



UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL MICROBIOLOGY

**Investigating GLASS priority pathogens associated with urinary tract
infection among patients at 37 Military Teaching Hospital**

BY

ABUBAKARI ISSAKA

(ID # 10934891)

**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER
OF PHILOSOPHY MICROBIOLOGY DEGREE**

OCTOBER, 2024

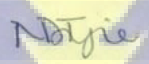
Declaration

I, Abubakari Issaka, do hereby declare that the work presented in this research proposal entitled, “Investigating GLASS priority pathogens associated with urinary tract infection among patients at 37 Military Teaching Hospital” is my original work not part of it has been presented for another degree in this university or elsewhere.

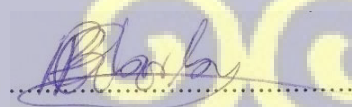
Candidate’s Signature  Date28/ 10 / 2024...

Name: Abubakari Issaka

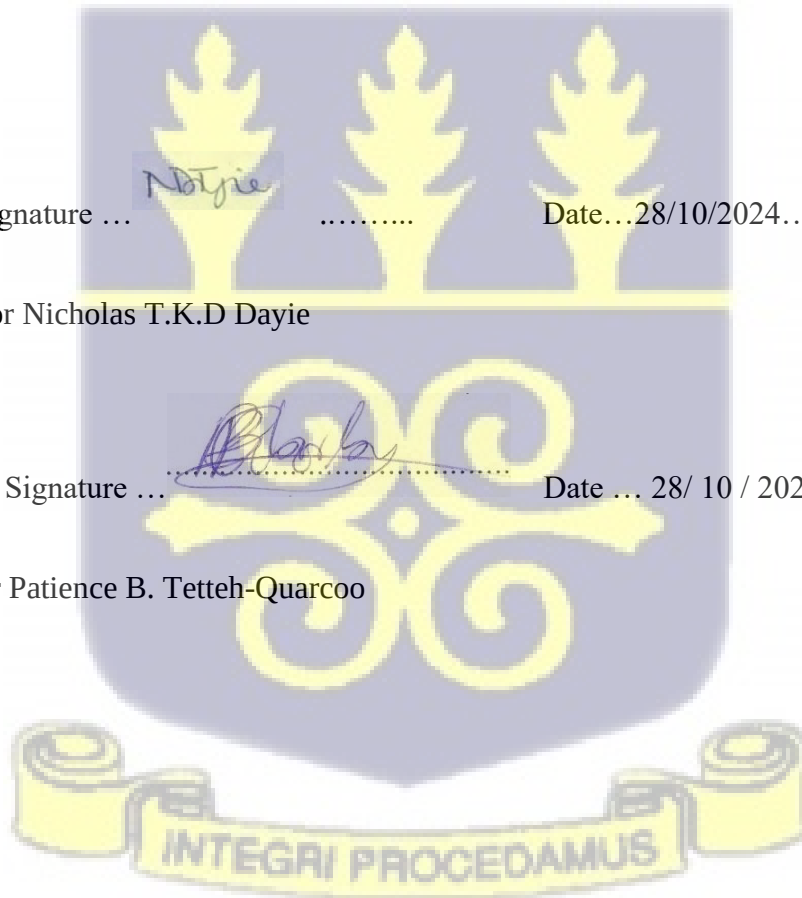
Supervisors’

Supervisor’s Signature ...  Date...28/10/2024.....

Name: Professor Nicholas T.K.D Dayie

Co-Supervisor’s Signature ...  Date ... 28/ 10 / 2024...

Name: Professor Patience B. Tetteh-Quarcoo



DEDICATION

I dedicate this work to the Almighty Allah and my entire family



ACKNOWLEDGEMENT

To the entire lecturers and staff of the Department of Medical Microbiology, University of Ghana Medical School, it took their effort for me to get to this stage of my educational carrier. I must admit I am every grateful to my supervisors for their contribution through to the success of this study.

To the entire staff of Department of Medical Microbiology of 37 Military Hospital Pathology Division, for your support. It worth nothing to say thank you so much to Lt Col Ackah, Maj Tetteh, Flt Lt Sevor and his wife, Flt Lt Acquah, and CE Ama for your immense assistance and moral support.

I acknowledge the support and understanding of my wife Hajia Ramatu Seidu-Issaka and my entire family for been there for me when the pressure was at its exponential height.

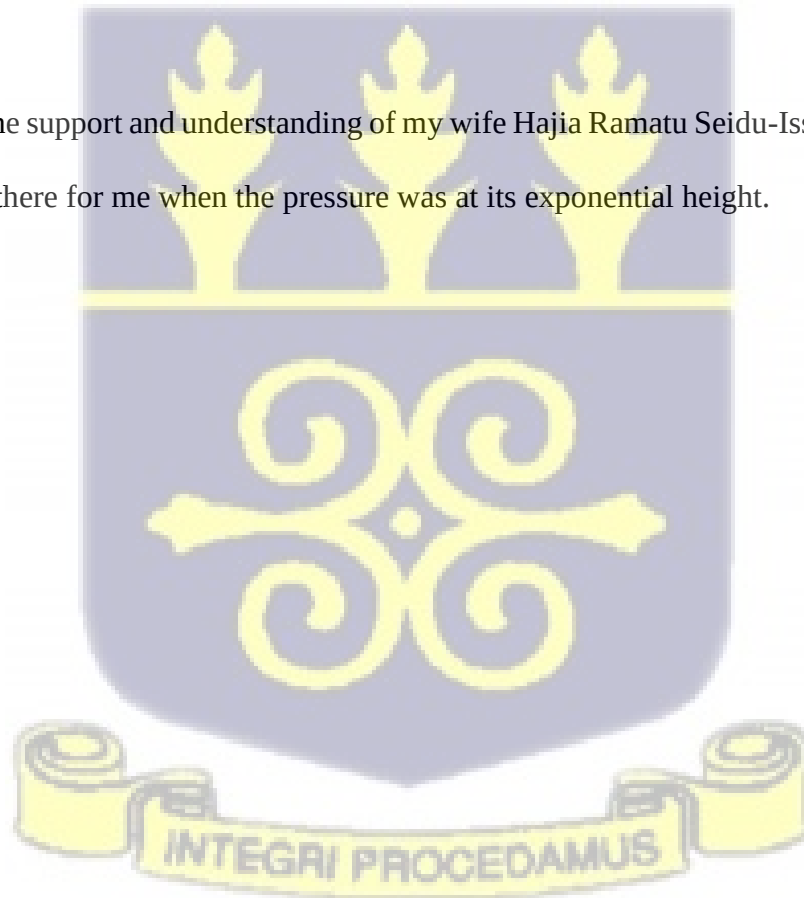


Table of Contents

Declaration	Error! Bookmark not defined.
Table of Contents	v
Abstracts	vii
1.0 Chapter One	1
1.1 Introduction	1
1.2 Problem statement	3
1.3 Justification	3
1.4 Aim	7
1.5 Objectives	7
1.6 Hypothesis	8
Chapter Two	9
2.0 Literature Review	9
2.1.0 Definition	9
2.1.1 Antibiotic Resistance	9
2.1.2 Multi-drug resistance	9
2.1.3 Extensively-Drug Resistance	10
2.1.4 Pan drug resistance	10
2.2 Global Antimicrobial Resistance Surveillance System (GLASS)	11
2.3 Ghana AMR policy	12
2.4.0 What Caused the Current AMR Crisis?	14
2.4.1 Microbial adaptation and the emergence of resistance within microorganism	14
2.4.2 Human activity and the emergence of resistance within humans	17
2.4.3 Clinical usage	19
2.4.4 Agriculture practice and the emergence of antimicrobial resistance	20
2.4.5 AMR and the environment – a neglected area	21
2.5.0 Mechanism of Resistance	23
2.5.1.0 Antibiotic destruction — chemical and enzyme	23
2.5.1.2 Destruction of the antibiotic molecule	24
2.5.2 Efflux pump	26
2.5.3 Modification of drug targets site	27
2.5.4 Limiting drug uptake	29
2.6.0 Extended Spectrum β-lactamase (ESBL); responsible of breaking down certain antibiotics	30

2.6.1 Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) causing UTIs	31
2.6.2 Carbapenem-Resistant Enterobacteriaceae (CRE)	33
Chapter three	36
3.0 Methodology	36
3.1 Study Site	36
3.2 Study design	36
3.3 Sample technique/size	37
3.4 Sample collection	38
3.5 Sample procedure	38
3.6 Purity of isolate and Gram staining	38
3.7 Identification of bacterial isolates (ID) and antimicrobial susceptibility testing (AST)	39
3.8 Detection of Extended-Spectrum β-Lactamases and carbapenamase producing Gram-isolate	40
3.9 DNA Extraction and whole genome sequencing	41
3.10 Bioinformatic analysis	42
3.11 Data analysis	42
3.12 Inclusion Criteria	43
3.13 Exclusion Criteria	43
3.14 Dissemination of Study Results	43
3.15 Ethical approval	43
Chapter four	44
4.0 Results	44
Chapter five	58
5.0 Discussion	58
Conclusion	74
Reference	76



Abstracts

Urinary tract infections (UTIs) are a common infection that is frequently encountered in settings such as hospitals and communities. It is the most frequent bacterial infection and the second most prevalent form of infection in humans. Currently, the rapidly increasing global spread of antibiotic resistance poses a serious challenge for caregivers. In 2015, the Global Antimicrobial Resistance Surveillance System (GLASS) program was introduced by the World Health Organization (WHO) with the aim of fostering global collaboration and establish standardized surveillance of antimicrobial resistance (AMR). GLASS priority pathogen causes a lot of infections that are very difficult to treat due to their ability of resisting multi-drugs. The use, misuse and overuse of antibiotic to manage UTIs have contributed to the surge of extended-spectrum beta- lactam (ESBL) and carbapenemase producing uropathogens.

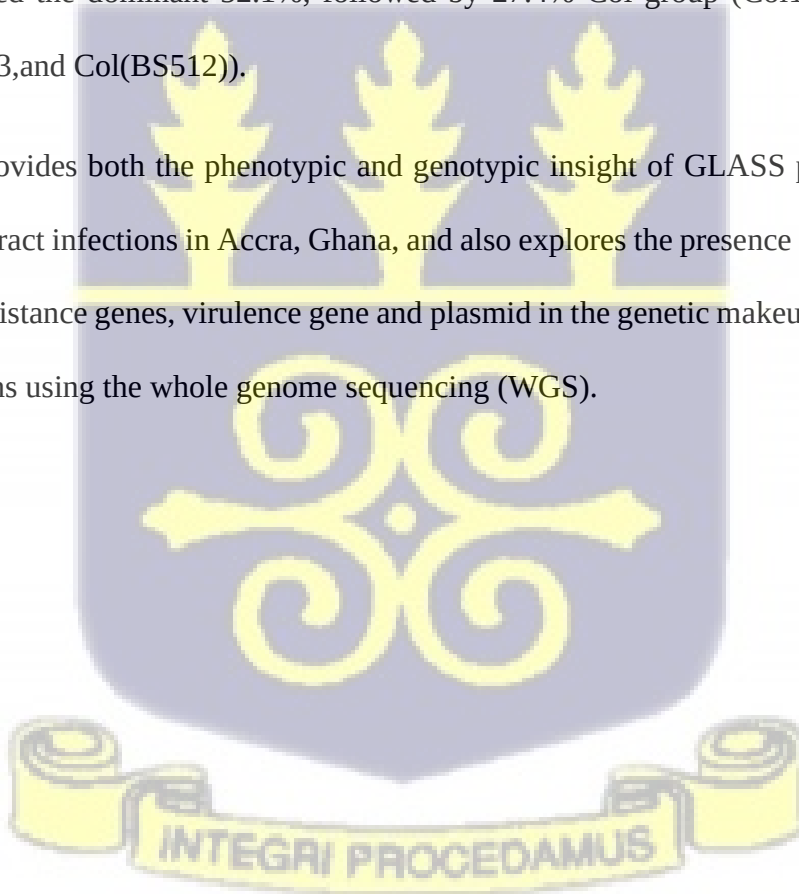
A cross-sectional study design was carried out to determine the prevalence of GLASS priority pathogens with the resistance genes they possess causing UTIs and determine the antibiogram from the third largest tertiary hospital in Ghana over the period of January 2023 – June 2023.

Out of 500, mid-stream urine presented, 147 had significant growth with 63.2% (93/147) of the isolates recovered from CA-UTI and 38.1% (56/147) from HA-UTI. Four different GLASS priority pathogens were identified. The majority of isolates came from Enterobacteriaceae, with *Escherichia coli* being the most commonly identified uropathogen isolated 84(57.1%) followed by *Klebsiella pneumoniae* 53(36.1%), *Acinetobacter baumannii* 7(4.8%) and *Staphylococcus aureus* 3(2.0%). The antibiotics with the highest percentage of resistance include ampicillin, cefuroxime (72%) and ciprofloxacin (45%) while the least resistance includes amikacin (2%), imipenem

(13%), meropenem (9%) and tigecycline (13%) which are all part of WHO watch and reserve category.

Fifteen different sequence types (STs) were identified, including international clonal ones of *E. coli* such as ST131 and ST 410 and hypervirulent ones of *K. pneumoniae* such as ST464 and ST147. The isolates harbored β -lactamase resistance genes (variants of *bla*_{CTX-M} (78.6%), *bla*_{SHV} (35.7%), *bla*_{TEM} (50.0%), as well as carbapenemase resistance genes (OXA-1 (64.3%) and OXA-232 (7.1%). The isolates carried virulence-associated genes including adhesins, toxins, anti-host defense factors, iron acquisition systems, and more. Overall, the plasmid group IncF (FII, FIA, and FIB) emerged the dominant 52.1%, followed by 27.4% Col group (Col156, Col(MG828), Col4401, ColKp3, and Col(BS512)).

This research provides both the phenotypic and genotypic insight of GLASS priority pathogens causing urinary tract infections in Accra, Ghana, and also explores the presence of sequence types, antimicrobial resistance genes, virulence gene and plasmid in the genetic makeup of *E. coli* and *K. pneumoniae* strains using the whole genome sequencing (WGS).



Keywords: Urinary tract infection; WGS; GLASS; Virulence; Plasmid, ESBL; Antimicrobial Resistance Gene (ARG);

Abbreviations

(UTIs) - Urinary tract infections

(GLASS) - Global antimicrobial resistance surveillance system

(AMR) - antimicrobial resistance

(MDR) - multi-drug resistance

(ESBL) - extended-spectrum beta- lactam

(CRE) - carbapenem resistant enterobacteriaceae

(WHO) - World Health Organization

(STG) - Standard Treatment Guidelines

(LMICs) - Low- and middle-income countries



1.0 Chapter One

1.1 Introduction

Urinary tract infections (UTIs) are a common infection that is frequently encountered in settings such as hospitals and communities (Kibret & Abera, 2010; Al-Zidan, *et al.*, 2024; Sirijatuphata, *et al.*, 2020; Carrasco, *et al.*, 2022). UTIs develop when microbial organisms colonize the urinary tract (Prah *et al.*, 2019). A urinary tract infection mostly affects the lower urinary tract, but if left untreated, it can further move to the upper region of the urinary tract (the ureters and kidneys) and seriously harm the kidneys. UTIs cause infections such as bladder infections (cystitis), urethral infections (urethritis), kidney infections (pyelonephritis), and ureter infections (ureteritis) (Muthulakshmi & Gopalakrishnan 2017). Approximately 40% of women and 12% of men may have at least one symptomatic UTI over their lives, with up to 40% of affected women experiencing recurrent UTIs (Kant *et al.*, 2017). It is the most frequent bacterial infection and the second most prevalent form of infection in humans after respiratory infection (Forsyth *et al.* 2020). In 2014, UTI accounted for 0.8% of all hospital admissions among Ghanaians (Prah *et al.*, 2019).

Again, the incidence of bacteria resisting antibiotics and causing urinary tract infections (UTIs) is increasing globally (Sirijatuphata, *et al.*, 2020). There are several reports on antibiotic resistance caused by pathogens associated with UTIs (Donkor *et al.*, 2019; Adu-Poku & Addo, 2020; Asamoah, *et al.*, 2022). *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella species* are some pathogens causing UTIs and are capable of developing strategies against antibiotic actions (Tao *et al.*, 2015).

Currently, the rapidly increasing global spread of antibiotic resistance poses a serious challenge for caregivers (Khameneh, 2016). Unfortunately, the sub-Saharan Africa which largely consist of Low and middle-income countries (LMICs) bear the utmost burden of UTIs, with possibly the

fewest resources and limited information regarding the prevalence and impact of antibiotic resistance (Iskandar *et al.*, 2021). Based on a recent comprehensive study published in *The Lancet* estimating death associated with AMR stated that areas within the sub-Saharan Africa had the greatest mortality rates involving all categories of ages, attributed and related to bacterial antimicrobial resistance (AMR) (Hein *et al.*, 2022).

Although, appropriate antimicrobial drug for treating UTIs is fundamental for accomplishing a good treatment outcome. However, it is becoming more difficult to select appropriate antibiotics for UTI treatment due to the rising incidence of antibiotic resistance (Sirijatuphata, *et al.*, 2020).

Moreover, antimicrobial resistance (AMR) emergence is a natural occurrence accelerated by improper use and overuse of antibiotics in agriculture, humans, and animals (Newman *et al.*, 2011; O'Neill J. 2016; WHO, 2018). AMR has resulted in adverse consequences such as prolonged hospital stays, prolonged antimicrobial therapy, increased medical expenses for patients and their families, overburdened public health systems, and increased mortality (WHO, 2016).

Also, multi-drug resistance (MDR) among Gram-positive and Gram-negative bacterial typically results in failed infection treatment with mainstay antibiotics, and even the reserved antibiotics are becoming inactive (Chellat, 2016; Zhang *et al.*, 2020). To monitor the potency of this antibiotic; AMR surveillance reports have been widely utilized to monitor trends, provide guidance and recommendations regarding empirical treatment, estimate the burden of AMR, and evaluate the effect of regional, national, and international (Cherry *et al.*, 2021).

Thus, in 2015, the Global Antimicrobial Resistance Surveillance System (GLASS) program was introduced by the World Health Organization (WHO) with the aim of fostering global collaboration and establish standardized surveillance of antimicrobial resistance (AMR) (WHO,

2017). The areas GLASS aims to standardized includes collection of AMR data, analyzing and sharing the data base on countries on a common platform. The program shares similar objective with the Global Action Plan (WHO, 2017)

Unfortunately, this is impeded by limited data from Africa due to inadequate diagnostic microbiology infrastructure on the continent (WHO, 2017). Nonetheless, few of the AMR studies reported include reports from Ethiopia (Kibret & Abera 2010), Nigeria (Dada-Adegbola & Muili 2010), and Ghana (Edoh & Alomatu 2007, Newman, *et al.*, 2011, Opintan, *et al.*, 2015) all presenting increasing level of antibiotics. The majority of these findings indicate that bacterial are resistant to widely used, reasonably priced antibiotics like ampicillin, cotrimoxazole, and tetracycline, which have long been the mainstay antimicrobials used several years in Africa (Newman, *et al.*, 2011).

Consequently, the emergence of GLASS priority pathogens presents a new paradigm for the effectiveness of antibiotics used. Pathogen occurrence and antimicrobial susceptibility patterns are considerably different from one geographical area to another as well as differences in various populations and environments (Kibret & Abera 2010).

1.2 Problem statement

UTIs are the second most predominant bacterial human infection in developed countries, and after pneumonia and digestive tract infection, it's the third most prevalent form of infection in human medicine globally (Rafalsky & Dovgan, 2017; Gajdács *et al.*, 2020).

Again, UTIs are one of the most common diseases that clinicians encounter in developing nations, with an estimated yearly occurrence of no less than 250 million cases globally. Up to 8.3 million symptomatic UTIs visits outpatient clinics (Mechal, *et al.*, 2021), 1 million emergency room

consultations, and 100,000 hospital admissions annually worldwide (Muthulakshmi & Gopalakrishnan, 2017; Worku et al., 2017).

On the other hand, antibiotics have been an essential medicine in saving many lives since their discovery in the 1950s (both individuals with fully functioning immune systems and those with weakened immune systems), and they have made it feasible to develop sophisticated medical procedures and specialties that would not have been conceivable before (Van Duin, & Paterson, 2016). The development and the subsequent utilization of antimicrobial agents can be regarded as a monumental milestone in the field of medicine (Erdem, et al., 2011). It has fundamentally changed the way we care for patients who might have otherwise passed away from widespread or serious bacterial infections (Lobanovska, & Pilla, 2017). These remarkable medications have greatly reduced the occurrence of conditions like skin infections, bacteremia, urinary tract infections, respiratory tract infections, gastrointestinal infections, and many more (Gajdács, et al., 2021). Despite its efficacy, the greatest challenge is the emerging resistance.

The emergence of antimicrobial resistance and spread could be attributed to bacterial evolutionary response to harmful agents such as target modification, efflux pump and horizontal genes transfer. Another factor that contributes the uplift of antibiotic resistance among uropathogen is the production of degradative enzyme (carbapenemase-producing and extended-spectrum beta-lactamase (ESBL) (Valenzuela-Valderrama, et al., 2020). Generally, microbes that produce carbapenemase and ESBL are resistant to multi-drugs. As a result of this, clinicians have no option than to utilized antibiotics classified under Watch and Reserve categories in the WHO's AWaRe system (Asamoah, et al., 2022). These Watch and Reserve classes of antibiotics are administered parenterally and may necessitate hospitalization of individuals (Kaye et al., 2017). The rise of AMR to these medications (antibiotics) has, however, proven to be the most worries of the

millennium with regard to public health and a threat to the global economy with an estimation of more than 6 billion U.S. dollars (Kant et al., 2017).

Furthermore, the effect of antimicrobial resistance uropathogen on patients include morbidity and mortality, the high cost of medical treatment, the possibility of losing job, patient-family association reduced with increased hospitalization time, and also the emotional effect of having a resistant infection (Cosgrove, & Carmeli, 2003; WHO 2016).

Lastly, some doctors often treat UTIs empirically by administering broad-spectrum antibiotics, while they wait for the results of the laboratory's culture and sensitivity tests. Though the idea of administering broad-spectrum antibiotics was for early treatment to curtail the pain and frustration of UTI patients, the indiscriminate consumption of these antibiotics is a major factor contributing the rise of multi-drug resistant species of bacterial pathogens and antimicrobial resistance in Ghana.

Thus, this study will investigate the prevalence of GLASS priority pathogen causing UTI and the resistance genes they confer.

1.3 Justification

AMR is rising throughout Africa, according to the WHO's Global Report on Surveillance (WHO, 2014), with limited treatment options (Donkor *et al.*, 2019). The Sustainable Development Goals (SDGs) of the World Health Organization (WHO) include achieving sustainable economic growth, promoting healthy lifestyles and well-being, and eliminating poverty and hunger. (Gajdács, *et al.*, 2021) are 3 out of 17 goals targeted to be achieved by 2030. Unsurprisingly, the rising levels of AMR are threatening the achievement of SDG because this phenomenon has substantial influence on societal and healthcare developments (WHO, 2017).

