and the effectiveness and functioning of these plants should be regulated by the authorised bodies. In fact, it is recommended that wastewater from hospital settings is pretreated, even prior to arrival in local treatment plants (Paulus *et al*., 2019).

6.2 Recommendations

Findings from this study suggest that studies of this nature should be done routinely and expanded to other hospitals in the country. Extensive research is to be conducted to identify efficient and cost-effective ways to remove ARBs from hospital effluents. Furthermore, hospitals should not be built without treatment plants and more so, the effectiveness and functioning of these plants should be regulated by the authorised bodies.

Finally, per the results of this study, it can be suggested that regular monitoring and surveillance of water quality including antibiotic-resistant bacteria of all water bodies, should be taken up as a priority in National and Global actions plans as these can have adverse impacts on the build-up of antibiotic resistance.

6.3 Limitations

One limitation this study has is that it was conducted in only one hospital, and focused on only two units of the hospital, and hence the results may not be representative of all the departments in the hospital or all the hospitals in the country. A study of the presence of antibiotic residues in the waste water and the pattern of microbial resistance in human specimens form these units would have provided further insight. Also the source of contamination could have been exogenous.

7.0 REFERENCES

Afriyie, D. K. (2014). Full Length Research Paper A description of the pattern of rational drug use in Ghana Police Hospital. *3*(1), 143–148.

Ahiabu, M. A., Tersbøl, B. P., Biritwum, R., Bygbjerg, I. C., & Magnussen, P. (2016). A retrospective audit of antibiotic prescriptions in primary health-care facilities in Eastern Region, Ghana. *Health Policy and Planning*, *31*(2), 250–258. https://doi.org/10.1093/heapol/czv048

Al Qarni, H., Collier, P., O'Keeffe, J., & Akunna, J. (2016). Investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. *Environmental Science and Pollution Research*, *23*(13), 13003–13014. https://doi.org/10.1007/s11356-016-6389-7

Ali, M., Wang, W., Chaudhry, N., & Geng, Y. (2017). Hospital waste management in developing countries : A mini review. (June). https://doi.org/10.1177/0734242X17691344

Amarasiri, M., Sano, D., & Suzuki, S. (2020). Technology Understanding human health risks by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water : Current knowledge and questions to be answered. *Critical Reviews in Environmental and Technology*, *50*(19), 2016–2059. ://doi.org/10.1080/10643389.2019.1692611

Anafo RB, Atiase Y, Dayie NTKD, Kotey FCN, Tetteh-Quarcoo PB, Duodu S, Osei M-M, KJ, Donkor ES (2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) infection of foot ulcers at a tertiary care hospital in Accra, Ghana. *Pathogens*, 10 (8), 937. Doi: .3390/pathogens10080937.

Anafo RB, Atiase Y, Kotey FCN, Dayie NTKD, Tetteh-Quarcoo PB, Duodu S, Osei M-M, KJ, Donkor ES (2021). Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal among patients with diabetes at the Korle Bu Teaching Hospital. *PLoS One*, 16 (9), Doi: 10.1371/journal.pone.0257004.

Anssour, L., Messai, Y., Estepa, V., Torres, C., & Bakour, R. (2016). Characteristics of ciprofloxacin-resistant enterobacteriaceae isolates recovered from wastewater of an Algerian hospital. *Journal of Infection in Developing Countries*, *10*(7), 728–734. https://doi.org/10.3855/jidc.6727

Appiah, V. A., Pesewu, G. A., Kotey, F. C. N., Boakye, A. N., Duodu, S., Tette, E. M. A., … Donkor, E. S. (2020). *Staphylococcus aureus* Nasal Colonization among Children with Sickle Cell Disease at the Children ' s Hospital , Accra : Prevalence , Risk Factors , and Antibiotic Resistance.

Asfaw, T. (2019). Review on hospital wastewater as a source of emerging drug resistance pathogens. (January). https://doi.org/10.14303/jrest.2018.020

Asfaw, T., Negash, L., Kahsay, A., & Weldu, Y. (2017). Antibiotic Resistant Bacteria from Treated and Untreated Hospital Wastewater at Ayder Referral Hospital, Mekelle, North Ethiopia. *Advances in Microbiology*, *07*(12), 871–886. https://doi.org/10.4236/aim.2017.712067

Assanasen, S. (2014). Microbial and Heavy Metal Contamination of Treated Hospital Wastewater in Thailand.