Moreover, AMR alone is claiming the lives of people more than traffic and cancer accidents in total, with 700,000 individuals battling their lives annually and at least 10 million are projected to die by 2050 if nothing is done to minimize its menace (Taneja & Sharma, 2019). This implies that individual in every 3 seconds may die due to AMR. According to He, *et al.*, 2023, the impact of AMR would not only affect human health but also would cost the global economy about \$100 trillion by 2050. Low and middle-income countries, in which Ghana is no exception, will suffer the most as a result of this economic harm and will be most affected by poverty (Adeyi *et al.*, 2017).

Again, researchers have discovered that certain regularly used, reasonably priced medications, such as ampicillin, tetracycline, and cotrimoxazole which have been the primary antimicrobials for therapy in Africa for generations have started developing bacterial resistance (Newman, *et al.*, 2011; Donkor *et al.*, 2019; Asamoah, *et al.*, 2022). Due to the higher cost of second and third-line regimens compared to first-line medications, increased resistance has significant economic ramifications. Doctors are compelled to use antimicrobials, even though they might be costly, more harmful to patients, and less effective when first- and second-line antibiotic treatment choices are reduced by resistance (Payumo *et al.*, 2021).

Most of the people who indulge in purchasing over-the-counter antibiotics without a prescription; have less or no knowledge of the impact of AMR on their health, costs their jobs due to prolonged admission at the hospital, and costs them financially as well as costs their lives.

According to Singh, *et al.*, 2021 the etiology of UTIs, as well as antimicrobial susceptibility patterns, vary dramatically between different geographical areas and periods. These trends may vary over time (Prah *et al.*, 2019). Thus, investigating local bacterial etiology and resistance

patterns are essential information to detect on time any changes that might have occurred to inform and implement suitable recommendations for optimal empirical treatment for UTIs (Kibret & Abera 2010). Though clinical and AMR susceptibility data is available at the 37 Military Hospital such information has not been evaluated to estimate the burden of GLASS-priority pathogens causing UTIs.

Hence, this study's objective is to evaluate the etiology and antibiogram of GLASS-priority pathogens causing UTIs among patients receiving treatment at the 37 Military Hospital, Accra - Ghana. The results of the study will provide information to guide treatment decisions in the hospitals and inform policymakers on the rational utilization of antibiotics necessary to prevent the spread of AMR microorganisms of public health concern.

1.4 Aim

This study aims to evaluate the etiology and antibiogram of GLASS-priority pathogens causing UTIs among patients receiving treatment at 37 Military Hospital, Accra - Ghana.

1.5 Objectives

1. To determine the prevalence of GLASS-priority pathogens causing hospital acquire and community acquire UTI at the 37 Military Hospital.
2. To determine the antimicrobial susceptibility patterns of the isolated pathogens and to associate the genetic basis for the phenotypic resistances observed.
3. To determine the sequence types, virulence genes and plasmid of the isolated pathogens using whole genome sequence.

1.6 Hypothesis

There are prevalence of GLASS-priority pathogens causing urinary tract infections among patients at 37 Military Hospital.



Chapter Two

2.0 Literature Review

2.1.0 Definition

2.1.1 Antibiotic Resistance

Antibiotic resistance is a subgroup of AMR which is simply referred to as bacteria resistant to antibiotics. Antibiotic resistance occurs when a bacterial pathogen ceases to respond to an antibiotic which was previously susceptible (WHO, 2014). Mutation of viruses, bacterial, parasites, and fungi is one of the factors that occurs when conventional drugs are becoming less effective in treating the illnesses they cause, this phenomenon is known as antimicrobial resistance (AMR) (Hartwig & Kliesow 2020). These terms are mostly used interchangeably. We concentrate on antibiotic resistance because antibiotics, which are used to treat bacterial infections, are more widely used than antiviral drugs.

2.1.2 Multi-drug resistance

Multi-Drug Resistance (MDR) refers “to the acquisition of resistance to at least one agent in three or more class of antimicrobial” (Basak *et al.*, 2016; Hartwig & Kliesow 2020). Due to ongoing selection pressure from various medications, organisms have evolved several genetic resistance mechanisms over time, leading to the development of multidrug resistance (MDR). In this scenario, "successful" antibiotic-resistant bacteria have the ability to distribute their features vertically to their offspring and horizontally to other close bacterial species, if they can act as a donor for mobile genetic elements (MGEs) like transposons and plasmids (Woodford, *et al.*, 2011; Navon-Venezia *et al.*, 2017).

2.1.3 Extensively-Drug Resistance

“Extensively-Drug Resistance (XDR) is defined as bacterial that are non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories)” (Basak *et al.*, 2016; Hartwig & Kliesow 2020).

2.1.4 Pan drug resistance

“Pan drug resistance (PDR) is defined as bacterial non-susceptibility to all agents in all classes of antimicrobial (i.e., no agents tested as susceptible for that organism)” (Magiorakos *et al.*, 2012; Hartwig & Kliesow 2020). More resistances mean more challenges in treating and controlling these bacteria, as well as a greater threat. (Hartwig & Kliesow 2020).

Microorganisms exposed to antimicrobial substances naturally experience resistance, an inevitable phenomenon whose traits might be shared by several kinds of bacteria (Davies & Davies, 2010). This natural tendency has been accelerated, nevertheless, by the improper use of antibiotics and inadequate infection and prevention control practices (Davies & Davies, 2010).

2.1.5 Hospital and community acquired UTI

“Hospital acquired UTI is defined as the onset of UTI in patients that has been in admission after 48 hours, whereas community-acquired UTI refers to infections that occur before hospital admission and not within 10 days of the patient's has been discharged” (Donkor *et al.*, 2019).

2.1.6 Uncomplicated UTIs

“Uncomplicated UTIs is defined as are those infections among non-pregnant persons without any structural or functional abnormalities within the urinary tract” (Owusu *et al.*, 2022).

2.2 Global Antimicrobial Resistance Surveillance System (GLASS)

The issue of antimicrobial resistance is increasingly becoming a significant public health concern worldwide. International health experts prioritize enhancing and standardizing worldwide AMR surveillance (Adeyi *et al.*, 2017). In 2015, the Global Antimicrobial Resistance Surveillance System (GLASS) was established by the WHO to gather clinical and epidemiological data on AMR in collaboration with national and regional surveillance systems (Islam *et al.*, 2019). GLASS aims to maintain a watchful eye over the condition of national AMR surveillance systems, both newly formed and already existing ones. Its objective is to offer a uniform methodology for data collection, analysis and dissemination for AMR data across countries (WHO, 2018; Sirijatuphata, *et al.*, 2020). They also seek to improve positive patient outcome.

In addition, 13 pathogens have been identified by WHO to be at risk of developing multiple resistances in response to the rising levels of resistance (Hartwig & Kliesow, 2020). However, 7 out of these 13 are categorized as GLASS priority pathogens. These pathogens includes *E. coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Shigella* spp, *Klebsiella pneumoniae*, and *Salmonella* spp, (WHO, 2015; Balaji & Kamini 2019). WHO compiles diagnostic findings from clinical samples like urine, stool, blood and as well as urethral and cervical samples that are regularly sent for medical microbiological tests in order to get this AMR data of certain priority pathogens that infect humans (Sirijatuphata, *et al.*, 2020).

Again, in order for GLASS to avoid data duplication, microbiological data sets are also de-duplicated. “This means if the same priority bacterial is isolated from two sample type (i.e., blood and urine sample) of the same individual, only one data will be considered and other ignored” (WHO, 2015; Sirijatuphata, *et al.*, 2020).

Last but not least, Ghana has implemented an action plan and national policy based on the worldwide action plan on AMR after realizing the threat poses. Since 2021, Ghana has joined the GLASS program as a reporting member nation (Asamoah, *et al.*, 2022).

2.3 Ghana AMR policy

According to a recent study, if no policy changes are made; worldwide antibiotic usage might increase by 200% by 2030 from 2015 levels (Li *et al.*, 2023).

Regulations and initiatives pertaining to the fight against AMR in Ghana begun since 1998 after Ghana implemented the “Rational Use of Medicines Programme (RUM)”, which completed the Essential Drugs List and National Formulary with Therapeutic Guidelines (which had been in place and had been frequently revised since 1988), eventually it was acknowledged and endorsed as Ghana’s “Standard Treatment Guidelines and Essential Medicines List” (Hein *et al.*, 2022). The Standard Treatment Guidelines were created by Ghana's Ministry of Health (MOH) to help ensure that medications, particularly antibiotics, are used properly (MoH, 2004). These guidelines aid medical practitioners in choosing the best medications to treat infectious diseases that affect people (Adeapena, 2021).

These efforts have been under the supervision of the “Ghana National Drugs Programme (GNDP)”, a pharmaceutical policy division of the Ministry of Health (MoH), since 2000 (Koduah, *et al.*, 2019) until 2013, the right to prescribe drugs was governed by the Health Professions Regulatory Body Act.

Furthermore, in most Ghanaian teaching, regional, and district hospitals, Drugs and Therapeutic Committees (DTCs) were established as part of the RUM program, which promoted the rational use of medications. Their responsibilities included keeping an eye on how often their facilities

used antibiotics. As a result, the percentage of Outpatient Departments (OPDs) prescribing antibiotics dropped unprecedented from 56.3% in 1999 to 41.4% in 2012, due to the RUM program (Hein *et al.*, 2022). The DTC is typically in charge of formulary management, which includes ensuring that formulary medications adhere to ethical standards and are compliant with nationally recognized standard treatment guidelines (STGs). The various hospitals' drug selection procedures are handled by this committee (Domfeh, 2021).

Moreover, with the establishment of the “National Policy platform for AMR (NPAR)” in 2011, Ghana's success story officially began. The NPAR meetings were presided over by the director of pharmacy, who was active in a number of AMR advocacy efforts (Gyansa-Lutterodt, 2013). Ghana's AMR policy was made possible by the leadership exercised by the Ministry of Health and donor funding provided by the “Swedish International Development Agency through Action on Antibiotic Resistance (ReAct)” (Opintan, 2018). Members from a range of industries, including non-governmental organizations (NGOs), business, the media, regulatory agencies, veterinary and animal industries, and the environmental sector, joined the AMR platform in the years that followed. In the years between 2015 and 2019, approximately 190 members of the previously stated organizations actively participated in the NPAR activities (Koduah, *et al.*, 2021).

Eventually, organizations including the regional FAO, “the Ministry of Fisheries and Aquaculture Development, the Ministry of Environment, Science, Technology and Innovation, and the Ministry of Food and Agriculture” joined the fight on AMR. As a result, talks at the table led to the beginning of awareness creation, which is one of GAP's strategic objectives (Opintan 2018).

Last but not least, Danish study collaboration project called "Antibiotic Drug use, Monitoring and Evaluation of Resistance in Ghana—A research capacity building project (ADMER)" that ran from

2009 to 2015 in collaboration with the “University of Ghana Medical School (UGMS) in Greater Accra and the Kwame Nkrumah University of Science and Technology (KNUST)” in Greater Kumasi was the catalyst for the conversation on AMR that resulted in the promotion of the Ghanaian NAP (Ghana NAP, 2017) and its accompanying policy document on April 11, 2018 (Opintan, 2018; Hein *et al.*, 2022). Both documents were launched by the President of the republic of Ghana “His Excellency Nana Addo Dankwa Akufo-Addo”, and they attracted a lot of media attention (Opintan, 2018).

2.4.0 What Caused the Current AMR Crisis?

In spite of the fact that there have been many epidemics across the world, including the bubonic plague, influenza, SARS Cov2, our current scenario is the crisis of AMR which poses a significant danger to human health. The current situation AMR is a result of an inescapable coincidence brought about by the convergence of several elements within the human ecology and health practices.

2.4.1 Microbial adaptation and the emergence of resistance within microorganism

The capacity of microbes to swiftly and efficiently adapt to environmental change is closely related to microbial survival and hence extends its life-span on our planet (Michael *et al.*, 2014). Increasingly, our understanding of the diverse and enduring processes that support this microorganism resilience is growing. Microorganisms have created strong defenses to resist numerous hazardous compounds through a process of Darwinian selection (Osmania *et al.*, 2018). Mutation is one factor contributing to genetic variability. In general, mutations, which are the result of a random change in an organism's genetic code, have no impact on the organism unless it is deleterious (Michael *et al.*, 2014). The possibility of a rare positive alteration occurring, becomes

inevitable when many species are involved (Michael *et al.*, 2014). According to Shor *et al.*, 2019, accumulation of mutations from different environments is one of the factors causing evolution, and in the case of pathogenic bacteria, mutations can result in the emergence of extremely hazardous strains that can resist numerous classes of antibiotics.

Antimicrobial resistance genes (ARGs), which have been described by Wright as "resistome" of antibiotics as set of genes found in a certain environment or bacteria that may confer resistance to a particular antibiotic (Wright, 2010).

Again, the vast "reservoirs" of adaptable genes serve as the second source of microbial diversity, which can be "mobilized" within and between different microbial species. (Gillings, 2017). A small proportion of these adaptable genes has been recognized as being acquired by human pathogens through horizontal gene transfer (HGT). The overall composition of resistomes is linked to their evolutionary relationships, suggesting that most of the resistance genes found in microbiomes are part of the intrinsic resistome (Baquero *et al.*, 2021). These discoveries align with prior studies on bacterial pathogens intrinsic resistome, suggesting that as much as 3% of the bacterial genome could potentially lead to antimicrobial resistance (Forsberg *et al.*, 2014). Several researches have also revealed that each ecosystem has its unique set of genes that have the ability of transmitting resistance to a different bacterial host (Munck *et al.*, 2015; Yang *et al.*, 2016).

Furthermore, a larger proportion of antimicrobial medications are either modified versions of microorganisms, such as environmental fungi and saprophytic bacteria, or they are produced naturally by these organisms. Only a small number of antimicrobial medications, such as sulphonamides and fluoroquinolones, are entirely synthetic (Holmes, *et al.*, 2015). These microorganisms also develop defensive mechanism to oppose the actions of these antimicrobial

agents by blocking the medication's entry or exit from the body, creating enzymes that break down or modify the antibiotic, and altering the antibiotic's target (Dowling *et al.*, 2017).

Moreover, functional meta-genomic investigation of microorganisms from soil has shown a diverse range of genetic factors causing antibiotics resistance (Lima, *et al.*, 2019). Even though very few have been reported in human pathogens, the development of resistance against β -lactam antimicrobial medications, wherein these antibiotic compounds are inactivated by enzymes (β -lactamases) which have been present for millions of years, and is one resistance mechanism that occur naturally and has an impact on the health of human (Aslam, *et al.*, 2018; Cascella & Pandey, 2019).

Also, it was believed initially that saprophytic organisms' production of antimicrobial molecules impeded the development of nearby organisms, giving them a competitive edge in the immediate environment. However, a number of recent studies point to a more complex connection. First, it is proposed that the number of antimicrobial compounds in the soil is much less than previously believed and might not be enough to stop neighboring bacteria from growing (Holmes, *et al.*, 2015; Aslam *et al.*, 2018). Second, study indicates that antimicrobial drugs at modest doses can greatly affect the physiological functions of microorganisms and the formation of adaptive evolution. They may also serve as effective signaling molecules that can activate the expression of resistance genes in microorganisms (Andersson & Hughes, 2014; Khurshid *et al.*, 2017).

Another key note is that some saprophytic bacteria are producers of carbapenems, an essential class of broad-spectrum antibiotic. Several genes implicated in carbapenem production might potentially be linked in biofilm development or quorum sensing (Holmes, *et al.*, 2015; Aslam *et al.*, 2018). These findings raise further questions about the unpredicted impact of antimicrobial

substance; beyond their inhibitory activity, our thinking of their potential effect on microorganism is still limited (Morita *et al.*, 2014).

Lastly, the increase in resistance does not only occurs to natural antimicrobials but also to antimicrobials made synthetically. The resistance to fluoroquinolones has been well demonstrated, where resistance to fluoroquinolone is presently at 10-40 percent in *Escherichia coli* isolates European patients (European Centre for Disease Prevention and Control, 2013; Ma, *et al.*, 2024). Numerous resistance mechanisms against drugs have also emerged, such as DNA-gyrase modification or alteration of the target, increased efflux (microbe export antibiotics), target protection by DNA-binding proteins (known as Qnr), and by aminoglycoside N-acetyltransferase inactivation of fluoroquinolone (Redgrave *et al.*, 2014).

2.4.2 Human activity and the emergence of resistance within humans

There are around seven billion people on Earth and its surrounding space, according to estimates from the United Nations Population Division in 2014. Factors related to public health that influence the incidence of microbial resistance among humans include vaccination uptake rates, various healthcare systems, the effects of migration and tourism, sanitation, and population density (Holmes *et al.*, 2014). Early in the twenty-first century, the dynamic phenomena of human population urbanization achieved a major turning point in human history (Michael *et al.*, 2014). Humans have had an influence on environments in which they have existed. However, due to a number of historical transformations during the past 10,000 years, interactions between humans and ecosystems have grown more significant; (i) the establishment of local settlements, crop farming, and livestock domestication; (ii) regional contact through trade; (iii) globalization, urbanization, and climate change; and (iv) intercontinental exploration, imperialism, and

industrialization (Hassell *et al.*, 2017). A significant proportion of the population living in close spaces creates a huge possibility for infectious disease to spread quickly.

Furthermore, humanity as a whole is now essentially a single biological population, due to increasing urbanization and population growth. Owing to that, people may visit the majority of the planet's areas in a day or two, and in about a week, they can essentially reach every other human population and environment on earth (Michael *et al.*, 2014). This means that humans have ability to rapidly move from one place to the other, their accompanying bacterial may likewise travel the world quickly without difficulty. Such quick movement can transfer infected persons around the globe several times before even the first symptoms of infection appear, allowing pathogens to spread globally (Hassell *et al.*, 2017).

Again, the large and globalized human population has made all people vulnerable to microorganisms both present and potential, from the different surroundings they come into contact with. (Michael *et al.*, 2014). On the other hand, every ecosystem that has been researched to date including Antarctica, the sea, the soil, drinking water, and numerous food items has been found to harbor antimicrobial-resistant bacteria (Holmes, *et al.*, 2015; Browne, *et al.*, 2017). Some of these microorganisms' most especially commensal microbiome (bacteria that are usually found at a body site without causing illness such as *E. coli* in the gastrointestinal tract) that develops at the earlier age of life (Li *et al.*, 2018). At a compromised condition, some of these microorganisms become pathogenic and require antimicrobial drugs to inhibit their growth. Nonetheless, the administration of antibiotics places selective pressure on pathogens and commensal human microflora that there is a higher chance that microorganism develops resistant (Gillings *et al.*, 2017). Antimicrobial exposure exerts a selection pressure that facilitates the survival and

proliferation of microbes possessing innate resistance or recently acquired mutations or resistance genes (Holmes *et al.*, 2015; Gillings, *et al.*, 2017).

Last but not least, microbes are acquired by newborns during and after delivery from both vertical sources (the mother) and horizontal sources (other people or the environment) (Perez-Muñoz *et al.*, 2017). At birth neonates begin to be rapidly colonized by *Enterobacteriaceae*, regardless of whether they are fed by breast or not (Ma, *et al.*, 2020). In an Indian study of breast-fed babies, it was noticed that 14.3% had harbored *Enterobacteriaceae* that included extended-spectrum β -lactamase (ESBL), (an enzyme that inactivates β -lactam medicines) and after 60 days the percentage has elevated to 41.5% (Kothari *et al.*, 2013).

In sum, humans have become a vast and available target for microbial predators all throughout the world (WHO, 2014).

2.4.3 Clinical usage

Although antibiotics in human medicine are most the frequent prescribed medications, but up to fifty percent of all antibiotic prescribed are often inappropriate and deemed unnecessary (CDC, 2015; Gualano *et al.*, 2015). Clinicians' extensive empirical antibiotics utilization could be the primary cause of antibiotic misuse in clinical practice. For example, it typically takes 2-3 days to diagnose UTIs using a urine culture for pathogen identification (ID) and an AST for antibiotic susceptibility. This delay leads to empirical treatment, which in turn fuels antibiotics overuse and the rise of bacteria with increased resistance (Liao *et al.*, 2017). Understandably, a patient who exhibits symptoms that might be fatal necessitates treatments right away (Michael *et al.*, 2014).

Furthermore, the recurrent administrations of antibiotics by clinicians are much more frequent than treating really ill patients empirically. This circumstance occurs when a patient seeks medical

attention for a condition that is due infectious pathogen. The physicians frequently administer or prescribe antibiotics based on prior experience and local epidemiology rather than always testing for pathogen and antibiotic susceptibility (Kuehn, 2013; Michael *et al.*, 2014).

Another practice of use, misuse, and overuse is self-medication. Self-medication according to “World Health Organization (WHO) can be defined as the use of drugs to treat self-diagnosed disorders or symptoms, or the intermittent or continued use of a prescribed drug for chronic or recurrent diseases or symptoms”. Self-medication includes getting medications without a prescription, utilizing an existing prescription to get new medications, sharing medications with family or friends, or using leftover medications that have been stored at home (WHO, 2000; Rather, *et al.*, 2017). Self-medication with antibiotics can have harmful consequences, including antibiotic resistance, unsuccessful treatments, and drug toxicity (Alhomoud *et al.*, 2017).

Again, one of the elements influencing the use, misuse, and overuse of antibiotics (Laxminarayan *et al.*, 2013) is unapproved advertisements. Advertising antibiotics through various media channels like television, radio, and print, as well as recommendations from family and friends, are the main factors that lead people to self-medicate (Rather, *et al.*, 2017).

Nonetheless, the same medium of advertisement if positively made can create awareness of the negative impact of AMR. Prescriber education plays a critical role in combating the overuse or abuse of antibiotics and has proven to be successful in both primary and secondary healthcare settings (Holmes, *et al.*, 2015).

2.4.4 Agriculture practice and the emergence of antimicrobial resistance

The application of antimicrobial agent to overcome disease causing pathogenic microorganism is not only for human but also applied in the agriculture sector. Agriculture sector in every country

is a significant area that contribute to national development (Salimova *et al.*, 2020). To sustain the production of these agriculture products to enable curtail the fear of food insecurity, farmers use antimicrobial agents as growth hormones and for sanitation in food-animal production (Hartwig & Kliesow 2020). Many studies have testified to the fact that, food production uses more antimicrobials than human being consumption (Holmes, *et al.*, 2015; Hartwig & Kliesow 2020). Globally, there is an expectation that the use of antibiotics in the production of food animals will rise by 67% by the year 2030 (Islam *et al.*, 2019; Hartwig & Kliesow 2020). The indiscriminate application of this antimicrobial on these farm animals exerts selective pressure which is evidence enough to bring about antimicrobial resistance (Gillings *et al.*, 2017).

2.4.5 AMR and the environment – a neglected area

One area which is overlooked at as a significant driver of antimicrobial resistance is the environment and substance that evolve it. Antimicrobial drugs are frequently used in many different types of agriculture, not just that involving land animals but also crop farming, for growth or preventive measures (Hartwig & Kliesow, 2020), leaving their residues spread all over the environment. There is a possibility that the sewage and waste processing in the environment could also be a key contributor of AMR.

In the 1990s, numerous monitoring and efficacy studies that were related to the environmental dangers of various antibiotic products were first conducted (Ågerstrand *et al.*, 2015; Ondon *et al.*, 2021). Meanwhile, antibiotics were created to be effective in minimal doses, have a brief duration in the body, and be removed through urine and feces in their original, active form (Wang *et al.*, 2016). Animal feces could contaminate the environment, resulting in higher levels of antibiotic residue and the development of antibiotic-resistant bacteria (Arun *et al.*, 2017). This antimicrobial resistance bacterial (ARBs) can cause serious illnesses such as carbapenem-resistant, multi-drug

resistant gut bacteria of the *Enterobacteraceae* family, methicillin-resistant *S. aureus* that may cause pneumonia, skin infections, toxic shock syndrome, and endocarditis and Vancomycin-resistant *Enterococcus*, (Hazra, *et al.*, 2022; Li *et al.*, 2023).

Furthermore, pharmaceutical residue is one of the areas that contribute to AMR. Islam *et al.*, 2019 claimed that, the widespread occurrence of pharmaceutical residues in the environment can be attributed to their inadequate elimination through activated sludge systems and other wastewater treatment methods that are widely employed globally. The quantity of waste produced by these companies has increased equally with the growth of the pharmaceutical industry. This waste flows into bodies of water and acts as a continuous AMR source in the environment because of inadequate regulation and legal enforcement (UNESCO, 2019; Taneja & Sharma 2019). This indirectly exposes people who depend solely on these water bodies as source of drinking water to antimicrobial resistance. Studies have also shown that people who drink from the tap within that environment are equally not save, since low doses of antibiotics can ultimately wind up in distribution networks and finished drinking (tap) water (Lubbert *et al.*, 2017; Islam *et al.*, 2019).

Additionally, even when present at levels below the minimum inhibitory concentration (MIC), antibiotic remnants in the environment can cause genetic alterations in bacteria, resulting in the development and proliferation of resistance at high levels (Singer *et al.*, 2016; Wistrand-Yuen *et al.*, 2018). Several studies have revealed that antimicrobial resistance genes ARGs related to human illnesses are derived from the environment (Waseem *et al.*, 2017; Rousham *et al.*, 2018; Manyi-Loh *et al.*, 2018).

High levels of antibiotic residues discharged into the environment by the pharmaceutical industry have been associated with the spread of bacteria that produce carbapenemase and ESBL (Lubbert, *et al.*, 2017).

2.5.0 Mechanism of Resistance

2.5.1.0 Antibiotic destruction — chemical and enzyme

2.5.1.1 Modification of the chemical structure of antibiotics.

The development of enzymes that can induce chemical modifications to the antimicrobial molecule is a widely recognized mechanism of acquired antibiotic resistance in both Gram-negative and Gram-positive bacteria.

Bacteria produce enzymes that alter vulnerable areas on antibiotics by attaching chemical groups, preventing them from attaching to specific proteins and rendering them useless (Wilson, 2014). This leads to the development of a resistance to specific antibiotics.

Enzymes like acetyltransferases, nucleotidyltransferases, and phosphotransferases, (Jian *et al.*, 2021) which make up a wide and diversified family of antibiotic-resistant enzymes, can transfer a range of various chemical groups (including acyl, phosphates, and nucleotides) to the susceptible regions (Peterson & Kaur, 2018; Jian *et al.*, 2021). Biochemical reactions including *i*) acetylation (aminoglycosides, chloramphenicol, streptogramins), *ii*) adenylation (aminoglycosides, lincosamides), and *iii*) phosphorylation (aminoglycosides, chloramphenicol) are catalyzed by these modifying enzymes (Munita & Arias, 2016; Peterson & Kaur, 2018). These enzymes are commonly located within mobile genetic elements (MGEs), however, in certain bacterial species, genes encoding for aminoglycoside-modifying enzymes (AMEs) have also been discovered as part of the chromosome. This has been observed in some aminoglycoside acetyltransferases in the

species *Serratia marcescens*, *Enterococcus faecium*, and *Providencia stuartii* (Munita & Arias, 2016). Aminoglycosides are intricate molecules that are large and have many hydroxyl and amide groups exposed, making them vulnerable to alterations. This leads to a high rate of antibiotic resistance (Jian *et al.*, 2021).

An illustration of resistance brought about by the drug modification is demonstrated by the existence of aminoglycoside-modifying enzymes (AMEs). These enzymes execute a covalent modification of the hydroxyl or amino groups on the aminoglycoside molecule, which weakens its capacity to bind to its intended target. This results in reduced effectiveness and higher drug resistance. Currently, multiple AMEs has been documented, and they have established themselves as the most prevalent mode of aminoglycoside resistance on a global scale. These developments highlight the importance of ongoing study in creating novel methods to combat bacterial resistance to antibiotics (Munita & Arias, 2016; Peterson & Kaur, 2018).

2.5.1.2 Destruction of the antibiotic molecule

One of the methods by which bacteria defend themselves is through the modification of the antibiotic (Peterson & Kaur, 2018).

Bacteria possess the capability to inactivate antibiotics through hydrolysis via enzymatic activity. Through extensive research, a significant number of enzymes capable of breaking down and modifying a wide range of antibiotics including, aminoglycosides, macrolides, β -lactams and phenicols, have been identified (Jian *et al.*, 2021). Some enzymes are capable of breaking down antibiotics belonging to the same class, such as β -lactam antibiotics, which consist of carbapenems, cephalosporins, monobactams, and penicillins. These antibiotics can be degraded through the action of various β -lactamases (Jian *et al.*, 2021). These enzymes break down the amide bond of

the β -lactam ring, causing the antimicrobial to lose its effectiveness. The discovery of β -lactamases dates back to the 1940s, prior to the widespread use of penicillin. Evidence of their existence, however, extends back millions of years (Munita & Arias, 2016).

Again, in the 1960s, a novel plasmid-encoded β -lactamase enzyme that had the ability to degrade ampicillin was identified within gram-negative bacteria. This enzyme was named TEM-1, after the patient (Temoneira) in whom it was initially found (Giedraitienė *et al.*, 2011). Since then, the introduction of new generations of β -lactams has consistently been met with the emergence of enzymes that can break them down, showcasing antibiotic-driven adaptive evolution in bacteria. This occurrence is a perfect illustration of how bacteria have changed in reaction to the discovery of new medicines (Munita & Arias, 2016).

Furthermore, the genes encoding for β -lactamases are typically referred to as "bla" followed by the specific enzyme name (e.g. blaKPC). These genes can be located either as part of the accessory genome within mobile genetic elements (MGEs) or in the chromosome (Giedraitienė *et al.*, 2011; Munita & Arias, 2016). β -lactamase encoding genes are transferable through transposons and may also be present in integrons, contributing to their widespread dissemination. Their expression can be either constitutive or induced by an external signal (Giedraitienė *et al.*, 2011).

Also, β -Lactamases are widely prevalent enzymes that are classified into two primary systems: Ambler and Bush-Jacoby-Medeiros (Alekhun & Levy 2007; Giedraitienė *et al.*, 2011; Munita & Arias, 2016). The Ambler classify β -lactamases into 4 groups (A, B, C, and D) according to the identification of amino acid sequence. In contrast, the Bush-Jacoby classification categorizes β -lactamases into 4 categories, each with subgroups, based on their biochemical function, primarily substrate specificity (Munita & Arias, 2016).

2.5.2 Efflux pump

Even when intact antibiotic successfully enters through the cell wall of the bacterial and drug targets are easily reachable, active drug efflux mechanisms may become activated. The genes that encode efflux pumps are located on the bacteria chromosomes; some of these genes are expressed continuously, while others are activated or overexpressed when subjected to particular environmental triggers or the presence of the right substrate (mutations that modify the transport channel are usually the source of elevated resistance) (Reygaert 2018).

Regardless of the bacteria cell structure either Gram-positive or negative bacteria, efflux pump can be present (Garcia *et al.*, 2022). However, compared to Gram-positive bacteria, the disparities of structural and resistance capabilities through efflux pumps make Gram-negative bacteria more complicated (Varela *et al.*, 2021; Garcia *et al.*, 2022). Efflux pumps play crucial function in guaranteeing the survival of bacterial and the emergence of resistant species of clinically significant bacterial which includes *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, MDR Mycobacterium TB, and methicillin-resistant *Staphylococcus aureus* (MRSA), (Garcia *et al.*, 2022).

Again, antibiotics are among the many substrates that Efflux proteins aggressively pump out of cells and into the environment (Bharadwaj *et al.*, 2022). Microbes with trans-membrane proteins called multidrug (MDR) efflux pumps primary help them to survive in hostile conditions by removing toxic substances of metabolism (Reygaert 2018), maintenance of homeostasis and to increase their resistance to antibiotics (Kumawat *et al.*, 2023). Transport is greatly reliant on bacterial genes that encode efflux pumps, which is a major factor in bacteria's resistance to antibiotics (Kotwani, *et al.*, 2021; Varela *et al.*, 2021).

Furthermore, based on their structure and energy source, bacteria's efflux pumps may be further classified into six primary groups: (a) ATP-binding cassette (ABC) family, (b) Major facilitator superfamily (MFS), (c) Multidrug and toxic compound extrusion (MATE) family, (d) Small multidrug resistance (SMR) family, (e) Proteobacterial antimicrobial compound efflux family (PACE) and (f) Resistance-nodulation-cell division (RND) family (Garcia *et al.*, 2022). Although the ABC family relies solely on ATP hydrolysis in the cytoplasm to transport substrates like metabolites across the cytoplasmic membrane, most of these efflux pump families (MATE, MFS, PACE, SMR, and RND) utilize protons for their driving force (Garcia *et al.*, 2022).

2.5.3 Modification of drug targets site

To avoid being identified by an antimicrobial agent, certain resistant bacteria modify their target sites. Antimicrobial agent can specifically target a number of different parts within the cell of bacterial, and the bacteria can also alter some of those targets in order to develop resistance against the antimicrobial agent (Shaikh *et al.*, 2015; Abushaheen *et al.*, 2020). One mechanism through which Gram-positive bacteria predominantly utilized in developing resistance to β -lactam medicines is by changing the structure or the quantity of penicillin-binding proteins (PBPs) (Shaikh *et al.*, 2015; Varela *et al.*, 2021; Nayak & Roy 2023). Peptidoglycan is a well-known instance of such a target. Many bacterial species depend on peptidoglycan for growth and survival, and the chemical structure of peptidoglycan differs from that of the mammalian hosts that they infect (Varela *et al.*, 2021; Ijaz *et al.*, 2024). PBPs are enzyme called transpeptidases that contribute to the construction of the peptidoglycan, which forms the cell wall (Reygaert 2018; Nayak & Roy 2023). A mutation in the gene responsible for encoding penicillin-binding proteins (PBPs) results in the production of these enzymes, which are normally anchored to the cytoplasmic membrane of bacterial cell walls and work in the assembly and regulation of the latter stages of

cell wall formation (Santajit & Indrawattana, 2016). Some of the Gram-positives also produce PBP2a. For example, in contrast to the native PBPs (PBP1-4), *S. aureus* expresses a distinct PBP2a, which is the most prevalent PBP in the MRSA cell (Santajit & Indrawattana, 2016).

Furthermore, *S. aureus* is able to withstand high doses of β -lactam antibiotics, such as methicillin, due to the low affinity of PBP2a for all β -lactam medications (Mamman, 2021). Changes in the quantity of PBPs can impact the ability of antibiotics to bind to the specific target (more PBPs can lead to less effective drug binding, whereas fewer PBPs can maintain usual drug binding levels). A structural alteration (for instance the *mecA* gene's acquisition of PBP2a in *S. aureus*) may make an antibiotic less likely to attach to it or completely prevent it from doing so (Reygaert 2018).

Another method by which bacteria changes their target site is by replacement and bypass technique. The development of resistance through replacement and bypass techniques is exemplified by the crucial case of vancomycin resistance. Glycopeptides, including vancomycin and teicoplanin, work in a comparable way to β -lactam antibiotics by blocking the formation of bacterial cell walls (Munita & Arias, 2016) and daptomycin is a lipopeptide that operates by inducing depolarization of the cell membrane (Reygaert 2018). In contrast to β -lactams, glycopeptides do not have a direct interaction with penicillin-binding proteins (PBPs). Instead of directly interacting with PBPs, glycopeptides attach to the end of D-alanine-D-alanine (D-Ala-D-Ala) of the pentapeptide portion present in forming peptidoglycan precursors (lipid II), inhibiting the synthesis of cell walls by blocking PBP-mediated cross-linking. The primary outcome of vancomycin binding to D-Ala-D-Ala-ending precursors that come from the cytoplasm is thought to be a change of transglycosylation (likely caused by steric hindrance), this stops any additional cell wall development and leads to mortality of the bacterial (Munita & Arias, 2016; Santajit &

Indrawattana, 2016). Glycopeptides have the ability to block the formation of methicillin-resistant Gram-positive bacterial cell walls (Santajit & Indrawattana, 2016).

2.5.4 Limiting drug uptake

Antimicrobial medications may be blocked from entering bacteria, contrary to drug efflux mechanism in which resistance through export is a viable strategy. One important factor contributing to bacterial resistance to antimicrobial drugs is blocking the ability of drug from entering the internal environment of pathogenic cells (Masi *et al.*, 2017; Winterhalter, 2021). Species of Gram-negative bacterial that adopt these strategies of resisting antimicrobial agents include *Vibrio cholerae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Escherichia coli* are especially problematic (Varela *et al.*, 2021). These Gram-negative species are capable of preventing antibiotics with the tendency of inhibition from entering into them due to their complex structural nature of the lipopolysaccharide (LPS) layer of their cell wall (Ghai, & Ghai, 2018; Varela *et al.*, 2021; Winterhalter, 2021). This provides those bacteria with a natural ability to resist certain types of antibiotics. Mycobacteria, for example, contain a large amount of lipids in their outer layer, meaning that drugs like rifampicin and fluoroquinolones, which are hydrophobic, can easily enter the cell, while hydrophilic medications have restricted entry (Reygaert, 2018). Hence, bacteria like Mycoplasma and related species that do not have a cell wall are naturally immune to antibiotics that aim at the cell wall, like β -lactams and glycopeptides (Masi *et al.*, 2017; Reygaert, 2018).

Another molecular strategy of resistance through the bacterial cell wall is the reduction of porins, which are proteins that have become integral to the outer membrane and have water-filled channels that allows the transport of molecules with specific big sizes and charges (Masi *et al.*, 2017; Varela

et al., 2021). In general, porin channels in gram negative bacteria enable the entry of hydrophilic substances. Porin alterations can restrict the entry of drugs by reducing porin levels and altering the porin channel's selectivity due to mutations (Reygaert, 2018; Winterhalter, 2021). Enterobacteriaceae members develop resistance by reducing the overall amount of porins they produce (and, in some instances, completely ceasing production of some porins). Carbapenem resistance in some extent is due to this mechanism (Reygaert, 2018; Winterhalter, 2021).

Last but the least, one widely recognized system for antimicrobial resistance due to porins is found in *E. coli*, which includes the cell wall proteins PhoE, OmpF, and OmpC. Two significant pathogen that have been extensively investigated on their poring structures are *Acinetobacter baumannii* and *P. aeruginosa* (Winterhalter, 2021; Varela *et al.*, 2021).

2.6.0 Extended Spectrum β -lactamase (ESBL); responsible of breaking down certain antibiotics

Over the last 20 years, hospital settings and the community have seen an upsurge of extended-spectrum β -lactamase (ESBL)-producing bacteria transmission, which has become a severe public health concern (Karaiskos, & Giamarellou, 2020; Jackson, *et al.*, 2021). Among the most often given antibiotics globally are β -lactam antibiotics, such as penicillins, monobactams, cephalosporins, and carbapenems (Peirano, & Pitout, 2019). These antibiotics binds to and block bacterial enzymes (known as penicillin-binding proteins or PBPs) that are in charge of cell wall formation (Varela *et al.*, 2021). To prevent the destruction by this agent, bacterial produces β -Lactamases, an enzyme that deactivate β -lactam antibiotics through hydrolysis, making the antibiotics ineffective (Peirano, & Pitout, 2019). These bacterial enzymes are called extended spectrum beta-lactamase (ESBL).

Moreover, extended spectrum beta-lactamase belongs to the class of enzymes that confer resistance to the majority of beta-lactam antibiotics, such as third-generation cephalosporins (such as cefotaxime, ceftazidime, ceftriaxone, cefuroxime, and cefepime) and monobactams (such as aztreonam), and they are frequently seen in conjunction with other resistance mechanisms, leading to multidrug resistance (Peirano, & Pitout, 2019). However, cephamycins and carbapenems, such as imipenem, meropenem, doripenem, and ertapenem, mostly promote resistance to ESBL enzymes (Peirano & Pitout, 2019; Karaiskos, & Giamarellou, 2020). Although Cephamycin is efficient against the ESBL enzyme, it is ineffective against AmpC cephalosporinases and porin mutations (Karaiskos & Giamarellou, 2020).

Again, β -lactamases are categorized by two systems; the Ambler molecular classification scheme and the Bush-Jacoby-Medieros functional classification system. They are categorized based on amino acid homology or substrate and inhibitor profile (Shaikh *et al.*, 2015; Munita & Arias, 2016; Chastain, et al., 2017). Most ESBLs belong to Ambler class A and consist of the sulfhydryl reagent variable (SHV), Temoniera (TEM), and cefotaxime-M (CTX-M) (Peirano, & Pitout, 2019; Karaiskos, & Giamarellou, 2020). Ambler class A β -lactamases, are very vulnerable to inhibition by beta-lactamase inhibitors (BLIs), such as clavulanate, sulbactam, and tazobactam (Chastain, *et al.*, 2017).

2.6.1 Extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-PE) causing UTIs

The production of β -lactamases by Gram-negative bacteria continues to be the principal cause of β -lactam resistance, its increasing prevalence, and its ongoing development.

Extended-spectrum β -lactamase-producing Enterobacteriaceae (ESBL-PE) causes UTIs and threatens treatment accounting for longer hospital admission, more healthcare costs, and high-rate mortality and morbidity (Ouchar *et al.*, 2019; Jackson, *et al.*, 2021). *Escherichia coli* and *Klebsiella pneumoniae* are the two most common ESBL-producing microorganisms globally (Peirano, & Pitout, 2019). Even though the frequency of this enzyme is lower, they have been identified in some members of the *Enterobacteriaceae* family, such as *Citrobacter spp.*, *Proteus spp.*, *Morganella morganii.*, and *Enterobacter spp* (Ouchar *et al.*, 2019).

Unfortunately, treatment and management of UTIs is now more difficult owing to the dramatic rise in antimicrobial resistance (AMR) in both the community and the hospital settings (Mazzariol, *et al.*, 2017). This is becoming increasingly common as a result of the spread of pathogenic microorganism that shows phenotype resistance to extended-spectrum cephalosporins, like ESBL and plasmid-mediated AmpC (pAmpC)-producing Enterobacteriaceae (Jackson, *et al.*, 2021).

Furthermore, the treatment alternative for ESBL and pAmpC-producing Enterobacterales can be narrow, since they are mostly involved in multidrug-resistant (MDR), thereby having the tendency of resisting majority of first-line antibiotic used for empiric treatment of UTI, including the nitrofurantoin, fluoroquinolones, trimethoprim/sulfamethoxazole, aminoglycosides, and tetracycline. (Kresken *et al.*, 2014; Tulara, 2018; Ouchar *et al.*, 2019; Tacconelli *et al.*, 2019). However, for the treatment of Enterobacteriaceae that produce ESBLs (ESBL-PE), carbapenems have been regarded as the "gold standard" therapy and has yielded better results (Karaikos & Helen 2020).

For the treatment of Enterobacteriaceae that produce ESBLs (ESBL-PE), carbapenems have been regarded as the "gold standard" therapy and have been linked to better results (Karaiskos & Helen 2020).

Again, ESBLs are carried by a variety of genes found within genetic mobile elements (GME), like plasmids, which assist in their transmission across different bacterial species (Ouchar et al, 2019). The most common ESBLs belong to the CTX-M, SHV and TEM families (Karaiskos, & Giamarellou, 2020). Hitherto, there are over 220 distinct enzymes within the CTX-M- β -lactamases, categorized into five subfamilies (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) based on their amino acid sequences (Peirano, & Pitout, 2019).

Finally, study has shown that the CTX-M family, particularly CTX-M-15, has emerged globally and is currently the predominant type of ESBL in both hospitals and the community (Ouchar *et al.*, 2019; Donkor *et al.*, 2019).

2.6.2 Carbapenem-Resistant Enterobacteriaceae (CRE)

Globally, carbapenem-resistant Enterobacteriaceae (CRE)-induced bacterial infections are a significant factor in illness and death and present a danger to public health (Van, 2017; Shields, *et al.*, 2021). The World Health Organization in 2017 reported that among drug resistant bacterial, CRE is identified one of the top three classes of drug-resistant bacterial in the world that urgently require the discovery of new antibiotics (Ma *et al.*, 2023). The term "carbapenem resistance" (CR) describes a bacteria's capability to resist and grow when exposed to clinically meaningful amounts of carbapenems class of antibiotics (Durante-Mangoni, *et al.*, 2019). Thus, CRE can be defined as *Enterobacteriaceae* that has the ability resist at least one carbapenems in vitro (Ma *et al.*, 2023).

In the meantime, the US centers for disease control and prevention (CDC) describe carbapenem-resistant Enterobacteriaceae (CRE) as enterobacteria that are resistant to any carbapenem (with an MIC of ≥ 4 mg/L for meropenem, doripenem, or imipenem, or ≥ 2 mg/L for ertapenem) or known to produce a carbapenemase (CDC, 2015).

Carbapenems are effective antimicrobials that belong to the β -lactam class and are structurally similar to penicillins (Codjoe & Donkor 2017). These classes of antibiotic impede bacterial cell wall synthesis by binding and inhibiting penicillin-binding protein (PBP) (Ma *et al.*, 2023). Carbapenems have proven and known to be the alternate and the “gold standard treatment” of severe pathology infected by *Enterobacteriaceae* extended spectrum β -lactamase (ESBL) producing bacteria (Codjoe & Donkor 2017). Examples of these classes of antibiotics are meropenem, ertapenem, imipenem, doripenem, panipenem and biapenem. The frequent use of these antibiotics owing to increasing resistance to cephalosporin in the *Enterobacteriaceae* group contributed to its resistance (Codjoe & Donkor 2017).