Aththanyaka, W., Asanthi, H., & Maithreepala, R. (2014). An assessment of the effects of hospital wastes released to Nilwala river, Matara. *Journal of the University of Ruhuna*, *2*(1–2), 33. https://doi.org/10.4038/jur.v2i1-2.7852

Azanu, D., Styrishave, B., Darko, G., Juhl, J., & Clement, R. (2018). Science of the Total Environment Occurrence and risk assessment of antibiotics in water and lettuce in Ghana. *Science of the Total Environment*, *622–623*, 293–305. https://doi.org/10.1016/j.scitotenv.2017.11.287

Beattie, R. E., Skwor, T., & Hristova, K. R. (2020). Survivor microbial populations in post-chlorinated wastewater are strongly associated with untreated hospital sewage and include ceftazidime and meropenem resistant populations. *Science of the Total Environment*, *740*, 140186. https://doi.org/10.1016/j.scitotenv.2020.140186

Bediako-bowan, A. A. A., Kurtzhals, J. A. L., Mølbak, K., Labi, A., Owusu, E., & Newman, M. J. (2020). High rates of multi-drug resistant gram- negative organisms associated with surgical site infections in a teaching hospital in Ghana. *1*, 1–9.

Bhattacharya, S. S. A. (2017). Drinking water contamination and treatment techniques. *Applied Water Science*, *7*(3), 1043–1067. https://doi.org/10.1007/s13201-016-0455-7

Breathnach, A. S., Cubbon, M. D., Karunaharan, R. N., Pope, C. F., & Planche, T. D. (2012). Multidrug-resistant Pseudomonas aeruginosa outbreaks in two hospitals: Association with contaminated hospital waste-water systems. *Journal of Hospital Infection*, *82*(1), 19–24. https://doi.org/10.1016/j.jhin.2012.06.007

Carlet, J., Collignon, P., Goldmann, D., Goossens, H., Gyssens, I. C., Harbarth, S., … Voss, A. (2011). Society's failure to protect a precious resource: Antibiotics. *The Lancet*, *378*(9788), 369–371. https://doi.org/10.1016/S0140-6736(11)60401-7

Colque, P., Sullivan, K. O., Paulshus, E., Kühn, I., & Roland, M. (2019). Diversity and antibiotic resistance among Escherichia coli populations in hospital and community wastewater compared to wastewater at the receiving urban treatment plant. *161*, 232–241.

https://doi.org/10.1016/j.watres.2019.05.102

Costa, O. Y., & Kuramae, E. E. (2021). The influence of agar brands and micronutrients in the growth optimization of Granulicella sp. (Acidobacteriota). *Journal of Microbiological Methods*, 181, 106148.

Croxatto, A., Prod'hom, G., & Greub, G. (2012). Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiology Reviews*, *36*(2), 380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x

Croxen, M. A., Law, R. J., Scholz, R., Kristie, M., Wlodarska, M., Finlay, B. B., Finlay, B. B. (2013). *Recent Advances in Understanding Enteric Pathogenic Escherichia coli*, *26*(4). https://doi.org/10.1128/CMR.00022-13

Daoud, Z., Farah, J., Salem, E. S., Kfoury, K. El, & Dahdouh, E. (2017). Multidrug-Resistant Enterobacteriaceae in Lebanese Hospital Wastewater: Implication in the One Health Concept. 00(00), 1–9. https://doi.org/10.1089/mdr.2017.0090

Dayie, N. T. K. D., Osei, M., Opintan, J. A., Tetteh-quarcoo, P. B., Kotey, F. C. N., Ahenkorah, J., … Donkor, E. S. (2021). Nasopharyngeal Carriage and Antimicrobial Susceptibility Profile of *Staphylococcus aureus* among Children under Five Years in Accra.

Diwan, V., Tamhankar, A. J., Khandal, R. K., Sen, S., Aggarwal, M., Marothi, Y., … Lundborg, C. S.-. (2010). *Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain , India*.

Donkor ES, Nortey T, Opintan JA, Dayie N, Akyeh ML. Antimicrobial susceptibility of *Salmonella typhi* and *Staphylococcus aureus* and the effect of some media on the susceptibility testing results. *Internet Journal of Microbiology* 2007; 4:2.

Donkor ES, Jamrozy D, Mills RO, Dankwah T, Amoo PK, Egyir B, Badoe EV, Twasam J, Bentley SD. A genomic infection control study for *Staphylococcus aureus* in two Ghanaian hospitals.