Furthermore, CRE infections are very difficult to treat and are often observed in both clinic and communities (Lupande-Mwenebitu *et al.*, 2020). MDR infections caused by CRE, specifically *Escherichia coli* and *Klebsiella spp.*, are challenging to treat (Mitgang, *et al.*, 2018; Shields, *et al.*, 2021). These bacteria are frequently identified as the main causes of urinary tract infections (UTI) in individuals and are frequently reported (Van, 2017).

Also, carbapenem resistance may occur due to the breakdown of the β -lactam ring by carbapenemase enzymes, or it can be as result of mutations in efflux pumps or porins that alter membrane permeability, combined with the expression of extended-spectrum β -lactamases (ESBLs) (Potter, *et al.*, 2016).

Again, carbapenem-resistant genes are horizontally transferable by plasmids or transposons. Therefore, resistance genes can be transferred from carbapenem-resistant *K. pneumoniae* (CRKP) and carbapenem-resistant *Escherichia coli* (CREco) to *Salmonella* to make them antibiotic resistant which could explain why resistance has spread to other *Enterobacteriaceae* (Ma *et al.*, 2023).

Finally, Ambler class B metallo- β -lactamases (MBLs) and Ambler class A or D serine β -lactamases are the sources of carbapenemases. Among the carbapenemase-producing *Enterobacteriaceae* (CPE), the most common types of carbapenemases are *K. pneumoniae* carbapenemase (KPC - Class A), Verona integron-encoded metallo- β -lactamase (VIM - Class B), New Delhi metallo- β -lactamase (NDM - Class B), Imipenemase metallo- β -lactamase (IMP - Class B), and Oxacillinase-48 (OXA-48 - Class D) variants (Potter, *et al.*, 2016).

Conclusion

In conclusion, the lack of clean water access, open sewage systems instead of closed ones, differences in healthcare infection-control methods, limited access to antimicrobials and diagnostics, farming systems with inadequate antimicrobial regulation, and high population densities greatly affect the spread of antimicrobial-resistant microbes globally among humans, animals, and the environment. Although some of these issues are present in settings with plentiful resources, they are likely the primary reasons for antibiotic resistance in low- and middle-income countries (Holmes, *et al.*, 2015).

Chapter three

3.0 Methodology

3.1 Study Site

The 37 Military Teaching Hospital is located in the Greater Accra region of Ghana. Currently, it is the main military base hospital and the second largest hospital after the Korle-Bu Teaching Hospital in the Republic of Ghana and also a national referral center. The hospital was set up in the beginning to care for soldiers injured during World War II, but it was eventually made public. Military people still make up the majority of the hospital's workforce. At the moment, the hospital can accommodate around 400 beds in total. It includes a pharmacy and accident and emergency room open all the time. The Laboratory is accredited with ISO 15189; 2012 certificate. The hospital has several departments and wards which includes pediatrics, dental, ENT, polyclinic, and veterinary among the other departments. Additionally, the hospital also functions as a teaching facility for students pursuing postgraduate medical practices. This site was selected because the medical microbiology department where the research was conducted has established quality assurance protocols during the pre-analytical, analytical, and post-analytical phases and also it is well equipped for the study.

3.2 Study design

According to Creswell (2009) quantitative research methods facilitate the precise measurement and systematic analysis of data, enabling the establishment of statistical associations between independent and dependent variables, and enhancing the objectivity of research findings. For these reasons, a quantitative cross-sectional design was adopted for this study. The study was carried for a period of six months (January, 2023 - June, 2023).

3.3 Sample technique/size

A random sampling technique was adopted to collect clean-catch midstream urine samples from study participants. Sample size formular ($n = [Z^2 \times P \times (1 - P)] / d^2$) (Pourhoseingholi *et al.*, 2013). was used to determine the appropriate sample size. Where;

n = required sample size

Z = Z-score corresponding to the desired confidence level

P = expected prevalence

d = precision or margin of error

For this study:

Z = 1.96, which corresponds to a 95% confidence level.

P = 0.225 (22.5%), based on the expected prevalence of the condition (e.g., UTI or antimicrobial resistance) from prior studies or literature (Donkor *et al.*, 2019).

d = 0.05 (5%), indicating the desired precision, meaning the estimate should be within $\pm 5\%$ of the true prevalence.

The values into the formula: $n = 1.96^2 \times 0.225 \times (1 - 0.225) / 0.05^2$ n = 267

This sample size ensures that the study can estimate the true prevalence of the condition within a 5% margin of error at a 95% confidence level, assuming an expected prevalence of 22.5%. However, to maximize the power of the study 500 midstream urine was collected from study participants.

Case Definition: "HA-UTI" refers to patient's whose onset of UTI occurs 48 hours after admission, whereas "CA-UTI" describes UTI that occur before visiting the hospital and not within ten days following the patient's discharge.

3.4 Sample collection

Samples were collected from study participants from the outpatient and inpatient department (wards) that have consented to take part in the study. They were asked to provide about 5–10 milliliters of mid-stream urine clean catch, which should be hygienically collected while trained personnel (nurses), collect urine samples from study participants admitted at the various wards. The urine samples were transported to the Medical Microbiology Laboratory on an ice pack for Culture and Antimicrobial Susceptibility Testing (AST) aseptically.

3.5 Sample procedure

Swiftly the received mid-stream urine specimen were cultured on Cysteine Lactose Electrolyte Deficient (CLED) media (Oxoid, UK) and incubated at $37^{\circ}\text{C} \pm 2$ for 18-24 hours in ambient air and observed for significant bacteriuria at the Department of Medical Microbiology, 37 Military Hospital. Isolated bacterial colonies were counted after incubation, and a count of greater than or equal to 10^5 (cfu / ml) were considered as significant uropathogen.

3.6 Purity of isolate and Gram staining

Positive cultures were isolated and those with mixed growth are purified by performing purity plating. Gram staining was performed on each bacterial isolate to help select the appropriate panel (either negative minimum inhibitory concentration (NMIC) or positive minimum inhibitory concentration (PMIC) panel) for accurate identification and antimicrobial susceptibility testing (AST).

3.7 Identification of bacterial isolates (ID) and antimicrobial susceptibility testing (AST)

Identification of bacterial isolates (ID) and antimicrobial susceptibility testing (AST) was conducted on pure significant bacterial isolates using the fully automated BD phoenix M50 ID/AST analyzer (Becton Dickinson, Maryland, USA) guided by Clinical Laboratory Standard Institute guidelines (CLSI). The following are the list of antibiotics that were tested against significant isolates; amikacin, Amoxicillin/Clavulanic, ceftriaxone, piperacillin-Tazobactam, imipenem, ceftazidime, ciprofloxacin, gentamicin, levofloxacin, meropenem, nitrofurantoin, penicillin, vancomycin, trimethoprim-Sulfamethoxazole, and cefuroxime. Bacterial inoculums were prepared in sterile ID broth and a turbidity of 0.5 McFarland standard was measured using a BD nephelometer (PhoenixSpec™). A 25 ul of the standardized inoculum was measured with the aid of well calibrated pippete and inoculated into the AST broth (mixed with AST indicator). Both inoculums prepared in the ID broth and the AST broth were transfer into the respective wells of well label panels that were selected based on the bacterial Gram status. These panels were further loaded onto the BD phoenix M50 analyzer for bacterial isolates identification (ID) and antimicrobial susceptibility testing (AST). The results were obtained and interpreted from an Epicenter according to manufactures instruction after 16-18 hours. Only GLASS priority uropathoens were followed up

To establish the proportion of bacteria resistant to individual antibiotic, intermediate and resistant isolates were categorized in this study as resistant (Alves *et al.*, 2014). Quality control was conducted using a control strain of *Escherichia coli* (ATCC 25922).

3.8 Detection of Extended-Spectrum β -Lactamases and carbapenemase producing Gram-isolate.

All the positive Gram-negative GLASS isolates detected as a potential ESBLs or carbapenem resistance by BD phoenix M50 (exhibiting resistance to cephalosporins or carbapenem class of antibiotics) were selected and screened for phenotypic confirmation of Extended Spectrum β -lactamases (ESBLs) and carbapenemase producing organism using antibiotic double discs synergy diffusion and modified Hodge's method respectfully.

3.8.1. Phenotypic confirmatory tests for ESBL production: Double disc synergy diffusion test (DDST) A- Double disc diffusion test with clavulanic acid

Pure bacterial solution was adjusted to a turbidity of 0.5 McFarland standards using a BD nephelometer (PhoenixSpec™) with the aid of cotton swabs was use to uniformly streaked on a well dry Muller-Hinton agar (Oxoid, UK). Amoxicillin-clavulanic acid (Oxoid, UK), was placed in the middle of the medium 20 mm (center to center) along with 30 μ g antibiotic discs of cefotaxime, ceftriaxone, and ceftazidime (Becton Dickinson, BBL) disc. The plates were incubated at 37°C for 18-24 hours. An observation of a clear extension of the edge of the antibiotic's inhibition zone diameter > 5 toward the disc containing clavulanate was interpreted as synergy, confirms the presence of an ESBL producing bacterial according to CLSI guidelines (Thabit, *et al.*, 2011; Gebremedhin, *et al.*, 2023).

3.8.2. Phenotypic confirmatory tests for Carbapenemase producing bacterial using Modified Hodge Test (MHT)

Modified Hodge Test (MHT) was the method use to confirm phenotypic cabapenemase producing bacterial. Standardized inoculums of *E. coli* ATCC 25229 susceptible to meropenem (indicator

organism) were prepared in BD identification broth after it was mixed homogeneously with a vortex. The turbidity was measured to 0.5 McFarland standards using a BD nephelometer (PhoenixSpec™). A well dry Muller Hinton agar (Oxoid, UK) plates were streaked on with the standardized inoculums by swabbing to obtain confluent growth post incubation. The plates were left at room temperature for ten minutes and 10 µg meropenem (Becton Dickinson, BBL) antimicrobial discs were aseptically put in the center of the test region after drying the plate for 10 minutes. The bacterial to be tested was streaked in a straight line from the edge of the plate to the edge of the disk using a sterile loop. The plates were incubated for 18-24 hours at 37°C ±2 under aerobic conditions recommended by Clinical Laboratory Standard Institute guidelines 2016. A positive carbapenemase producing bacteria showed a clover leaf-like indentation of the *E. coli* ATCC 25229 growing along the test organism growth streak within the disk diffusion zone diameter (Pasteran *et al.*, 2016; Gebremedhin, *et al.*, 2023).

3.9 DNA Extraction and whole genome sequencing

Genomic DNA extraction was performed on sixteen selected positive isolates exhibiting multi-drug resistance phenotypically for WGS. Extraction of DNA was performed using the QIAamp DNA Mini Kit (Qiagen Inc., GmbH, Holden, Germany) according to manufacturer's recommendations. Extracted DNA was evaluated for quality and quantified using using a Qubit™ 4.0 Fluorometer (Invitrogen, Thermo Fischer Scientific, Singapore) (Bruzek *et al.*, 2020; Tettey *et al.*, 2024). Sequencing libraries was prepared using the Illumina DNA Library Prep (M) Tagmentation Library Prep Kit (Illumina, San Diego, CA, USA). The fragmented DNA amplified libraries were assessed for quality and concentration using an Agilent 2100 bioanalyzer system (Agilent, Santa Clara, CA, USA) and quantitative PCR (qPCR) using the Kapa Sybr Fast qPCR kit (Kapa Biosystems, USA) respectively. Libraries were pooled and loaded on the Illumina 2 x

300 cycle for sequencing on the Mi-Seq machine (Illumina Inc., San Diego, CA, USA) (Tettey *et al.*, 2024) at the Noguchi Memorial Institute for Medical Research, Accra.

3.10 Bioinformatic analysis

The raw sequence reads were processed for quality filtering and trimming with the help of FASTQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and Trimmomatic V0.39. The minimum quality threshold was defined as Q20 (Andrews, 2010). Following trimming, the reads were assembled de novo using the Unicycler V0.4.8 assembler with default settings. Contigs with a length exceeding 200 bp were the only ones kept for further analysis.

Utilizing default settings, sequence types (STs) of the assembled genomes were examined for on the MLST 2.0 database offered by the Center for Genomic Epidemiology (CGE) (<http://cge.cbs.dtu.dk/services/MLST/>). The obtained resistance gene types were examined using default settings in ResFinder 4.1, which is hosted by CGE (<https://cge.food.dtu.dk/services/ResFinder/>). The ClermonTyping database (<http://clermonttyping.iame-research.center/>) was used with default settings to determine the phylogroups of *E. coli* isolates.

3.11 Data analysis

Data were initially entered into Microsoft Excel and imported into Statistical Package for the Social Sciences - SPSS for data analysis. With the study variables, descriptive analysis was performed, which involved calculating arithmetic means, frequencies, and percentages. Univariable relations were established between uropathogen and the other study variables.

3.12 Inclusion Criteria

Patients visiting the OPD and diagnosed or suspected to have UTI and not after 10 days discharge from the hospital.

Patients admitted at the wards and diagnose UTI after 48 hours of admission.

3.13 Exclusion Criteria

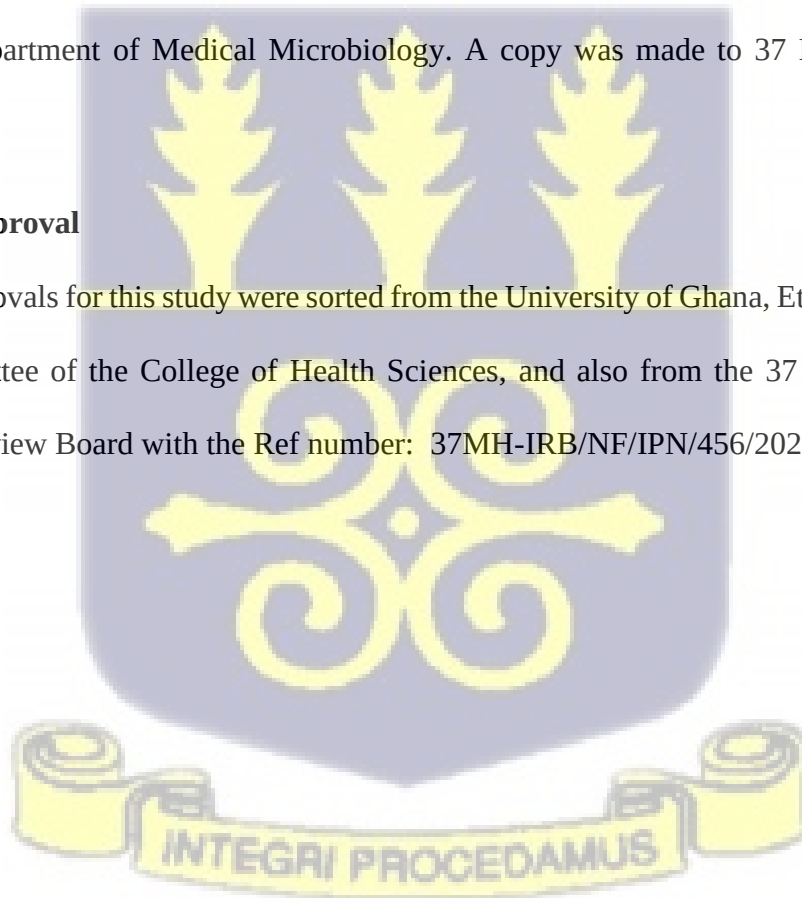
Patients admitted at the wards and diagnose UTI before 48 hours of admission.

3.14 Dissemination of Study Results

The findings of this research work were submitted to the Graduate school of University of Ghana through the Department of Medical Microbiology. A copy was made to 37 Military Teaching Hospital.

3.15 Ethical approval

The ethical approvals for this study were sorted from the University of Ghana, Ethical and Protocol Review Committee of the College of Health Sciences, and also from the 37 Military Hospital Institutional Review Board with the Ref number: 37MH-IRB/NF/IPN/456/2021 (appendix 1).



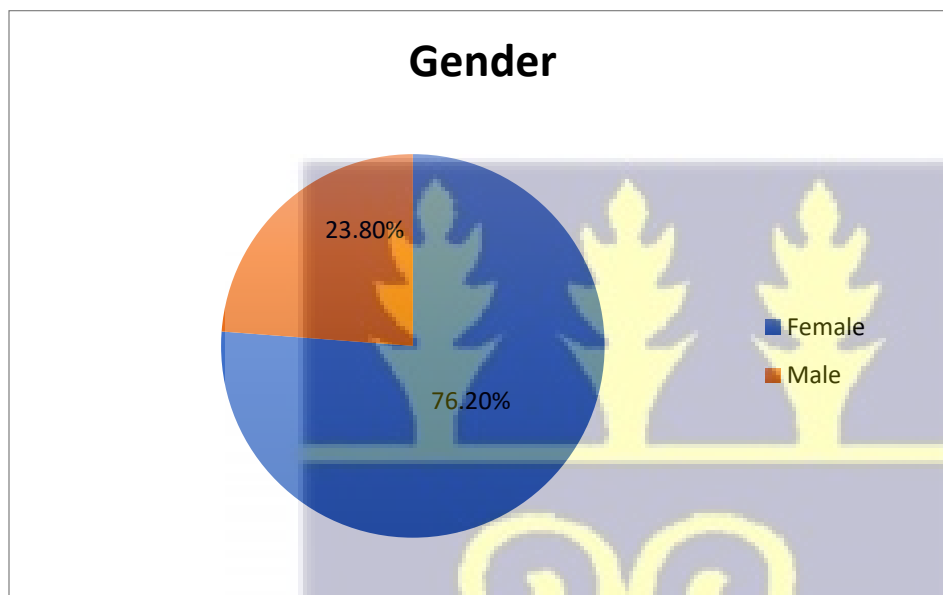
Chapter four

4.0 Results

Demographics

The majority of the positive culture was observed in urine sample of the female participant 112(76.2%) and 35(23.8%) for the male.

Fig 1. shows the gender and the frequency of positive culture.



Etiological GLASS priority pathogen of UTIs and antibiotic resistance

A total of 500 participants mid-stream urine samples were obtained from the outpatient patient department (OPD) and wards of 37 Military Teaching Hospital. Out of these, 147(500) had significant growth and the rate of UTI was 61.9% (91/147) for community patients and 38.1% (56/147) for hospitalized patients (Table 1).

Four different GLASS priority pathogens were identified from the mid-stream urine specimens of the study participants. The isolates were predominantly Enterobacteriaceae. *Escherichia coli* was

the most frequent uropathogen isolated 84(57%) followed by *Klebsiella pneumonia* 53(36%), *Acinetobacter baumannii* 7(5%) and *Staphylococcus aureus* 3(2%) (Table1).

The antibiogram of the GLASS priority uropathogens recovered from the positive urine specimen is presented in Table 2. Most of the bacterial isolated where resistance to ampicillin 91% followed by cefuroxime 71%, trimethoprim sulfamethoxazole 70%, ceftriaxone 65% and 61% amoxicillin/clavulanic acid for the Gram negative bacterial and for the Gram positive the resistance ranges from ciprofloxacin (0%) to Ampicillin (100%).

The overall prevalence of antibiotic resistance among Gram negative bacterial isolates varied from amikacin (2%) to ampicillin (91%). Antibiotic resistance was particularly high for (penicillin) β -lactam antibiotics (fig 1).

In this study, *E. coli* highest reported resistance was against the penicillin drug Ampicillin 88%, Amoxicillin /Clavulanic 63%, followed closely by Trimethoprim-Sulfamethoxazole 77%, cephalosporins third generation drugs ceftazidime 57%, ceftriaxone 69%, aminoglycosides drugs gentamycin 37%, fluoroquinolone ciprofloxacin was 51%, Levofloxacin 51%. *E. coli* had the lowest resistance against aminoglycosides drugs (2% amikacin), glycylicycline (7% tigercycline), carbapenems (11% meropenem, and 16% imipenem) and Furadantins (Nitrofurantoin was 20%).

Klebsiella pneumoniae was most resistance against the penicillin drug Ampicillin 100%, Amoxicillin/ Clavulanic 64%. The resistance pattern was similar to *E. coli* with a 0-4% difference against aminoglycoside and cephalosporins. The exclusions to these similarities were Trimethoprim-Sulfamethoxazole, nitrofurantoin, Levofloxacin, imipenem and meropenem were 69%, 60%, 27%, 10%, and 6% respectively. The lowest resistance exhibited was on amikacin 2%

followed by the carbapenems (6% meropenem and 10%, imipenem) and glycylicycline (7% tigecycline).

Acinetobacter baumannii shows the highest resistance to several class of antibiotics, although it is the less gram-negative bacteria and the only *Enterobacterales* isolated according to our study data. They absolutely resisted most of the Penicillin, and nitrofurantoin 100% respectively as well as show high resistance to the cephalosporin antibiotics (Cefuroxime 86%, ceftazidime 57%, and ceftriaxone 47%) followed by tigecycline 71%, Trimethoprim-Sulfamethoxazole 57%, Gentamicin 43%, and Ciprofloxacin 29%. *Acinetobacter baumannii* shows 0% resistance to Amikacin and less resistance to Piperacillin-Tazobactam 14%, Levofloxacin 14%, imipenem 14% and 14% meropenem.

Staphylococcus aureus was the only Gram-positive GLASS priority pathogen isolated from this study. They completely resisted ampicillin 100% followed by 66.30% of gentamicin, cefotaxime, penicillin and clindamycin. *Staphylococcus aureus* shows 0% resistance to amoxicillin/clavulanic, ciprofloxacin, nitrofurantoin, ceftazidime and less resistance to gentamicin Trimethoprim-Sulfamethoxazole 33.30%, and Vancomycin 33.30% (figure 2).

Table1: The overall distribution of urinary GLASS isolates according to their infection onset whether community or hospital-acquired.

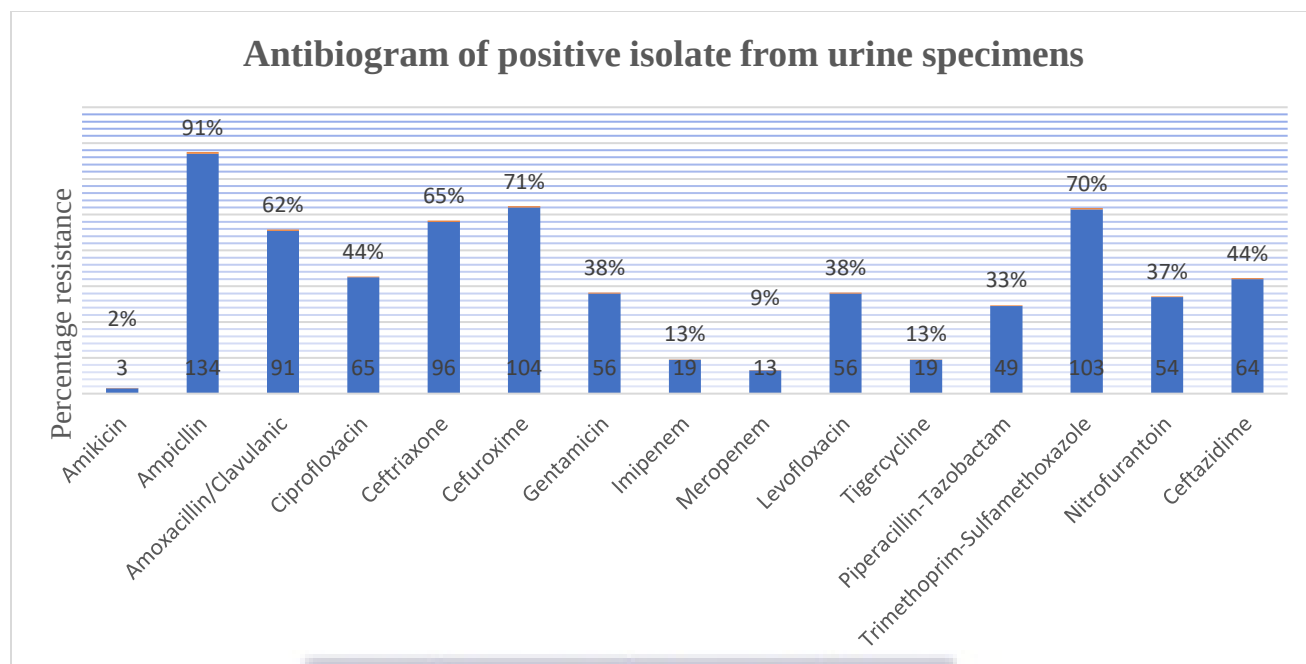
Isolates (n=147)	Community-acquired (n=91)	Hospital-acquired (n=56)
<i>E. coli</i> (n=84)	51(60.7%)	33 (39.3%)
<i>K. pneumoniae</i> (n=53)	33(62.3%)	20(37.7%)
<i>Acinetobacter baumannii</i> (n=7)	5(71.4%)	2(28.6%)
<i>S. aureus</i> (n=3)	2(66.7%)	1(33.3%)

Table 2: The frequency and proportion of antimicrobial resistance among isolated GLASS priority pathogen isolated.

Antibiotics	<i>E. coli</i> n (%)	<i>K. pneumoniae</i> n (%)	<i>A. baumannii</i> n (%)	<i>S. aureus</i> n (%)
Amikacin	2(2%)	1(2%)	0(0%)	NT
Ampicillin	74 (88%)	52 (100%)	7(100%)	3(100%)
Amoxicillin/Clavulanic	50 (63%)	39(64%)	7(100%)	0(0)
Ciprofloxacin	43 (51%)	20(38%)	2(29%)	0(0)
Ceftriaxone	58(69%)	35(66%)	3(43%)	NT
Cefuroxime	61(73%)	47(70%)	6(86%)	NT
Gentamicin	31(37%)	20(38%)	3(43%)	2(66.6%)
Imipenem	13(16%)	5(10%)	1(14%)	NT
Meropenem	9(11%)	3(6%)	1(14%)	NT
Levofloxacin	41(51%)	14(27%)	1(14%)	1(33.3%)
Tigecycline	6(7%)	10(19%)	5(71%)	NT
Piperacillin-Tazobactam	30(36%)	18(34%)	1(14%)	NT
Trimethoprim-Sulfamethoxazole	63(77%)	35(69%)	4(57%)	1(33.3%)
Nitrofurantoin	16(20%)	31(60%)	7(100%)	0(0)
Ceftazidime	4(57%)	25(57%)	4(57%)	NT
Vancomycin	NT	NT	NT	1(33.3%)
Cefotaxime	NT	NT	NT	2(66.6%)
Penicillin	NT	NT	NT	1(33.3%)
Cefoxitin	NT	NT	NT	0(0)
Clindamycin	NT	NT	NT	2(66.6%)

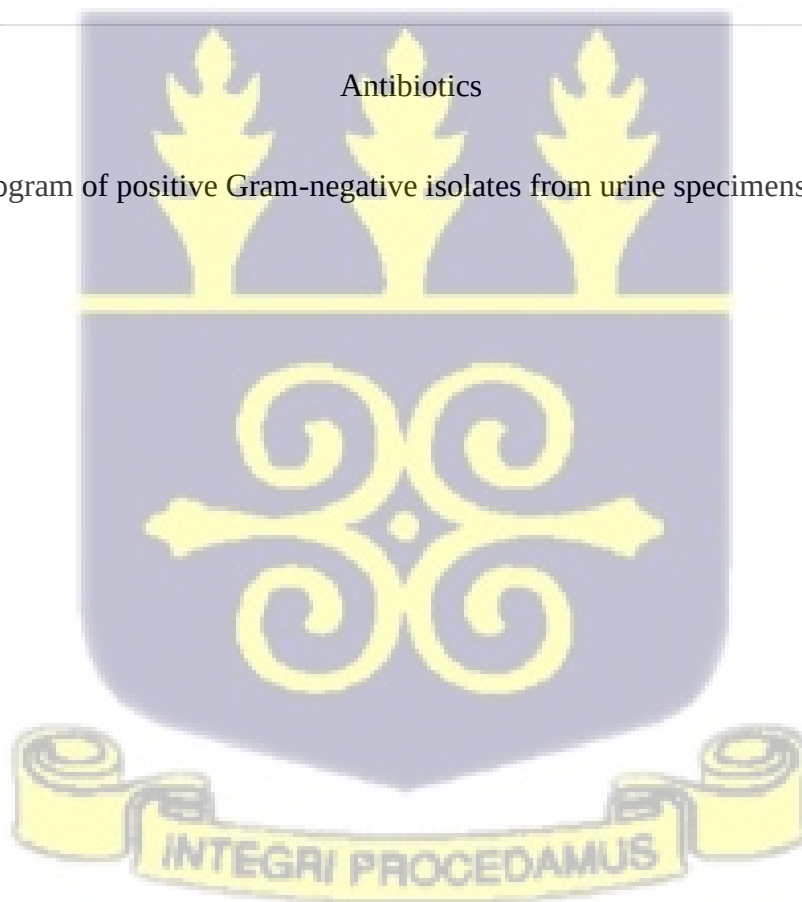
n: frequency of resistant isolate **NT:** not tested **%:** proportion of resistance calculated using the total frequency of specific isolates as denominator.





Antibiotics

Figure 2. Antibiogram of positive Gram-negative isolates from urine specimens



Overall prevalence of resistance among the antibiotics tested on the Gram-positive isolate

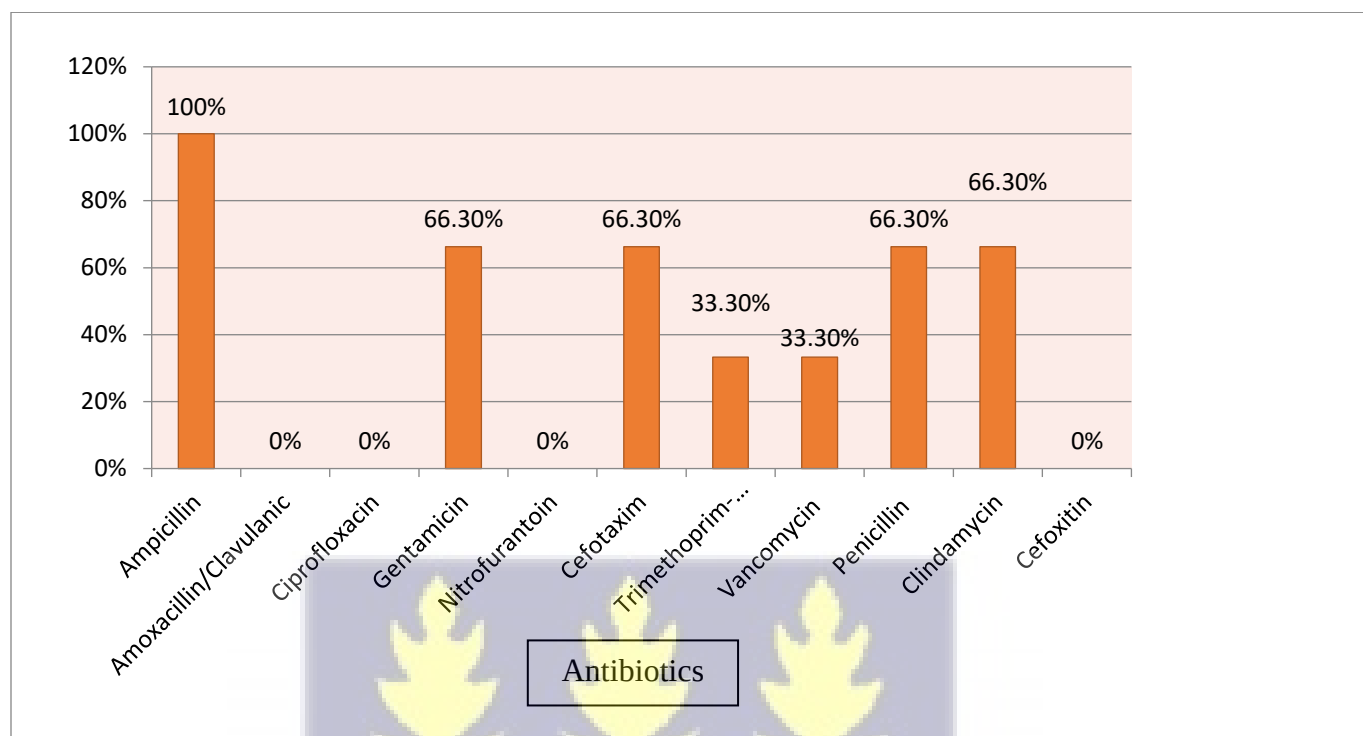


Figure 3. Antibigram of positive Gram-positive isolate from urine specimens

Phenotypic confirmatory tests for ESBL production: Double disc synergy diffusion test (DDST)

Bacteria Isolate	Number of isolate (N)	ESBL N %
<i>E. coli</i>	84	55 (65.5)
<i>Klebseilla pneumoniae</i>	53	36 (67.9)
<i>Acinetobacter baumannii</i>	7	4 (57.1)

Table 3 Phenotypic confirmatory tests for ESBL production

ESBLs phenotypically, were found among some Gram-negative isolates tested. The overall prevalence of ESBL producing Gram negative isolates was 95 (66.0%).

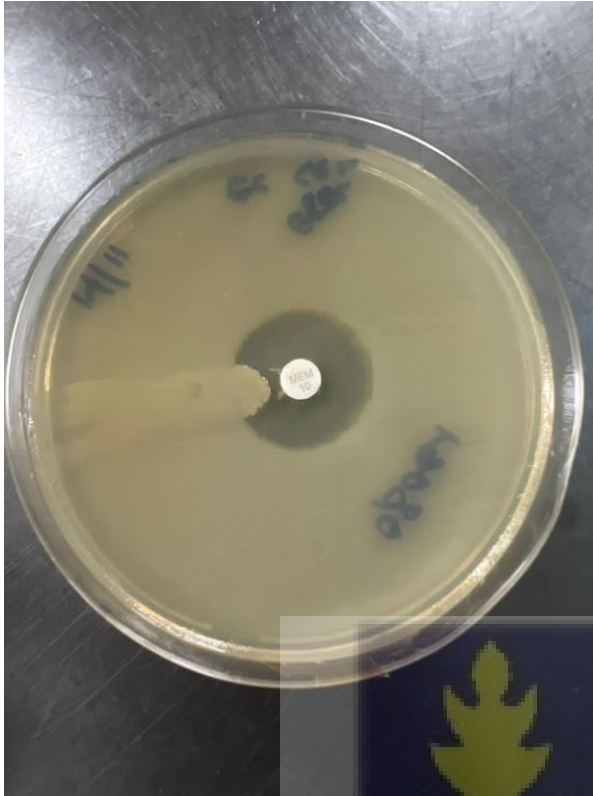
Phenotypic confirmatory tests for carbapenem resistance bacterial

Bacteria Isolate	Number of isolate (N)	N %
<i>E. coli</i>	84	14 (16.6)
<i>Klebseilla pneumoniae</i>	53	6 (11.3)
<i>Acinetobacter baumannii</i>	7	1 (14.3)

Table 4 Phenotypic carbapenemase activity was detected in 21 (14.3%) of the 144 GLASS isolate.

Figure 4. pictorial exhibition of Phenotypic ESBL and CRE of gram-negative GLASS priority pathogen.





(a)



(b)

(a) Phenotypic confirmatory tests for carbapenemase producing.

(b) Phenotypic confirmatory tests for ESBL producing bacteria.

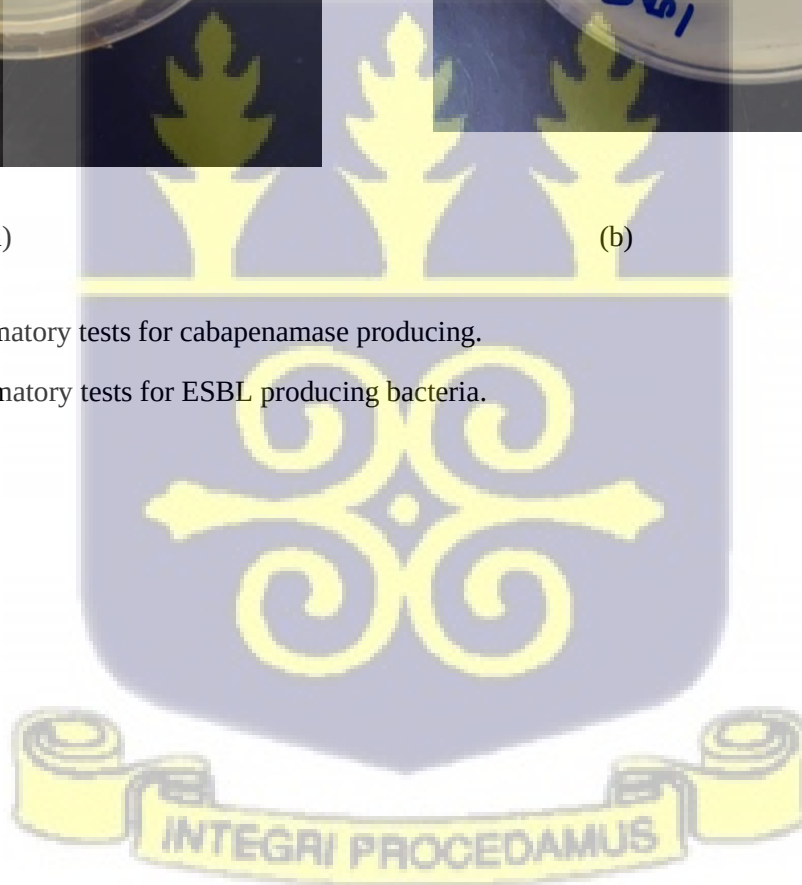


Table 5; Prevalence of Gram-negative GLASS priority pathogen isolated from patients with CA-UTI and HA-UTI and their respective frequency of ESBL and Carbapenemase production at 37 Military Hospital, Ghana from January 2023 to June 2023 (n=144).

GLASS isolate (n)	Frequency (n, %)		ESBL (n, %)		CRE (n, %)	
	CA-UTI	HA-UTI	CA-UTI	HA-UTI	CA-UTI	HA-UTI
<i>E. coli</i> (84)	51(60.7)	33 (39.3)	21(25)	34(40.1)	8(9.5)	6(7.1)
<i>K. pneumoniae</i> (53)	33(62.3)	20(37.7)	21(39.6)	15(28.3)	4(7.5)	2(3.8)
<i>Acinetobacter baumannii</i> (7)	5(71.4)	2(28.6)	1(14.3)	3(42.9)	1(14.3)	-
Total	89(61.8)	55(38.2)	43(48.3)	52(94.5)	13(14.6)	8(14.5)

Accurate species identification and misidentification

Traditional diagnostic methods incorrectly identified certain isolates as either *E. coli* or *K. pneumoniae*. Nineteen MDR isolates were selected after using BD phoenix to identify them as ten *E. coli*, eight *K. pneumoniae* and one *A. baumannii*. These isolates were further confirmed using MALDI-TOF MS (Matrix-assisted laser desorption ionization–time of flight mass spectrometry) before sequencing.

Post-sequencing verification using bioinformatics tools (MLST and Pathogenwatch) revealed eight true *E. coli*, six *K. pneumoniae*, one *K. quasipneumoniae*, and one *A. baumannii*, with additional species including *Enterobacter cloacae* and *Citrobacter freundii*. Two isolates were not sequenced successfully. The following genomic analysis concentrated on the 15 GLASS priority

uropathogen isolates that were confirmed, with 8 being *E. coli*, 6 being *K. pneumoniae*, and 1 being *A. baumannii*.

Resistome, mobilome, and virulome of Gram-negative GLASS priority pathogen

The genotypic prediction deduced from the phenotypic characteristics showed a perfect concordance. Numerous resistance genes were recovered from the selected GLASS uropathogen. Nonetheless, according to Resfinder and the NCBI AMR Finder, the majority of the Gram-negative GLASS priority pathogens (n = 10/15) conversed the *bla*_{CTX-M-15} gene. The *bla*_{CTX-M-1} and *bla*_{CTX-M-27} (variant of *bla*_{CTX-M-15} gene) was also detected in one of the selected isolates. The second most frequent ESBL genes detected were *bla*_{OXA-1} (9/15) followed by *bla*_{TEM-1B} (7/15) whereas the following genes *bla*_{SHV-11}, *bla*_{SHV-67}, *bla*_{SHV-35}, *bla*_{SHV-38}, *bla*_{SHV-56}, *bla*_{SHV-28}, *bla*_{SHV-40}, *bla*_{SHV-85}, *bla*_{SHV-79}, *bla*_{SHV-89}, *bla*_{SHV-106}, *bla*_{OXA-232}, *bla*_{CYM-138}, *bla*_{CYM-14}, *bla*_{CYM-149}, *bla*_{CYM-15}, *bla*_{CYM-16}, *bla*_{CYM-42}, *bla*_{DHA-1}, were found in at least one of the sequenced isolates (Table 6).

Certain resistome of ESBL which includes Aminoglycoside resistance genes (*aac*(3)-IIa, *aac*(6')-Ib-cr, *aadA16*, *aph*(6)-Id), Folate pathway antagonists (*sul1*, *sul2*, *OqxB1*, *dfrA27*, *dfrA15*, *dfrA14*, *dfrA17*, *dfrB4*), Tetracyclines (*tet* (A), *tet*(B) *tet*(D), and Fluoroquinolones (*aac*(6')-Ib-cr, *qnrS1*, *qnrB4*, *qnrB2*, *qnrB6*, *qepA2*), Rifampicin resistance gene (*ARR-3*), Fosfomycin (*fosA*), Macrolide (*erm*(B)) and Chloramphenicol (*catA1*, *catB3*) were also recovered in this study.

Moreover, several virulence genes were detected in the *E. coli* and *Klebsiella pneumonia* isolates. Out of all the isolates, the most conserved virulence gene was: FimH (mediate adhesion), *csgA* (mediated biofilm formation), *gad* (acid resistance), *lpfA* (long polar fimbriae) *cea* (Colicin E1), *hlyF* (hemolysin F), *cvaC* (Microcin C), *iss* (Increased serum survival), *celb* (Endonuclease colicin E2), *sitA* (iron transport protein), *papC* (Outer membrane usher P fimbriae), *tsh* (temperature-

sensitive hemagglutinin) irp2(iron-acquisition) and iucC (Aerobactin synthetase). Several other were seen in some of the isolates air (Enteroaggregative immunoglobulin repeat protein), terC (Tellurium ion resistance protein), astA (EAST-1 heat-stable toxin), and traT (Outer membrane protein complement resistance) (Table 6)

All fifteen of the sequenced isolates had different sequence types (STs) with some isolates belonging to the same phylogroup. By using multi locus sequence typing (MLST), the eight isolates of *E. coli* were categorized into eight known sequence types (STs), ST 354, ST 1284, ST 10, ST 131, ST 315, ST 6332, ST 1722, and ST 410. Some of these *E. coli* strains belongs to the same phylogroup; GLS059 and GLS060 (phylogroup A), GLS037 and GLS059 (phylogroup F), and GLS063 and GLS138 (phylogroup C). Phylogroup B2 and D were also recovered in this study (Table 7). STs 464 (2/6) was the most prevalent *K. pneumoniae* detected among the six identified. *Acinetobacter baumannii* was also identified as ST 2220.