Infection and Drug Resistance 2018; 11:1757-1765.

Donkor ES. Nosocomial pathogens: An in-depth analysis of the vectorial potential of cockroaches. *Tropical Medicine and Infectious Disease* 2019; 4(1). pii: E14.

Donkor, E. S., Dayie, N. T. K. D., & Tette, E. M. A. (2019). *Methicillin-Resistant Staphylococcus aureus in Ghana: Past, Present, and Future 1 1*. *25*(5). https://doi.org/10.1089/mdr.2018.0115

Donkor, E. S., & Kotey, F. C. N. (2020). *Methicillin-Resistant Staphylococcus aureus in the Oral Cavity : Implications for Antibiotic Prophylaxis and Surveillance*. https://doi.org/10.1177/1178633720976581

Egyir, B., Bentum, J., Attram, N., Fox, A., Obeng-nkrumah, N., Appiah-korang, L., … Letizia, A. G. (2021). *Whole Genome Sequencing and Antimicrobial Resistance of Staphylococcus aureus from Surgical Site Infections in Ghana*. 1–13.

Epa, U. S. (2015). *Drinking Water Contaminants*. (October), 21–23.

Fekadu, S., Merid, Y., Beyene, H., Teshome, W., & Gebre-Selassie, S. (2015). Assessment of antibiotic- and disinfectant-resistant bacteria in hospital wastewater, South Ethiopia: A cross-sectional study. *Journal of Infection in Developing Countries*, *9*(2), 149–156. https://doi.org/10.3855/jidc.4808

Fianko, S. K. (2019). *Clinical Liquid Waste Management in three Ghanaian healthcare facilities-A case study of Sunyani Municipality*. (June).

Garcia-Vello, P., Brobbey, F., Gonzalez-Zorn, B., & Saba, C. K. S. (2020). A cross-sectional study on antibiotic prescription in a teaching hospital in Ghana. *Pan African Medical Journal*, *35*, 1–7. https://doi.org/10.11604/pamj.2020.35.12.18324

Gheethi, A. A. Al, Bala, A. N. E. J. D., Abdel, I. N. M. O., & Ab, M. M. O. (2018). Removal of pathogenic bacteria from sewage - treated effluent and biosolids for agricultural purposes. *Applied Water Science*, *8*(2), 1–25. https://doi.org/10.1007/s13201-018-0698-6

Gupta, A. (2017). *Water pollution-sources , effects and control water pollution-sources , effects and control*. (January 2016)

Heudorf, U., Kempf, V., Reinheimer, C., Exner, M., Schmithausen, R. *et al.* (2018). *Klebsiella pneumoniae* KPC-3 Ausbruch am Universitätsklinikum Frankfurt am Main – teil II: weitere umwelthygienische Erkenntnisse (*Klebsiella pneumoniae KPC-3 outbreak at the University Hospital Frankfurt am Main - Part II: further environmental hygiene knowledge)***.** *Gesundheitswesen*, 80, 383.

Hocquet, D., Muller, A., & Bertrand, X. (2016). What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. *Journal of Hospital Infection*, *93*(4), 395–402. https://doi.org/10.1016/j.jhin.2016.01.010

Husain, A., Khan, N. A., Ahmed, S., Dhingra, A., Pratap, C., Ullah, S., … Ali, I. (2020). *Application of advanced oxidation processes followed by different treatment technologies for hospital wastewater treatment*. *269*. https://doi.org/10.1016/j.jclepro.2020.122411

Ibrahim, C., Hassen, A., Pothier, P., Mejri, S., & Hammami, S. (2018). Molecular detection and genotypic characterization of enteric adenoviruses in a hospital wastewater. *Environmental Science and Pollution Research*, *25*(11), 10977–10987. https://doi.org/10.1007/s11356-018-1399-2

Itzel, F., Jewell, K. S., Leonhardt, J., Gehrmann, L., Nielsen, U., Ternes, T. A., Tuerk, J. (2018). Comprehensive analysis of antagonistic endocrine activity during ozone treatment of hospital wastewater. *Science of the Total Environment*, *624*, 1443–1454. https://doi.org/10.1016/j.scitotenv.2017.12.181

Jury, K., Biotech, T., & Vancov, T. (2010). *Antibiotic resistance dissemination and sewage treatment plants*. (January).