Table 6: Genomic analysis - Gram negative Glass Priority pathogens isolated from mid-stream urine (MSU)

ID	Species	Status	Class of Antibiotics	Plasmid	Resistance gene	Virulence gene
GLS26	<i>K.pneumoniae</i>	XDR	Aminoglycoside Amphenicol Cephalosporins Beta-lactam Folate pathway antagonist Fosfomycin Rifamycin Quinolone Tetracycline	IncFIA, IncFIB(AP001918)	ARR3,OqxA,OqxB,aac(3)-IIa,aac(6')-Ib-cr,aadA16,blaCTX-M-15,blaOXA-1,blaSHV-11,blaSHV-67,catA1,catB3,dfrA27,fosA,qnrB6,sul1,tet(D)	fimH,fyuA,irp2,iutA,mrkA:ABW83989,nlpI,traT
GLS037	<i>E. coli</i>	MDR	Quinolone beta-lactam folate pathway antagonist tetracycline	IncFIA, IncFIB(AP001918)	blaTEM-1B, catA1, dfrB4, qepA2, sul1, tet(B)	AslA, air,anr,chuA,csgA,dhaK,eilA,espY2:000868321,

						fimH,fyuA,gad,hlyE,hra,ibeA,irp2,iucC,iutA,kpsE,kpsMII,lpfA,nlpI,papA_F13,papC,sitA,terC,usp,yehA,yehB,yehC,yehD,yfcV
GLS059	<i>E. coli</i>	XDR	Aminoglycoside Beta-lactam Cephalosporins Folate pathway antagonist Aminocyclitol Macrolide Quinolone Tetracycline	Col(MG828),Col(pHAD28),IncFIA,IncFIB(AP001918),IncFII	aac(6')-Ib-cr,aadA5,aph(3")-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-35,catB3,dfrA17,erm(B),mph(A),sul1,sul2,tet(B)	AslA,anr,csgA,fdeC,hlyE,iss,iucC,iutA,nlpI,sitA,terC,traJ,traT,yehA,yehB,yehC,yehD
GLS060	<i>E. coli</i>	DR	Amphenicol	IncFIA,IncFIB(AP001918),IncFII	catA1	AslA,anr,csgA,fdeC,fimH,fyuA,hha,hlyE,irp2,nlpI,papC,sitA,terC,traJ,yehA,yehB
GLS061	<i>E. coli</i>	XDR	Aminoglycoside Amphenicol Cephalosporins Beta-lactam folate pathway antagonist Macrolide Aminocyclitol Quinolone Tetracycline	Col(MG828),Col156,IncFIA,IncFIB(AP001918),IncFIB(H89-PhagePlasmid),IncI1-I(Alpha)	aac(6')-Ib-cr,aadA5,aph(3")-Ib,aph(6)-Id,blaCMY-138,blaCMY-14,blaCMY-149,blaCMY-15,blaCMY-16,blaCMY-2,blaCMY-4,blaCTX-M-15,blaOXA-1,catB3,dfrA17,mph(A),sul1,sul2,tet(A)	AslA,aamR:FN554766,afaA,afaB,afaC,afaD,afaE,chuA,csgA,fdeC,fimH,fyuA,gad,hra,iha,irp2,iss,iucC,iutA,kpsE,kpsMII_K5,nlpI,ompT,papA_F43,sat,senB,sitA,terC,usp,yehA,yehB,yehC,yehD,yfcV
GLS062	<i>E. coli</i>	XDR	Aminoglycoside Cephalosporins Beta-lactam Folate pathway antagonist Macrolide Quinolone Tetracycline	IncFIB(AP001918),IncFIB(K),IncFII,IncI1-I(Alpha),IncY	aac(3)-IIId,aph(3")-Ib,aph(6)-Id,blaCMY-42,blaCTX-M-15,dfrA14,erm(B),mph(A),qnrS1,sul2,tet(A)	AslA,air,capU,chuA,csgA,eilA,fdeC,fimH,gad,hlyE,iss,kpsE,kpsMII_K5,nlpI,sitA,terC,traT,yehB,yehC,yehD
GLS063	<i>E. coli</i>	XDR	Aminoglycoside Cephalosporins Beta-lactam Folate pathway antagonist Macrolide Quinolone Tetracycline Aminocyclitol	Col(MG828),IncFIA,IncFIB(AP001918),IncQ1,IncY	aac(3)-IIa,aac(6')-Ib-cr,aph(3")-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaTEM-1B,catB3,dfrA14,mph(A),sul2,tet(A)	anr,csgA,fdeC,fimH,fyuA,gad,hha,hlyE,irp2,iss,iucC,iutA,lpfA,nlpI,sitA,terC,traJ,traT,yehA,yehB,yehC,yehD
GLS071	<i>K. quasipneumoniae</i>	XDR	Aminoglycoside Amphenicol Cephalosporins Beta-lactam	Col440I,ColKP3,IncFIB(K)(pCAV1099-	OqxA,OqxB,aac(3)-IIa,aac(6')-Ib-cr,aph(3")-Ib,aph(6)-Id,blaCTX-M-15,blaOKP-B-	fimH,iutA,mrkA:ABW83989,nlpI,terC,traT

			folate pathway antagonist Fosfomycin Quinolone Tetracycline Carbapenem	114),IncFIB(pQil),IncFII(K),IncN	19,blaOXA-1,blaOXA-232,blaTEM-1B,catB3,dfrA14,fosA,qnrB1,sul2,tet(A)	
GLS084	<i>K.pneumoniae</i>	XDR	Aminoglycoside Cephalosporins Quinolone Beta-lactam Folate pathway Antagonist Tetracycline Fosfomycin Amphenicol	Col(MG828),Col440I,IncFIB(K)(pCAV1099-114),IncFIB(pKPHS1),IncFII(K),IncHI1B(pNDM-MAR),IncR	OqxA,OqxB,aac(3)-IIa,aac(6')-Ib-cr,aadA1,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaSHV-38,blaTEM-1B,catA1,catB3,dfrA15,fosA,qnrB2,sul1,sul2,tet(A)	fimH,iutA,mrkA:ABW83989,terC
GLS092	<i>K.pneumoniae</i>	XDR	Aminoglycoside Cephalosporins Quinolone Beta-lactam Folate pathway antagonist Tetracycline Fosfomycin Amphenicol	Col(MG828),Col(pHAD28),Col440I,IncFIA(HI1),IncFIB(K)(pCAV1099-114),IncHI1B(pNDM-MAR),pKP1433	OqxA,OqxB,aac(3)-IIa,aac(6')-Ib-cr,aadA1,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaSHV-106,blaSHV-28,blaTEM-1B,catB3,dfrA15,fosA,qnrB2,sul1,sul2,tet(D)	fimH,fyuA,irp2,iutA,mrkA:ABW83989
GLS109	<i>K.pneumoniae</i>	XDR	Cephalosporins Quinolone Beta-lactam Folate pathway antagonist Tetracycline Fosfomycin	Col440I,IncFIA(HI1),IncFIB(pKPHS1),IncR	OqxA,OqxB,blaSHV-40,blaSHV-56,blaSHV-79,blaSHV-85,blaSHV-89,fosA	fimH,fyuA,irp2,iutA,mrkA:ABW83989,nlpI
GLS111	<i>K.pneumoniae</i>	XDR	Aminoglycoside Cephalosporins Quinolone Beta-lactam Folate pathway antagonist Tetracycline Fosfomycin Amphenicol	Col(pHAD28),Col440I,IncFIB(K)(pCAV1099-114),IncFIB(pKPHS1),IncFII(K),IncHI1B(pNDM-MAR),IncR,IncX4	OqxA,OqxB,aac(3)-IIa,aac(6')-Ib-cr,aadA1,aph(3'')-Ib,aph(6)-Id,blaCTX-M-15,blaOXA-1,blaSHV-38,blaTEM-1B,catA1,catB3,dfrA15,fosA,qnrB2,sul1,sul2,tet(A)	fimH,iutA,mrkA:ABW83989,terC
GLS117	<i>E. coli</i>	XDR	Aminoglycoside Cephalosporins Beta-lactam Folate pathway antagonist Macrolide Quinolone Tetracycline Amphenicol	Col(MG828),Col8282,IncFIB(AP001918),IncFII,IncFII(pSE11),IncQ1	aph(3'')-Ib,aph(6)-Id,blaCTX-M-27,blaDHA-1,catA1,dfrA17,erm(B),mph(A),qnrB4,sul1,sul2,tet(B)	AslA,afaD,air,astA,chuA,csgA,eilA,espY2:000868321,fd eC,fimH,fyuA,gad,hlyE,hra,irp2,iss,kp sE,lpfA,nlpI,shiB,terC,yehA,yehB,yehC,yehD,yfcV
GLS138	<i>E. coli</i>	XDR	Aminoglycoside Cephalosporins Beta-lactam	Col156,IncFIA,IncFIB(AP001918),IncFII	aac(6')-Ib-cr,aadA5,blaCTX-M-15,blaOXA-	csgA,fdeC,fimH,fyuA,gad,hha,hlyE,irp2,iucC,iutA,lpfA,

			Folate pathway antagonist Macrolide Quinolone Tetracycline Aminocyclitol		1,catB3,dfrA17,mph(A), sul1,tet(A)	nlpI,senB,sitA,terC ,yehA,yehB,yehC,yehD
GLS140	<i>Acinetobacter baumannii</i>	ND	ND	ND	ND	ND

ND – Not detected

Table 6: Genomic diversity of sequenced – Gram negative Glass Priority pathogens isolated from mid-stream urine

ID	ST	Serotype	Phylogroup
GLS26	ST 147	K10	KpI
GLS037	ST 354	O1:H34	F
GLS059	ST 1284	O101: H21	A
GLS060	ST 10	O101: H10	A
GLS061	ST 131	O25: H4	B2
GLS062	ST 315	O7: H15	D
GLS063	ST 6332	O8: H21	C
GLS071	ST 5330	K103	KpII-B
GLS084	ST 464	K53	Kp1
GLS092	ST 15	K48	Kp1
GLS109	ST 111	K63	Kp1
GLS111	ST 464	K53	Kp1
GLS117	ST 1722	O11: H25	F
GLS138	ST 410	O ^a : H9	C
GLS140	ST 2220	-	-

Unknown O (somatic) antigen- a*



Chapter five

5.0 Discussion

Globally, drug-resistant bacterial are rapidly emerging as well as AMR posing threat to public health and needs to be dealt with locally, nationally and internationally basis. *In The lancet*, the report from Global Burden of Diseases (GBD) 2019 Antimicrobial Resistance Collaborators estimated that 7.7 million mortalities out of the estimated 13.7 million infection-related deaths in 2019 was caused by bacteria antimicrobial resistance (Ikuta, *et al.*, 2022). The infections were associated with five leading bacterial (*S aureus*, *E coli*, *S pneumoniae*, *K pneumoniae*, and *P aeruginosa*) of which 3 are included in this study. The most prevalence bacterial responsible for most deaths are *E. coli* followed by *Klebsiella pneumoniae* (Ahmed, *et al.*, 2019; Yassen, 2023).

To combat AMR, the World Health Organization (WHO) has initiated a program called Global Antimicrobial Resistance Surveillance System (GLASS) with the goal to establish standardize AMR and improve positive patient outcome worldwide (Islam *et al.*, 2019). Thus, this study was conducted to evaluate the etiology and antibiogram of GLASS-priority pathogens causing UTIs among patients receiving treatment at 37 Military Hospital, Accra - Ghana.

UTI is one of the most common infections, with a variety of etiological agents, affecting at least 250 million people each year and in complicated UTI cases causes the death of 150 million people globally (Mohiuddin & Nasirullah, 2019).

Females (76.2%) in this study shows higher significant bacteria growth in their urine sample than males (23.8%) similar results were reported in other studies with prevalence being generally higher in female than in males (Gyansa-Lutterodt, *et al.*, 2014; Ahmed, *et al.*, 2019; Yassen, 2023). The anatomical disparities between the male and female urethras and closeness of the female urethra

to the anal orifice may be the contributing factor of the relatively high UTI in female as cited by Ahmed, *et al.*, 2019. Other factors may include the capability of bacterial from the anal orifice to adhere to the uroepithelium as a result of colonization of the urogenital mucosa through adhesins, pili, fimbriae, and the P-1 blood group phenotypic receptor. (Bhargava, *et al.*, 2022).

Furthermore, to achieve successful treatment of UTI patients, it is imperative to accurately identify the causative bacteria and the antimicrobial resistance pattern to enable physicians' select efficacious and right antibiotics for management. In our study the most prevalent urinary tract Glass priority pathogen found among males and females was *E. coli* followed by *Klebsiella pneumoniae*. These findings were in line with several studies conducted in other part of the region in Ghana with regard to UTI related study (Gyansa-Lutterodt, *et al.*, 2014; Donkor *et al.*, 2019; Karikari, *et al.*, 2022; Asamoah, *et al.*, 2022). According to Owusu *et al.*, 2022, seventy five percent (75%) of pathogens causing UTI are *E. coli* and *Klebsiella pneumoniae*. These microorganisms dominate the gut flora of humans, which is recognized to be a prevalent source of autoinfection for UTIs (Asamoah, *et al.*, 2022). The findings of our study also largely agree with a study conducted in Northern India, which found that *E. coli* and *K. pneumoniae* were the two most common bacteria, with 55.0 % and 6.6% of cases, respectively (Bhargava, *et al.*, 2022). Similar findings were made by Regmi *et al.*, 2018 in another related investigation, which found that the majority of the isolates were *E. coli* (65.84%) and *K. pneumoniae* (12.42%). whereas in Iraq, Jalil, & Al Atbee, reported the prevalence of *E. coli* (68.3%) and *K. pneumoniae* (31.7%).

Also, out of the total 500 morning mid-stream urine collected, 147 positive isolates culture were identified and, among those, 61.9% (91/147) of the isolates were recovered from CA-UTI and 38.1% (56/147) from HA-UTI. This finding was similar with Perera, *et al.*, 2022 in Sri Lanka with 72% (302/422) from CA-UTI and 28% (120/422) were from HA-UTI. *E. coli* was the most

frequent isolated microorganism (60.7% in CA-UTI and 39.3% in HA-UTI), followed by *Klebsiella pneumoniae* (62.3% in CA-UTI and 37.7% in HA-UTI). This is in line with the findings of (Peñaranda *et al.*, 2020; Fourie, *et al.*, 2021; Gebremedhin, *et al.*, 2023). Apart from the high outcome of the positive isolates obtained from the CA-UTI, there was high prevalence of resistance of 2, 3 or more classes of antibiotics. These observed patterns of resistance from the communities could be explained by the habitual use of same antibiotics in treating recurrent UTIs and other form of bacterial infection with similar antimicrobial coverage to UTIs (Ku, *et al.*, 2023). Another reason may be that individual within the community use existing prescription to buy antibiotics and share left over medication among family or friends (Rather, *et al.*, 2017). These indiscriminate uses of antibiotics exert selective pressure on these pathogens and commensal human microflora to develop some mechanism of resistant as well as share their resistance genes to near-by bacterial (Gillings *et al.*, 2017). It could also be looked at from a different dimension where there may be a surge of resistance genes (i.e., ESBL and Carbapenemase) spreading within the community owing to inappropriate disposer of pharmaceutical waste in the environment and the water bodies (Islam *et al.*, 2019). According to Kubone *et al.*, 2020, the over diagnosis of UTIs leads to the prescription of these medications to people who weren't really in need of them, which is what may have created the observed resistance.

E. coli was most reported resistance against the penicillin drug Ampicillin 88%, Amoxicillin/Clavulanic 63%, followed closely by Trimethoprim-Sulfamethoxazole 77%, cephalosporins third generation drugs (cefuroxime 73%, ceftazidime 57%, ceftriaxone 69%), fluoroquinolone (ciprofloxacin was 51%, Levofloxacin, 51%, nitrofurantoin was 20%). However, *E. coli* had least resistance against aminoglycosides drug (2% amikacin and 37% gentamycin) and carbapenems (11% meropenem, 16% imipenem) similar results were found in (Donkor *et al.*,

2019; Ahmed, *et al.*, 2019; Hossain, *et al.*, 2020). Related study in Iraq (Yasen, 2023) shows a high resistance percentage of *E. coli* to amikacin which contradicted our findings. The resistance of the cephalosporins exhibited by *E. coli* may be as a result of conferring some resistance gene of ESBL.

Again, our study showed similar resistance pattern between *Klebsiella pneumoniae* and *E. coli*, these findings were also mentioned in (Yasen, 2023) besides the resistance percentages of ampicillin and nitrofurantoin has elevated for *Klebsiella pneumoniae* than *E. coli*. The similarity in *E. coli* and *klebsiella pneumonia* resistance patterns may probably be attributed to the fact that both isolates belong to the same family, that they are found in comparable environments, and that both have high rates of horizontal gene transfer (Yasen, 2023). The less resistant drug was amikacin and the carbapenems.

Acinetobacter baumani was highly resistant to majority of the antibiotics tested against in this study. Most resistance was to the Ampicillin (100%), Amoxicillin/Clavulanic (100%), nitrofurantoin (100%), followed by cefuroxime (86%), tigecycline (71%), trimethoprim-sulfamethoxazole (57%) and ceftazidime (57%) these findings were also similar in. The most susceptible antibiotics against these bacteria was amikacin (100%) and carbapenems (86%) in this study.

Staphylococcus aureus was the only Gram-positive bacteria identified among the Glass priority pathogen which constituted only 2.04% of significant microbial isolates in this study. This is in line with the findings of Gyansa-Lutterodt, *et al.*, 2014. This also agrees with the finding that Gram-negative bacteria are the predominant etiologic agents, responsible for over eighty-five percent of all UTI cases, and are part of the normal intestinal flora, particularly in the rectum,

which is proximal to the urethral orifice (Gyansa-Lutterodt, *et al.*, 2014; Fatima, & Mussaed 2017).

The high amoxicillin resistance rate of 91.0% found in our study reflects a well-established global pattern, including reports from Africa of rates above 70% (Mamuye, 2016). Study from international for instance, have documented *E. coli* resistance to amoxicillin at 85% in Ethiopia, 67.5% in Senegal, and 72% in India. (Aypak *et al.*, 2009; Krishna *et al.*, 2013; Majumder *et al.*, 2014). As one of the first penicillin's, its extensive use over years has led to significant resistance in approximately two-thirds of gram-negative rods. For this reason, it is not considered a first-line antibiotic for UTIs and should only be used if sensitivity is confirmed by culture. Similarly, Ampicillin, another early penicillin derivative known for its enhanced gram-positive coverage—showed a 100.0% resistance rate in our study.

Cefuroxime, a first-line antibiotic for lower UTIs per Ghanaian guidelines, showed a resistance rate of 71% in our study. This is comparatively lower than the 85% rate documented in Romania in 2019 (Golli A.L., *et al.*, 2019). Similar to our investigation, which observed substantially decreased sensitivity in gram-negative uropathogens, several studies have also reported resistance to β -lactams, cephalosporins, and fluoroquinolones (Sood & Gupta 2012; Sharma *et al.*, 2020; Malik *et al.*, 2021).

Again, mostly physicians manage uncomplicated UTIs by prescribing empirical antibiotics (Fourie, *et al.*, 2021). Uncomplicated UTIs are one of the main reasons for outpatient visits in Ghana, a country in West Africa (Donkor *et al.*, 2019). Individuals who have been initially diagnosed to uncomplicated UTIs on the basis of presenting their symptoms or regular urine screening are given empirical antibiotics while waiting for the AST results to confirm the diagnosis

and choose the susceptible antibiotics to curtail the UTIs (Owusu *et al.*, 2022). The selection of first-line antibiotics for a given illness is influenced by several factors in which every country has their own developed protocols. These recommendations are done base on the rate of resistance of etiological bacterial and the antibiotics therapeutic efficacy, availability and accessibility of the antibiotic's, potential side effects associated with antibiotics, the financial burden on the medical institution and the patient, as well as the capacity to produce novel antibiotics to counteract the selection pressure induced by antibiotic resistance (Kubone, *et al.*, 2020; Owusu, *et al.*, 2022). The management of uncomplicated UTIs was incorporated into the 7th edition of the Standard Treatment Guidelines (STGs) which was published by the Ministry of Health of Ghana in 2017(MoH, 2017). For uncomplicated UTI, oral ciprofloxacin or cefuroxime was recommended as the first line of antibiotics and to be taken for 5-7 days for females and 10–14 days for males whilst with complicated UTIs parenteral antibiotics such as gentamicin or ceftriaxone to be administered according to Ghana standard treatment recommendations (STGs) (MoH, 2017). However, findings of this study show some significant level of resistance to this first line of both the oral (ciprofloxacin 44% and cefuroxime 75%) and parenteral antibiotics (gentamicin 39% and ceftriaxone 67%) recommended by the Minister of Health (MoH). This is similar to the findings of earlier study conducted in Ghana by Asamoah, *et al.*, 2022; where among oral antibiotics, cefuroxime (60.2%) and ciprofloxacin (62.3%) exhibited highest resistance. These findings were also consistent with those of Owusu *et al.*, 2022. According to the findings of Owusu *et al.*, 2022, 89% of the physicians prescribe in accordance to STG and the prevalence antibiotics empirically prescribed for the treatment of UTIs were ciprofloxacin 31% and cefuroxime 54%. This implies that most physicians pay heed to the Ghana Standard Treatment Guidelines in the management of common infections such UTIs. This call for a major surveillance across the nation to revise the

national STGs in reference to the recommended treatment for both uncomplicated and complicated UTIs as to achieve the purpose of treatment and reduce the detrimental effect of AMR.

Furthermore, the recent change in the UTI susceptibility pattern could be possible due to the exponential growth of enterobacteria having a comparatively high prevalence of ESBL and Carbapenemase (Carrasco, *et al.*, 2022). ESBL and carbapenem resistance genes are harbored in plasmids, which are known of transporting MDR genes and affecting other nearby bacterial to be MDR bacterial (Gekenidis, *et al.*, 2020). These increasing resistances possibly require a change in the management of these infections not only empirically but also the rational use of antibiotics.

After phenotypic screening in our study, it was found that 65.5% (55/84) was *E. coli* followed by *K. pneumoniae* 67.9% (36/53), and *Acinetobacter baumannii* 57.1% (4/7) were ESBL producing organisms (figure 3). This is in line with the findings in Mexico, Sierra-Díaz, *et al.*, 2019 with 27% of *Klebsiella pneumoniae* strains and 49% of *E. coli* strains showed extended-spectrum beta-lactamase (ESBL) production. The study showed high proportion of ESBL producing *E. coli* from the HA-UTI 40.1% (34/84) than CA-UTI 25% (21/84) but *Klebsiella pneumonia* exhibited opposite with rate from CA-UTI 39.6% (21/53) higher than HA-UTI 28.3% (15/53). This finding agrees with ESBL producing *E. coli* (69% HA-UTI and CA-UTI 38%) in a study by Perera, *et al.*, 2022 in Sri Lanka but had different proportion in *Klebsiella pneumonia* (95% HA-UTI and 44% CA-UTI). Again, the ESBL producing bacterial inactivate β -lactam antibiotics by hydrolysis, which results in rendering the antibiotics ineffective (Mahmud *et al.*, 2020; Bhargava *et al.*, 2022). These enzymes (ESBLs) produce by such bacterial confer resistance to the majority of beta-lactam antibiotics, such as monobactams (aztreonam) and third-generation cephalosporins (like ceftriaxone, cefuroxime, ceftazidime, cefotaxime, and cefepime), and they are frequently seen in conjunction with other methods of resistance, leading to multidrug resistance

(Peirano, & Pitout, 2019). Similar to what we found in our study, some studies in Ghana have found resistance to the antibiotics in the β -lactam class, cephalosporins, and fluoroquinolones (Donkor *et al.*, 2019; Asamoah, *et al.*, 2022). The use, over use and misuse of broad-spectrum antibiotics are the main causative factors linked with MDR infections contributing to patient prolonged hospital stay, high cost of treatment and higher mortality and morbidity rates (Prestinaci *et al.*, 2015; Jackson, *et al.*, 2021). Research indicates that the occurrence of ESBL production and antimicrobial resistance is significantly greater in infections acquired in healthcare settings compared to those acquired in the community (Gebremedhin, *et al.*, 2023). This was confirmed further in the findings of our study, which found that isolates obtained from HA-UTIs had a significantly higher proportion of ESBL than isolates recovered from CA-UTI. It is also important to mention that the study period was post COVID-19 pandemic of which feverish patients where perhaps were treated with antibiotics empirically to avoid subsequent bacterial infections; however, the use of these antibiotics may have increased ESBL, Carbapenemase, and MDR among UTI patients (Adebisi *et al.*, 2021).

In clinical settings, carbapenems are frequently employed as a first-line therapy since ESBL-producing bacteria do not readily hydrolyze them (Bhargava *et al.*, 2022). According to Karaiskos, & Giamarellou, 2020, carbapenems are regarded as the "gold standard" therapy in the treatment of gram-negative pathogens that produce ESBL and have demonstrated better patient outcomes, even in cases when alternative β -lactams show in vitro activity. This concurs to the findings in this study as the carbapenems (imipenem and meropenem) exhibited much efficacy to most of the isolates that showed resistance to the 2nd and 3rd generation cephalosporins.

In all, antibiotic susceptibility pattern in this study revealed that ampicillin followed by cefuroxime, amoxicillin - clavulanic, ceftriaxone, ciprofloxacin and trimethoprim-

sulfamethoxazole were the most ineffective antibiotics against all identified Gram-negative Glass priority pathogens which also concord with the findings of (Islam *et al.*, 2022). Among the Gram-positive, ampicillin followed by cefotaxime, gentamicin, penicillin and clindamycin exhibited some rate of resistance.

Contrarily, amikacin, meropenem, imipenem, and tigecycline were the most susceptible antibiotics for Gram-negative bacteria. These antibiotic susceptibility findings were consistent with previous study conducted by Malik *et al.*, 2021, Haryana in India. Antibiotics such as amoxicillin – clavulanic, ciprofloxacin, nitrofurantoin and vancomycin were the most susceptible in Gram-positive in our study.

In line with several studies that showed amikacin to be very effective even against Gram negatives that produces ESBLs, amikacin in these studies has shown to be sensibly active against almost all the Gram-negative GLASS priority uropathogens (Gyansa-Lutterodt, *et al.*, 2014; Donkor *et al.*, 2019; Bhargava, *et al.*, 2022). Despite the fact that amikacin and other aminoglycosides appeared to be suitable as an empirical treatment for UTIs in this study, however, their nephrotoxicity renders them inappropriate, especially for individuals with renal impairment (Karikari, *et al.*, 2022). Because of this, aminoglycosides are carefully prescribed to UTI individuals (Karikari *et al.*, 2022). Another reason why amikacin showed less resistance was because of its high cost (Donkor *et al.*, 2019). This helps to explain why aminoglycosides such amikacin is comparatively more effective at inhibiting bacterial than other commonly used antibiotics, as shown by a number of epidemiological studies conducted in Ghana. Another effective old antibiotic is nitrofurantoin, but its side effects' arising from it's also limits its usage and it shows relatively low resistance in comparison to another antibiotics.

Genomic characterization of GLASS priority pathogen

The increase in UTIs caused by multi-drug resistant Gram-negative GLASS priority uropathogens producing ESBL poses a threat to public health concern. A thorough comprehension of a bacterial genetic variation is vital for understanding how it spreads, identifying its origins, and assessing the risk of hospital or community transmission (Ikhimiukor, *et al.*, 2022; Tettey *et al.*, 2024). As outlined by the Global Antimicrobial Resistance and Use Surveillance System (GLASS), the primary objective of epidemiological surveillance includes monitoring extended-spectrum β -lactamases (ESBL), colistin resistance, carbapenemases, and fluoroquinolones resistance (WHO, 2019). It was therefore significant to use Whole Genome Sequencing (WGS) as a crucial genomic surveillance tool for identifying the genetic profile of ARGs - producing Gram-negative GLASS uropathogens.

This study results revealed that most of the sequenced isolates were either multidrug-resistance (MDR) or extensively drug-resistance (XDR) and hydrolyzing several antibiotics classes (Table 6). The surge of MDR and XDR could be as a result of the strong antibiotics selective pressures imposed on bacteria in the hospital setting and the community making treatment difficult (Holt, *et al.*, 2015).

This study also revealed that most of the sequenced isolates genotypically harbour's one or more ARGs of CTX-M alleles and others including OXA, TEM, SHV and CYM. Likewise, in the research conducted by Deku *et al.*, 2021 in Ghana, on a set of *E. coli* isolates obtained from various clinical samples which include high vaginal swab, blood, pleural aspirates, ear swab, sputum, urine and wound, and reported that the prevalent genes identified among ESBL isolates were *bla*_{TEM-1} and *bla*_{CTX-M} (specifically *bla*_{CTX-M-1}, *bla*_{CTX-M-825}, and *bla*_{CTX-M-914}).

These findings could account for the significant levels of ampicillin and cefuroxime resistance observed in this present study.

The *bla*_{CTX-M-15} gene was the predominant ESBL gene. Similarly, several reports from studies in Ghana (Donkor *et al.*, 2019; Obeng-Nkrumah *et al.*, 2023; Tettey *et al.*, 2024), and international (Carvalho *et al.*, 2021) concurred to our findings. Donkor *et al.*, 2019 reported that the most prevalence ESBL gene in community acquire UTIs in Ghana was *bla*_{CTX-M-15}. There has also been a report of *bla*_{CTX-M-15} being the most prevalent ESBL gene recovered from animal husbandry most especially poultry (Alonso *et al.*, 2017). The present investigation's findings are consistent with other research, particularly those conducted in African nations where reports of the rising incidence of ESBL Enterobacteriaceae, particularly *bla*_{CTX-M-15}, have been made (Inwezerua, *et al.*, 2014; Ribeiro *et al.*, 2016; Founou *et al.*, 2017; Ogbolu, *et al.*, 2018; Muller-Schulte, *et al.*, 2020; Carvalho *et al.*, 2021). Under the current state of diagnosis and treatment, reports from underdeveloped nations, particularly in Africa, are concerning. This widespread dissemination of the *bla*_{CTX-M-15} ESBL gene could be largely attributed to horizontal gene transfer (HGT) among bacteria, facilitated by conjugative plasmids (Carvalho *et al.*, 2021). It may also be linked to the extensive and indiscriminate utilization of cephalosporins and other class antibiotics without proper prescription (Donkor *et al.*, 2019; Obeng-Nkrumah *et al.*, 2023). Because of its widespread distribution and connection to infections in human settings, this work provides more proof that the *bla*_{CTX-M-15} is still the most significant CTX-M enzyme in *E. coli* and *K. pneumoniae*.

Nevertheless, only the *E. coli* ST131 and ST315 isolates among those selected exhibited a variant of the *bla*_{CMY} gene. The primary cause of resistance to ceftriaxone is typically attributed to the presence of a cephalomycinase (CMY) enzyme, which is encoded by the *bla*_{CMY} gene family (Tóth *et al.*, 2024). The susceptibility test conducted in this study has verified the presence of a certain

degree of resistance to ceftriaxone (CRO) against these GLASS priority pathogens. Therefore, we can infer that resistance to CRO was driven by either *bla*_{CMY} or *bla*_{CTX-M}, depending on the sequence type.

Again, the variety of our isolates is apparent as we identified several narrow spectrum ARGs which include Aminoglycoside resistance genes (*aac*(3)-IIa, *aac*(6')-Ib-cr, *aadA16*, *aph*(6)-Id), Folate pathway antagonists (*sul1*, *sul2*, *Oqx*B1, *dfrA27*, *dfrA15*, *dfrA14*, *dfrA17*, *dfrB4*), Tetracyclines (*tet*(A), *tet*(B) *tet*(D)), and Fluoroquinolones (*aac*(6')-Ib-cr, *qnrS1*, *qnrB4*, *qnrB2*, *qnrB6*, *qepA2*), Rifampicin resistance gene (*ARR*-3), Fosfomycin (*fosA*), Macrolide (*erm*(B)) and Chloramphenicol (*catA1*,*catB3*). While these genes provide only minimal resistance, they could create conditions conducive to the emergence of extremely resistance factors, particularly in microorganisms carrying topoisomerase mutations (Jesumirhewe *et al.*, 2020). The extensive variety of the isolates and the concurrent presence of multiple clinically relevant Antibiotic Resistance Genes (ARGs) is concerning.

Furthermore, ARGs are frequently found in mobile genetic elements (MGE) like transposons, integrons, and plasmids which may also harbor certain resistance genes. This results in resistance to commonly used antibiotics in both humans and animals, such as sulfonamides, trimethoprim, fluoroquinolones, and aminoglycosides (Jesumirhewe *et al.*, 2020). The ESBL genes belonging to the SHV, OXA, TEM, and CTX-M families, along with other ARGs as mentioned earlier are disseminated on sizable conjugative plasmids (Mathers *et al.*, 2015). Conjugative plasmids are recognized as a primary driver in the widespread distribution of ESBL genes within Enterobacteriaceae (Jesumirhewe *et al.*, 2020). They are the key factor in effective gene spread. The Inc plasmid replicon types were found to be the most common type in this study, encompassing several distinct varieties of plasmids, such as IncN, IncQ1, IncI1-1(Alpha),

IncHI1B, Inc X4, IncY, IncR, and IncF groups in both *E. coli* and *K. pneumoniae*. Overall, the plasmid group IncF (FII, FIA, and FIB) emerged the dominant 52.1%, followed by 27.4% Col group (Col156, Col (MG828), Col4401, ColKp3, and Col (BS512)). The IncF replicons (FII, FIA, and FIB) exhibited reduced susceptibility to various antibiotics classes, such as Aminoglycoside, Cephalosporins, Sulfamethoxazole-trimethoprim, Tetracycline, carbapenem, Beta-lactam and quinolones. The narrow-host-range incompatibility group F (IncF) frequently carries several replicons (Mathers *et al.*, 2015). This data aligns with previous findings reported from sources in Africa and other regions around the world (Mathers *et al.*, 2015; Cherubini, *et al.*, 2022; Tettey *et al.*, 2024). The IncF group is an epidemic resistance plasmid featuring diverse replicon types like FII, FIA, and FIB, can acquire resistance genes and quickly spread among Enterobacteriaceae, particularly *E. coli* and *K. pneumoniae*. (Jesumirhewe *et al.*, 2020). The recent and significant rise in antimicrobial resistance worldwide may also be attributed to the unrestricted transfer of plasmid-borne genes, which may also be the cause of antibiotic resistance.

Additionally, Col-like plasmids also known as replicons, are mobile elements recognized for their role in facilitating the transfer of antibiotic resistance plasmids through horizontal gene transfer among members of the Enterobacteriaceae family (Cherubini *et al.*, 2022; Kondratyeva *et al.*, 2020). The presence of IncQ1, a plasmid linked to tetracycline resistance and carrying the tetA gene, as well as the Col(pHAD28) plasmid, which contains genes that resist quinolone associated with increased resistance to fluoroquinolones (such as ciprofloxacin and levofloxacin) and encoding the qnrB2 and qnrB6 gene were in line with findings of Raufu, *et al.*, 2021, in central Nigeria.

Moreover, our study finding shows diverse sequence types (ST) and clonal complexes. The MLST analysis of *E. coli* isolates identified eight distinct known sequence types (STs), including ST 354,

ST 1284, ST 10, ST 131, ST 315, ST 6332, ST 1722, and ST 410. Previous studies have documented ST131 as a prominent clinical clone with international dominance (Mottaghizadeh, *et al.*, 2019; Cherubini, *et al.*, 2022; Tettey, *et al.*, 2024). ST131 belongs to the phylogroup B2, is well recognized for its pathogenic in nature and its accountable for extraintestinal *E. coli* infections, particularly in humans (Cherubini, *et al.*, 2022; Tettey, *et al.*, 2024). According to Cherubini, *et al.*, 2022, these strains of *E. coli* have the ability to manifest their pathogenicity in the urinary tract and bloodstream. Due to their inherently pathogenic nature, a total of thirty-five virulence genes were identified in ST131 in this study which is relatively higher than the findings of Tettey, *et al.*, 2024 (23 virulence genes). Earlier study conducted by Tettey, *et al.*, 2024 in Ghana reported that the only ESBL producing *E. coli* ST131 classified as phylogroup B2 was isolated from Hospital effluent among the waste water collected from different environments in Accra. Similarly, this study isolated same strain of *E. coli* from HA-UTI. All of these findings suggest that these clonal lineages are commonly circulating, indicating their significant role in the spread of β -lactamases within hospital settings. In the research carried out by Mahazu *et al.*, 2022, which focused on *E. coli* recovered from various clinical samples, showed that ST131 was the dominant sequence type identified, accounting for 24.5% (25 out of 102 isolates).

Also, *E. coli* ST354 classified in the phylogroup F, an international clone was also detected in CA-UTI exhibiting MDR. This is in line with the findings of a study in Brazil (Soncini, *et al.*, 2022). ST410 has been detected around world in non-intestinal pathogens, showing resistance to 3rd generation cephalosporins, carbapenems, and fluoroquinolones (Runcharoen, *et al.*, 2017; Roer, *et al.*, 2018). *E. coli* strains belonging to the phylogroups A, C, and D, are commensal bacteria present in the aquatic environments and gastrointestinal of humans (Isaac, *et al.*, 2021; Tettey, *et al.*, 2024).

For *K. pneumoniae*, this study identified five distinct sequence types (ST147, ST5330, ST464, ST15, and ST111) known as major international high-risk *K. pneumoniae* clones and associated with different ESBL genes (Mileto, *et al.*, 2023; Zhou, *et al.*, 2023) out of six sequenced isolates. The prevalence among them was ST464 (n=2) which were isolated from the HA-UTI. In this work, the predominant hypervirulent *K. pneumoniae* clone identified was ST464 (n=2), an extensive drug resistance conferring several resistance genes (SHV 38, CTX-15 and OXA-1) and are also carried by several plasmids. As of now, the emergence of *K. pneumoniae* ST464 has been documented in multiple countries including Spain (Cañada-García, *et al.*, 2022), China (Hao, *et al.*, 2022), USA (Rakovitsky, *et al.*, 2020), France (Raquel, *et al.*, 2022) which covered 3 out of 7 continents. Most of these sequence types are rare in other research conducted in Ghana and Africa. Various sequence types (ST) of *K. pneumoniae* globally, including ST15 and ST147, have been documented to produce extensive drug resistance (Mileto, *et al.*, 2023; Zhou, *et al.*, 2023). Notably, ST147 is a high-risk international clone that led to a significant outbreak in Italy's Tuscany region from 2018 to 2021 (Martin, *et al.*, 2021; Di Pilato, *et al.*, 2022). Food animals commonly have ST111 identified in them (poultry, pigs and/or cows) (Franklin-Alming, *et al.*, 2021; Thongpan, *et al.*, 2021; Zhiyi, *et al.*, 2021).

ST5330 was identified in this study as *Klebsiella quasipneumoniae* subsp. *similipneumoniae* harboring several ARGs including *bla*_{OXA-232} a variant of *bla*_{OXA-48} (Findlay *et al.*, 2021). Similar to our study finding, the research conducted by Long *et al.*, 2017, indicates that the subspecies of *K. quasipneumoniae* have shown the ability to acquire carbapenem resistance through plasmids, potentially resulting in challenges for treatment methods.

Initially linked to environmental habitats, *Klebsiella quasipneumoniae* is now being increasingly reported to play a role in human infections (Venkitapathi *et al.*, 2021). Significant concerns were

raised by reports of *Klebsiella quasipneumoniae* subsp. *similipneumoniae* being identified as the causative agent of neonatal bacteremia outbreak at a tertiary hospital in Nigeria (Okomo *et al.*, 2020) and as the source of a neonatal septicemia in China (Perlaza-Jiménez *et al.*, 2020). These confirms the increase recognition of *K. quasipneumoniae* subspecies causing infections in humans (Imai *et al.*, 2019).

In a recent study conducted by Calland *et al.*, 2023 in Ghana, discovered that *K. quasipneumoniae* subspecies *similipneumoniae* was prevalent in the Northern region of Ghana, with 136 out of 573 cases (24%). The actual prevalence of *K. quasipneumoniae* subspecies *similipneumoniae* is uncertain, as it cannot be differentiated from *K. pneumoniae* in clinical labs that rely on matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) or other conventional methods for routine microbial identification (Rodrigues *et al.*, 2018).

In alignment with earlier research, our findings revealed that *K. quasipneumoniae* isolates are commonly misidentified by standard clinical microbiology diagnostics as *K. pneumoniae* and often lead to severe infections resembling those caused by *K. pneumoniae* (Long, *et al.*, 2017; Gorrie *et al.*, 2017; Gorrie *et al.*, 2021 Mills *et al.*, 2024).

The presence of *K. quasipneumoniae* subspecies *similipneumoniae* may be deemed as imported, given the close proximity and movement of people between Ghana and Nigeria. There is a fear for possibly outbreak of these subspecies of *K. pneumoniae* in Ghana in future if care is not taken.

These widely distributed sequence types are linked to the majority of hospital-acquired and community-acquired *K. pneumoniae* infections in humans including urinary tracts infection (Wyres, *et al.*, 2021).

Conclusion

Escherichia coli and *Klebsiella pneumoniae* were the most commonly identified GLASS uropathogen at the site of research with majority (61.9%) of the positive isolates recovered from CA-UTI. The outcome of this study demonstrated that the antibiotics with the highest percentage of resistance include ampicillin (93%), cefuroxime (72%) and ciprofloxacin (45%) whereas amikacin and carbapenems (imipenem, meropenem) remain the most effective agents, with tigecycline as a key option for multidrug-resistant cases.

We identified the precise genetic mechanisms fueling resistance, most notably the widespread presence of the extended-spectrum beta-lactamase (ESBL) gene *bla*_{CTX-M-15}, alongside other variants (*bla*_{OXA}, *bla*_{TEM}, *bla*_{SHV}). The high prevalence of IncF plasmids points to a highly mobile and transmissible genetic framework for these resistance genes.

Policy and Clinical Implications:

These findings necessitate further surveillance of local and national empiric treatment guidelines for UTIs. The high resistance rates render ampicillin, cefuroxime, and ciprofloxacin ineffective as first-line options, advocating for a shift towards amikacin or carbapenems in severe cases.

We demonstrate that whole-genome sequencing (WGS) is an indispensable tool for public health. National surveillance programs should adopt WGS to rapidly identify emerging resistance clones (like CTX-M-15-producing strains), track their transmission via IncF plasmids, and inform real-time outbreak response.

The rate of resistant, community-onset infections is a major public health concern. This study underscores the imperative to expand this genomic surveillance network nationally to understand the full scope of AMR spread and to develop targeted interventions beyond hospital settings.

In summary, this research moves beyond reporting resistance rates to revealing the genetic underpinnings of the AMR crisis in UTIs, providing an actionable evidence base for improving patient outcomes and crafting effective containment policies.

Recommendation

We recommend a nationwide surveillance to collect comprehensive data from all geographical area to enable policymakers to make informed decisions regarding the treatment of UTIs.

Antimicrobial stewardship program should be improved at the 37 Military Hospital and Ghana at large to promote the rational use of antibiotics in the hospitals and the communities.

Routine monitoring of antibiotics utilization at the various hospital settings should be intensified.

Limitations

While this study offered valuable molecular insights of GLASS priority pathogen causing urinary tract infections (UTIs) and the antibiogram at our specific study site, it is important to acknowledge its limitation. The study limitation is the inability to collect demographical data of the study participants to enable the evaluation of risk factors that has contributed to their UTIs for both CA-UTI and HA-UTI.



Reference

- Abushaheen, M. A., Fatani, A. J., Alosaimi, M., Mansy, W., George, M., Acharya, S., ... & Jhugroo, P. (2020). Antimicrobial resistance, mechanisms and its clinical significance. *Disease-a-Month*, 66(6), 100971.
- Access to Medicine Foundation (2018). Antimicrobial Resistance Benchmark, (Available from: https://accesstomedicinefoundation.org/media/uploads/downloads/5bc5edd8367eb_Antimicrobial-Resistance-Benchmark-2018.pdf)
- Adeapena, W., Afari-Asiedu, S., Najjemba, R., Griensven, J. V., Delamou, A., Ohene Buabeng, K., & Poku Asante, K. (2021). Antibiotic use in a municipal veterinary clinic in Ghana. *Tropical Medicine and Infectious Disease*, 6(3), 138.
- Adebisi YA, Alaran AJ, Okereke M, et al. (2021). COVID-19 and Antimicrobial Resistance: A Review. *Infectious Diseases: Research and Treatment*. doi:10.1177/11786337211033870
- Adeyi OO, Baris E, Jonas OB et al., (2017). Drug-resistant infections: a threat to our economic future (Vol. 2): final report (English). World Bank Group: Washington, DC, (Available from: <http://documents.worldbank.org/curated/en/323311493396993758/final-report>).
- Adu-Poku Maxwell and Matthew Glover Addo (2020). Antimicrobial Susceptibility Profile of Urinary Tract Pathogenic Infections in Diabetic Patients Attending a Health Facility in Kumasi, Ghana. *International Journal of Pathogen Research* 4(2): 37-45, 2020; Article no.IJPR.57489 ISSN: 2582-3876.
- Ågerstrand, M., et al., 2015. Improving environmental risk assessment of human pharmaceuticals. *Environ. Sci. Technol.* 49 (9), 5336–5345.
- Ahmed et al., (2019). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *International Journal of Health Science*. Vol 13 issue 2. ISSN: 1658-3639.
- Al-Mustapha I. A, et al., (2023). Antibiotic resistance genes, mobile elements, virulence genes, and phages in cultivated ESBL-producing *Escherichia coli* of poultry origin in Kwara State, North Central Nigeria
- Al-Zidan, R. N., Mahmood, S. M., & Younus, Z. M. (2024). The antibiotic susceptibility patterns of uropathogens among adults with recurrent urinary tract infection in Mosul, Iraq. *Pharmakeftiki*, 36(2).
- Alekshun MN, Levy SB (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*;128:1037-50.
- Alex, et al., (2012). Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. *E3 Journal of Medical Research* Vol. 1(6). pp. 074-083. <http://www.e3journals.org> ISSN 2276-9900 © E3 Journals 2012

- Alhomoud et al., (2017). Self-medication and self-prescription with antibiotics in the Middle East—do they really happen? A systematic review of the prevalence, possible reasons, and outcomes. *International Journal of Infectious Diseases* 57. 3–12
- Alonso, C., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama, K., Torres, C., 2017. Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. *Lett. Appl. Microbiol.* 64 (5), 318–334. <https://doi.org/10.1111/lam.12724>.
- Andersson DI, Hughes D. (2014). Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol.*;12(7):465–478.
- Arun, S., et al., 2017. A review on antibiotics consumption, physico-chemical properties and their sources in Asian soil. In: *Antibiotics and Antibiotics Resistance Genes in Soils*. Springer, pp. 39–53.
- Asamoah, B.; Labi, A.-K.; Gupte, H.A.; Davtyan, H.; Peprah, G.M.; Adu-Gyan, F.; Nair, D.; Muradyan, K.; Jessani, N.S. & Sekyere-Nyantakyi, P. (2022). High Resistance to Antibiotics Recommended in Standard Treatment Guidelines in Ghana: A Cross-Sectional Study of Antimicrobial Resistance Patterns in Patients with Urinary Tract Infections between 2017–2021. *Int. J. Environ. Res. Public Health*, 19, 16556. <https://doi.org/10.3390/ijerph192416556> PMID: 36016776
- Aslam B. et al., (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*. 1645—1658. doi: <https://doi.org/10.2147/IDR.S173867>
- Aypak C, Altunsoy A, & Düzgün N. (2009). Empiric antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: A prospective observational study. *Ann Clin Microbiol Antimicrob.*;8:27. doi: 10.1186/1476-0711-8-27.
- Baquero F, Martínez JL, F Lanza V, Rodríguez-Beltrán J, Galán JC, San Millán A, Cantón R, Coque TM. (2021). Evolutionary pathways and trajectories in antibiotic resistance. *Clin Microbiol Rev* 34:e00050-1
- Baharoglu Z, Garriss G, & Mazel D. Multiple pathways of genome plasticity leading to development of antibiotic resistance. *Antibiotics* (2013) 2:288–315. doi:10.3390/antibiotics2020288
- Balaji Veeraraghavan & Walia Kamini (2019). Antimicrobial susceptibility profile & resistance mechanisms of Global Antimicrobial Resistance Surveillance System (GLASS) priority pathogens from India. *Indian Journal of Medical Research* 149(2):p 87-96,. | DOI: 10.4103/ijmr.IJMR_214_18
- Basak, C., Fröllje, H., Lamy, F., Gersonde, R., Benz, V., Anderson, R., Molina-Kescher, M., & Pahnke, K., (2016): Neodymium isotope data from South Pacific sediment core ELT11.002-PC. PANGAEA, <https://doi.org/10.1594/PANGAEA.858964>,
- Bharadwaj A., Amisha R., Swadha P., Saurabh G., & Jagdip S.S., (2022). Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. *BioMed Research International* Volume 2022, Article ID 5419874, 17 pages <https://doi.org/10.1155/2022/5419874>