Kataki, S., Chatterjee, S., Vairale, M. G., Sharma, S., & Dwivedi, S. K. (2021). Concerns and strategies for wastewater treatment during COVID-19 pandemic to stop plausible transmission. *Resources, Conservation and Recycling*, *164*(September 2020), 105156. https://doi.org/10.1016/j.resconrec.2020.105156

Khan, N. A., Ahmed, S., Haq, I., Ali, I., Vambol, V., Changani, F., Ullah, S. (2020). *Trends in Analytical Chemistry Occurrence , sources and conventional treatment techniques for various antibiotics present in hospital wastewaters : A critical review*. *129*. https://doi.org/10.1016/j.trac.2020.115921

Kishida, N., Morita, H., Haramoto, E., Asami, M., & Akiba, M. (2012). One-year weekly survey of noroviruses and enteric adenoviruses in the Tone River water in Tokyo metropolitan area , Japan. *Water Research*, *46*(9), 2905–2910. https://doi.org/10.1016/j.watres.2012.03.010

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., & McArdell, C. S. (2012). Hospital wastewater treatment by membrane bioreactor: Performance and efficiency for organic micropollutant elimination. *Environmental Science and Technology*, *46*(3), 1536–1545. https://doi.org/10.1021/es203495dLabi A, Obeng-Nkrumah N, Addison N, Donkor ES. *Salmonella* blood stream infections in a tertiary care setting in Ghana. *BioMed Central Infectious Diseases* 2014; 14(1):697.

Laurens, C., Jean-Pierre, H., Licznar-Fajardo, P., Hantova, S., Godreuil, S. *et al.* (2018). Transmission of IMI-2 carbapenemase-producing Enterobacteriaceae from river water to human. *J Glob Antimicrob Re*, 15, 88–92.

Lee, T. H., Ng, C., Chen, H., Yi, X. Z., Koh, T. H., Barkham, T. M. S., … Gin, K. Y. H. (2016). Occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital wastewater in a tropical country. *Antimicrobial Agents and Chemotherapy*, *60*(12), 7449–7456. https://doi.org/10.1128/AAC.01556-16

Ledingham, K., Hinchliffe, S., Jackson, M., Thomas, F., & Tomson, G. (2019). Antibiotic resistance: using a cultural contexts of health approach to address a global health challenge. *World Health Organization Europe*, (2).

Lien, L. T. Q., Lan, P. T., Chuc, N. T. K., Hoa, N. Q., Nhung, P. H., Thoa, N. T. M., … Lundborg, C. S. (2017). Antibiotic resistance and antibiotic resistance genes in Escherichia coli isolates from hospital wastewater in Vietnam. *International Journal of Environmental Research and Public Health*, *14*(7), 1–11. https://doi.org/10.3390/ijerph14070699

Luczkiewicz, A., Kotlarska, E., Artichowicz, W., Tarasewicz, K., & Fudala-Ksiazek, S. (2015). Antimicrobial resistance of Pseudomonas spp. isolated from wastewater and wastewater-impacted marine coastal zone. *Environmental Science and Pollution Research*, *22*(24), 19823–19834. https://doi.org/10.1007/s11356-015-5098-y

Maheshwari, M., Yaser, N. H., Naz, S., Fatima, M., & Ahmad, I. (2016). Journal of Global Antimicrobial Resistance Emergence of ciprofloxacin-resistant extended-spectrum b - lactamase-producing enteric bacteria in hospital wastewater and clinical sources. *Integrative Medicine Research*, *5*, 22–25. https://doi.org/10.1016/j.jgar.2016.01.008

Marist Asia. (2018). 2016 Annual Report. *Ghana Health Service Reports*, 1–129. Retrieved from http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/131159%0Ahttps://maristasiafoundation.org/wp-content/uploads/.../maf-annual-report-2016. pdf

Moges, F., Endris, M., Belyhun, Y., & Worku, W. (2014). *Isolation and characterization of* *multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments , Northwest Ethiopia*. 2–7.

Murray, A., & Drechsel, P. (2011). Why do some wastewater treatment facilities work when the majority fail? Case study from the sanitation sector in Ghana. *Waterlines*, *30*(2), 135–149. https://doi.org/10.3362/1756-3488.2011.015

Newman MJ, Frimpong E, Donkor ES, Opintan J, Asamoah-Adu A. Resistance to antimicrobial drugs in Ghana. *Infection and Drug Resistance* 2011:4 215–220

Obeng-Nkrumah N, Labi AK, Acquah ME, Donkor ES. Bloodstream infections in patients with malignancies: implications for antibiotic treatment in a Ghanaian tertiary setting. *BioMed Central Research Notes* 2015; 8:742.

Okeyo, A. N., Nontongana, N., & Fadare, T. O. (2018). *Vibrio Species in Wastewater Final Effluents and Receiving Watershed in South Africa : Implications for Public Health*. 17. https://doi.org/10.3390/ijerph15061266

Opintan JA, Newman MJ, Arhin RE, Donkor ES, Gyansa-Lutterodt M, Mills-Pappoe W. Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana. *Infection and Drug Resistance*. 2015; 8:379-389.

Paulus, G. K., Hornstra, L. M., Alygizakis, N., Slobodnik, J., Thomaidis, N., & Medema, G. (2020). The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. *International Journal of Hygiene and Environmental Health*, *222*(4), 635–644. https://doi.org/10.1016/j.ijheh.2019.01.004

Petrovich, M. L., Zilberman, A., Kaplan, A., Eliraz, G. R., Wang, Y., Langenfeld, K., Wells, G. F. (2020). Microbial and Viral Communities and Their Antibiotic Resistance Genes Throughout a Hospital Wastewater Treatment System. 11(February), 1–13.

https://doi.org/10.3389/fmicb.2020.00153

Prado, T., Silva, D. M., Guilayn, W. C., Rose, T. L., Maria, A., Gaspar, C., & Miagostovich, M. P. (2010). Quantification and molecular characterization of enteric viruses detected in effluents from two hospital wastewater treatment plants. *Water Research*, *45*(3), 1287–1297. https://doi.org/10.1016/j.watres.2010.10.012

Prasad, G. (2018). Prevalence of Antibiotic Resistance among Bacterial Isolates from Hospital Environments and Effluents. (October).

Sakkas, H., Bozidis, P., Ilia, A., & Mpekoulis, G. (2019). *Antimicrobial Resistance in Bacterial Pathogens and Detection of Carbapenemases in Klebsiella pneumoniae Isolates from Hospital Wastewater*.

Stecchini, M. L., Del Torre, M., Donda, S., Maltini, E., & Pacor, S. (2001). Influence of agar content on the growth parameters of Bacillus cereus. *International journal of food microbiology*, *64*(1-2), 81-88

Rachel E. R.G., Shirley A.M., Shawn G. G., Johnnie A.D., Xin H., Ashish G., Lara M. K., Nicole A. S., Sampa M., Amir S., Sam W. J., Amy R.S.(2012). Methicillin-Resistant *Staphylococcus aureus* (MRSA) Detected at Four U.S. Wastewater Treatment Plants. *Environmental Health Perspectives*, volume 120 | number 11 | November 2012

Tamhankar, A. J., Diwan, V., & Sta, C. (2013). Seasonal and Temporal Variation in Release of Antibiotics in Hospital Wastewater : Estimation Using Continuous and Grab Sampling. *8*(7). https://doi.org/10.1371/journal.pone.0068715

Tesfaye, H., Alemayehu, H., Desta, A. F., & Eguale, T. (2019). Antimicrobial susceptibility profile of selected Enterobacteriaceae in wastewater samples from health facilities , abattoir, downstream rivers and a WWTP in Addis. *1*, 1–11.

Urase, T., Okazaki, M., & Tsutsui, H. (2020). Prevalence of ESBL-producing escherichia coliand carbapenem-resistant enterobacteriaceae in treated wastewater: A comparison with nosocomial infection surveillance. *Journal of Water and Health*, *18*(6), 899–910. https://doi.org/10.2166/wh.2020.014.

Voigt, A.M., Zacharias, N., Timm, C., Wasser, F., Sib, E., Skutlarek, D., Parcina, M., Schmithausen, R.M., Schwartz, T., Hembach, N., Tiehm, A., Stange, C., Engelhart, S., Bierbaum, G., Kistemann, T., Exner, M., Faerber, H.A., Schreiber, C. (2020). Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater - An evaluation of clinical influences. *Chemosphere*, 241, 125032. https://doi.org/10.1016/j.chemosphere.2019.125032.

Wang, Q., Wang, P., & Yang, Q. (2018). Science of the Total Environment Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. *Science of the Total Environment*, *621*, 990–999. https://doi.org/10.1016/j.scitotenv.2017.10.128