- Bhargava K, Nath G, Bhargava A, Kumari R, Aseri GK, Jain N. (2022). Bacterial profile and antibiotic susceptibility pattern of uropathogens causing urinary tract infection in the eastern part of Northern India. *Front Microbiol.* 9;13:965053.
- Browne, Hilary P., B. Anne Neville, Samuel C. Forster & Trevor D.L., (2017). Transmission of the gut microbiota: spreading of health Nat Rev Microbiol; 15(9): 531–543. doi:10.1038/nrmicro.2017.50
- Bruzek S., Grant V., Amanda L., Amorice L., & Suzane S.B., (2020). Whole Genome Sequencing on the Illumina iSeq 100 for Clinical and Public Health Laboratories
- Calland, J. K., Haukka, K., Kpordze, S. W., Brusah, A., Corbella, M., Merla, C., ... & Corander, J. (2023). Population structure and antimicrobial resistance among *Klebsiella* isolates sampled from human, animal, and environmental sources in Ghana: a cross-sectional genomic One Health study. *The Lancet Microbe*, 4(11), e943-e952.
- Cañada-García J.E, Grippo N, de Arellano E.R, Bautista V, Lara N, Navarro AM, Cabezas T, Martínez-Ramírez N.M, García-Cobos S., Calvo J., Cercenado E., Aracil B., Pérez-Vázquez M., Oteo-Iglesias J & the Spanish I.M.P Study Group (2022). Phenotypic and molecular characterization of IMP-producing Enterobacterales in Spain: Predominance of IMP-8 in *Klebsiella pneumoniae* and IMP-22 in *Enterobacter roggenkampii*. *Front. Microbiol.* 13:1000787. doi: 10.3389/fmicb.2022.1000787
- Carrasco Calzada, F., Aguilera-Correa, J. J., Cuadros González, J., Esteban Moreno, J., Roca Biosca, D., & Pérez-Tanoira, R. (2022). Urinary Tract Infection and Antimicrobial Susceptibility of Bacterial Isolates in Saint Joseph Kitgum Hospital, Kitgum, Uganda. *Antibiotics (Basel, Switzerland)*, 11(4), 504. <https://doi.org/10.3390/antibiotics11040504>
- Carvalho I, Chenouf NS, Carvalho JA, Castro AP, Silva V, Capita R, et al. (2021). Multidrug-resistant *Klebsiella pneumoniae* harboring extended spectrum β -lactamase encoding genes isolated from human septicemias. *PLoS ONE* 16(5): e0250525. <https://doi.org/10.1371/journal.pone.0250525>
- Cascella M & Pandey N., (2019). Beta Lactam Antibiotics. PMID: 31424895. <https://europepmc.org/article/nbk/nbk545311#impact> retrieved on 22/07/22 at 03:20pm
- Centres for Disease Control and Prevention (2013). Antibiotic resistance threats in the United States, Atlanta, 2013. <http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf> (accessed Jan 17, 2015)
- Centers for Disease Control and Prevention (CDC) (2015). Facility guidance for control of carbapenem-resistant Enterobacteriaceae (CRE). November 2015 update – CRE toolkit. Available at: <https://www.cdc.gov/hai/organisms/cre/cre-toolkit/index.html>.
- Chastain, D. B., White, B. P., Cretella, D. A., & Bland, C. M. (2017). Is It Time to Rethink the Notion of Carbapenem-Sparing Therapy Against Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae Bloodstream Infections? A Critical Review. *Annals of Pharmacotherapy*, 52(5), 484–492. doi:10.1177/1060028017748943

- Chellat, MF., Raguz L, & Riedl R. (2016). Targeting antibiotic resistance. *Angew Chem.*;55(23):6600–26
- Cherry L., et al (2021). Impact of low blood culture usage on rates of antimicrobial resistance. *Journal of Infection*. 82 355–362. Doi:10.1016/j.jinf.2020.10.040.
- Cherubini, S., Perilli, M., Azzini, A. M., Tacconelli, E., Maccacaro, L., Bazaj, A., Naso, L., Amicosante, G., Ltcf-Veneto Working Group, Lo Cascio, G., & Piccirilli, A. (2022). Resistome and Virulome of Multi-Drug Resistant *E. coli* ST131 Isolated from Residents of Long-Term Care Facilities in the Northern Italian Region. *Diagnostics (Basel, Switzerland)*, 12(1), 213. <https://doi.org/10.3390/diagnostics12010213>
- Chin Teh Li, Cliodna McNulty, Charles Beck and Alasdair MacGowan Antimicrobial resistance surveillance in urinary tract infections in primary care.
- Codjoe, F., & Donkor, E. (2017). Carbapenem Resistance: A Review. *Medical Sciences*, 6(1), 1. doi:10.3390/medsci6010001
- Cosgrove, S. E. & Carmeli Y. (2003). The Impact of Antimicrobial Resistance on Health and Economic Outcomes.
- Creswell, J. W. (2009). Research design: Qualitative, quantitative, and mixed method approaches. Thousand Oaks, CA: *Sage Publications, Inc.*
- Dada-Adegbola, H., & Muili, K. (2010). Antibiotic susceptibility pattern of urinary tract pathogens in Ibadan, Nigeria. *African journal of medicine and medical sciences*, 39(3), 173-179.
- Davies, J.; Davies, D (2010). Origins and Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. Rev.*, 74, 417–433.
- Deku, J.G.; Duedu, K.O.; Ativi, E.; Kpene, G.E.; Feglo, P.K. (2021). Occurrence and distribution of extended-spectrum β -lactamase in clinical *Escherichia coli* isolates at Ho Teaching Hospital in Ghana. *Ghana Med. J.* , 55, 298–307.
- Demilie T, Beyene G, Melaku S, Tsegaye W. (2012). Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in north west Ethiopia. *Ethiop J Health Sci*. 22(2): 121-128.
- Di Pilato V, Henrici De Angelis L, Aiezza N, Baccani I, Niccolai C, Parisio EM, et al. (2022). Resistome and virulome accretion in an NDM-1-producing ST147 sublineage of *Klebsiella pneumoniae* associated with an outbreak in Tuscany, Italy: a genotypic and phenotypic characterisation. *Lancet Microbe*. ;3(3):e224-34. [https://doi.org/10.1016/S2666-5247\(21\)00268-8](https://doi.org/10.1016/S2666-5247(21)00268-8) PMID: 35544076
- Domfeh, K. A. (2021). Pooled procurement program in the quality improvement of medicines of the National Catholic Health Service in Ghana: using the Donabedian model. *Journal of Pharmaceutical Health Services Research*, 12(2), 133-141.
- Donkor, E.S., Horlortu, P. Z., Dayie, N. TKD., Obeng-Nkrumah Noah and Labi Appiah-Korang (2019). Community acquired urinary tract infections among adults in Accra, Ghana. Infection and Drug Resistance.

- Dowling A., J. O' Dwyer and C.C. Adley (2017). Antibiotics: Mode of action and mechanisms of resistance. Antimicrobial research: Novel bioknowledge and educational programs (A. Méndez-Vilas, Ed.)
- Durante-Mangoni, E., Andini, R., & Zampino, R. (2019). Management of carbapenem resistant Enterobacteriaceae infections. *Clinical Microbiology and Infection*. doi:10.1016/j.cmi.2019.04.013
- Edoh, D., & Alomatu, B. (2007). Comparison of antibiotic resistance patterns between laboratories in Accra East, Ghana. *African Journal of Science and Technology*, 8(1), 1-7.
- Erdem, H.; Tetik, A.; Arun, O.; Besirbellioglu, B.A.; Coskun, O.; Eyigun, C.P (2011). War and infection in the pre-antibiotic era: The Third Ottoman Army in 1915. *Scand. J. Infect. Dis.*, 43, 690–695.
- European Centre for Disease Prevention and Control (2013). Antimicrobial resistance surveillance in Europe 2012. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm.
- Fatima, S. S., & Mussaed, E. A. (2017). Urinary Tract Infection. Bacterial Identification and Drug Susceptibility Patterns in Pregnant and Non-Pregnant UTI Patients, 1–22. doi:10.1007/978-981-10-4750-3_1
- Findlay, J., Poirel, L., Kessler, J., Kronenberg, A., & Nordmann, P. (2021). New Delhi Metallo- β -Lactamase-Producing Enterobacterales Bacteria, Switzerland, 2019–2020. *Emerging infectious diseases*, 27(10), 2628.
- Forsberg, K. J., Patel, S., Gibson, M. K., Lauber, C. L., Knight, R., Fierer, N., & Dantas, G. (2014). Bacterial phylogeny structures soil resistomes across habitats. *Nature*, 509(7502), 612–616. <https://doi.org/10.1038/nature13377>
- Forsyth VS, Himpls SD, Smith SN, Sarkissian CA, Mike LA, Stocki JA, Sintsova A, Alteri CJ, Mobley HLT. (2020) Optimization of an experimental vaccine to prevent Escherichia coli urinary tract infection. *mBio* 11:e00555-20. <https://doi.org/10.1128/mBio.00555-20>.
- Founou RC, Founou LL, Allam M, Ismail A, & Essack SY. (2019). Whole Genome Sequencing of Extended Spectrum β -lactamase {ESBL}-producing *Klebsiella pneumoniae* isolated from hospitalized patients in Kwa-Zulu-Natal, South Africa. *Sci. Rep.*; 9: 6266. <https://doi.org/10.1038/s41598-019-42672-2> PMID:31000772
- Fourie, J., Claassen, F.M., & Myburgh, J. (2021). Causative pathogens and antibiotic resistance in community-acquired urinary tract infections in central South Africa. *South African medical journal*, 111 2, 124-128.
- Franklin-Alming, F. V. et al. (2021). Exploring *Klebsiella pneumoniae* in healthy poultry reveals high genetic diversity, good biofilm-forming abilities and higher prevalence in turkeys than broilers. *Front Microbiol* 12, 2609. 6304, <https://doi.org/10.7883/yoken.JJID.2020.356>,

- Gajdács M., Ábrók M., Lázár A. & Burián K. (2020). Increasing relevance of Gram-positive cocci in urinary tract infections: a 10-year analysis of their prevalence and resistance trends. *Scientific Reports*, 10(1), 17658–. doi:10.1038/s41598-020-74834-y
- Gajdács, M.; Urbán, E.; Stájer, A.; Baráth, Z. (2021) Antimicrobial Resistance in the Context of the Sustainable Development Goals: A Brief Review. *Eur. J. Investig. Health Psychol. Educ.*, 11, 71–82. <https://doi.org/10.3390/ejihpe11010006>
- Garcia, L.A. et al., (2022). Microbial resistance: The role of efflux pump superfamilies and their respective substrates. *Life Sciences*. Volume 295, 120391. <https://doi.org/10.1016/j.lfs.2022.120391>.
- Ghai, I. & Ghai, S. (2018). Understanding Antibiotic Resistance via Outer Membrane Permeability. *Infect. Drug Resist.* 11, 523–530.
- Gebremedhin M.G. et al., (2023). Extended-Spectrum β -Lactamase and Carbapenemase-Producing Gram-Negative Bacteria and Associated Factors Among Patients Suspected of Community and Hospital-Acquired Urinary Tract Infections at Ayder Comprehensive Specialized Hospital, Tigray, Ethiopia. *Infection and Drug Resistance*.
- Gekenidis, M., Kläui, A., Smalla, K. & Drissner, D. (2020). Transferable Extended-Spectrum β -Lactamase (ESBL) Plasmids in Enterobacteriaceae from Irrigation Water. *Microorganisms*, 8, 978.
- Giedraitienė, A., Astra Vitkauskienė, Rima Naginienė, Alvydas Pavilonis (2011). Antibiotic Resistance Mechanisms of Clinically Important Bacteria. *Medicina (Kaunas)*.47(3):137-46.
- Gillings M. R., Ian T. Paulsen & Sasha G. Tetu (2017). Genomics and the evolution of antibiotic resistance. *Ann. N.Y. Acad. Sci.* ISSN 0077-8923
- Golli A.L., et al., (2019). Microbiological profile and antibiotic resistance pattern of bacterial uropathogens among hospitalized patients. *Farmacia*, Vol. 67, 1. <https://doi.org/10.31925/farmacia.2019.1.23>
- Gorrie CL, Mirceta M, Wick RR et al. (2017). Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. *Clin Infect Dis*; 65: 208–15. <https://doi.org/10.1093/cid/cix270>
- Gorrie CL, Mirčeta M, Wick RR et al. (2022). Genomic dissection of *Klebsiella pneumoniae* infections in hospital patients reveals insights into an opportunistic pathogen. *Nat Commun*; 13: 3017. <https://doi.org/10.1038/s41467-022-30717-6>
- Gualano R. Maria., Renata Gili, Giacomo Scaioli, Fabrizio Bert and Roberta Siliquini (2015). General population's knowledge and attitudes about antibiotics: a systematic review and meta-analysis. *pharmacoepidemiology and drug safety*; 24: 2–10. DOI: 10.1002/pds.3716
- Gyansa-Lutterodt, M., Afriyie, D.K., G. Asare, S.K. Amponsah, H. Abutiate and D. Darko (2014). Antimicrobial Use and Susceptibility Pattern of Uropathogens Associated with Urinary

- Tract Infections at the Ghana Police Hospital. *Global Journal of Pharmacology* 8 (3): 306-315, ISSN 1992-0075 DOI: 10.5829/idosi.gjp.2014.8.3.83193
- Hao J, Zhang B, Deng J, Wei Y, Xiao X and Liu J (2022). Emergence of a Hypervirulent Tigecycline-Resistant *Klebsiella pneumoniae* Strain Co-producing *bla_{NDM-1}* and *bla_{KPC-2}* With an Uncommon Sequence Type ST464 in Southwestern China. *Front. Microbiol.* 13:868705. doi: 10.3389/fmicb.2022.868705
- Hassell J. M., Michael B., Melissa J. W, & Eric M. F. (2017). Urbanization and Disease Emergence: Dynamics at the Wildlife–Livestock–Human Interface. *Trends in Ecology & Evolution*, Vol. 32, No. 1 <http://dx.doi.org/10.1016/j.tree.2016.09.012>
- Hartwig. R, and Kliesow. P. S. (2020) Antimicrobial Resistance between Lack of Access and Excess. *GIGA Focus | Global | Number 3 | ISSN 1862-3581*
- Hay SI, Rao PC, Dolecek C, Day NPJ, Stergachis A, Lopez AD, et al. (2018). Measuring and mapping the global burden of antimicrobial resistance. *BMC Med.*;16(1):78
- Hazra, M., et al., 2022. Antibiotics and antibiotic resistant bacteria/genes in urban wastewater: a comparison of their fate in conventional treatment systems and constructed wetlands. *Chemosphere* 303, 135148.
- He, S., Shrestha, P., Henry, A. D., & Legido-Quigley, H. (2023). Leveraging collaborative research networks against antimicrobial resistance in Asia. *Frontiers in Public Health*, 11, 1191036.
- Hein, W.; Aglanu, L.M.; Mensah-Sekyere, M.; Harant, A.; Brinkel, J.; Lamshöft, M.; Lorenz, E.; Eibach, D.; Amuasi, J. (2022). Fighting Antimicrobial Resistance: Development and Implementation of the Ghanaian National Action Plan (2017–2021). *Antibiotics*, 11, 613. <https://doi.org/10.3390/antibiotics11050613>
- Holmes, Alison H; Moore, Luke S P; Sundsfjord, Arnfinn; Steinbakk, Martin; Regmi, Sadie; Karkey, Abhilasha; Guerin, Philippe J; Piddock, Laura J V (2015). *Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet*, (), S0140673615004730–. doi:10.1016/s0140-6736(15)00473-0
- Holt, K. E., et al., (2015). Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proceedings of the National Academy of Sciences*, 112(27), E3574–E3581. doi:10.1073/pnas.1501049112
- Hossain, A. et al. (2020). Age and gender-specific antibiotic resistance patterns among Bangladeshi patients with urinary tract infection caused by *Escherichia coli*. *Science Direct Heliyon journal*. [www.cell.com/heliyon https://doi.org/10.1016/j.heliyon.2020.e04161](https://doi.org/10.1016/j.heliyon.2020.e04161)
- Ijaz, M., Hasan, I., Chaudhry, T. H., Huang, R., Zhang, L., Hu, Z., ... & Guo, B. (2024). Bacterial derivatives mediated drug delivery in cancer therapy: a new generation strategy. *Journal of Nanobiotechnology*, 22(1), 510.

- Ikuta KS, Swetschinski LR, Aguilar GR, Sharara F, Mestrovic T, Gray AP, et al. (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*;400 (10369):2221-48.
- Ikhimiukor, O., Odih, E., Donado-Godoy, P., & Okeke, I., (2022). A bottom-up view of antimicrobial resistance transmission in developing countries. *Nat. Microbiol.* 7 (6), 757–765. <https://doi.org/10.1038/s41564-022-01124-w>.
- Imai K, Ishibashi N, Kodana M, Tarumoto N, Sakai J, Kawamura T, Takeuchi S, Taji Y, Ebihara Y, Ikebuchi K, Murakami T, Maeda T, Mitsutake K, & Maesaki S. (2019). Clinical characteristics in blood stream infections caused by *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*: a comparative study, Japan, 2014–2017. *BMC*
- Inwezerua C, Mendonça N, Calhau V, Domingues S, Adeleke OE, Da Silva GJ. (2014). Occurrence of extended-spectrum beta-lactamases in human and bovine isolates of *Escherichia coli* from Oyo state Nigeria. *J Infect Dev Ctries*; 8: 774–779. <https://doi.org/10.3855/jidc.3430> PMID: 24916877
- Isaac, P. A. A., Thi T. H. N., Atsushi, I., Samiratu Mahazu, Wakana Sato, Takaya Hayashi, Shoji Yamaoka, Toshihiko, S., Shiroh I., Ablordey A., & Ryoichi S., (2021). Virulence Profiles of Diarrheagenic *Escherichia coli* Isolated from the Western Region of Ghana. *Japanese Journal of Infectious Diseases*. Volume 74, Issue 2, Pages 115-121, Released on J-STAGEOnline ISSN 1884-2836, Print ISSN 1344-
- Iskandar et al., (2021). Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. *Antimicrob Resist Infect Control*. 10:63
- Islam. S, Jared Aldstadt and Diana Aga (2019). Global antimicrobial resistance: a complex and dire threat with few definite answers. *Tropical Medicine and International Health*. volume 24 no 6 pp 658–662doi:10.1111/tmi.13230.
- Islam MA, Islam MR, Khan R, Amin MB, Rahman M, Hossain MI, Ahmed D, Asaduzzaman M, & Riley L.W. (2022). Prevalence, etiology and antibiotic resistance patterns of community-acquired urinary tract infections in Dhaka, Bangladesh. *PLoS One*. 15;17(9):e0274423. doi: 10.1371/journal.pone.0274423. PMID: 36107878; PMCID: PMC9477272.
- Jackson, N., Borges, C. A., Tarlton, N. J., Resendez, A., Milton, A. K., de Boer, T. R., ... Riley, L. W. (2021). A rapid, antibiotic susceptibility test for multidrug-resistant, Gram-negative bacterial uropathogens using the biochemical assay, DETECT. *Journal of Microbiological Methods*, 182, 106160. doi:10.1016/j.mimet.2021.106160
- Jalil, M.B.& Al Atbee, M.Y.N (2022). The prevalence of multiple drug resistance *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections. *J. Clin. Lab. Anal.*, 36, e24619.
- Jesumirhewe C, Springer B, Allerberger F, & RuppitschW (2020). Whole genome sequencing of extended-spectrum β -lactamase genes in *Enterobacteriaceae* isolates from Nigeria. *PLoS ONE* 15(4): e0231146. <https://doi.org/10.1371/journal.pone.0231146>

- Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L, et al. Antibiotic resistance genes in bacteria: occurrence, spread, and control. (2021). *J Basic Microbiol*;61:1049–1070. <https://doi.org/10.1002/jobm.202100201>
- Kant S, Lohiya A, Kapil A, & Gupta SK. (2017) Urinary tract infection among pregnant women at a secondary level hospital in Northern India. *Indian J Public Health*; 61:118-23.
- Karaiskos, I., & Giamarellou, H. (2020). Carbapenem-Sparing Strategies for ESBL Producers: When and How. 9, 61; doi:10.3390/antibiotics9020061
- Karikari, A. B., Saba, C.K.S & Yamik, D.Y (2022). Reported Cases of Urinary Tract Infections and the Susceptibility of Uropathogens from Hospitals in Northern Ghana. *Microbiology Insights* Volume 15: 1–9. DOI: 10.1177/11786361221106109
- Kaye, K.S., Nguyen, H.H., & Rybak, M.J. (2015) Clinical review guidelines for management and care transitions in the emergency. *J. Emerg. Med.* 48, 508–519.
- Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. (2016). Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. *Microb Pathog.*;95:32–42.
- Khurshid M, Rasool MH, Ashfaq UA, Aslam B, & Waseem M. (2017). Emergence of ISAbal1 harboring carbapenem-resistant *Acinetobacter baumannii* isolates in Pakistan. *Future Microbiol.*;12:1261–1269.
- Kibret, M., & Abera, B. (2010). Antimicrobial resistance trend of bacteria from clinical isolates: An 8-year retrospective study at Dessie Regional Laboratory, Northeast Ethiopia. *Ethiop Pharm J*, 28, 39-46.
- Koduah, A.; Gyansa-Lutterodt, M.; Hedidor, G.K.; Sekyi-Brown, R.; Asiedu-Danso, M.; Adu Asare, B.; Ama Ackon, A.; Andrews Annan, E. (2021). Antimicrobial resistance national level dialogue and action in Ghana: Setting and sustaining the agenda and outcomes. *One Health Outlook*, 3, 18.
- Koduah, A.; Asare, B.A.; Gavor, E.; Gyansa-Lutterodt, M.; Annan, E.A., & Ofei, F.W. (2019). Use of evidence and negotiation in the review of national standard treatment guidelines and essential medicines list: Experience from Ghana. *Health Policy Plan.*, 34, ii104–ii120.
- Kothari C, Gaiind R, Singh LC, et al. (2013). Community acquisition of β -lactamase producing Enterobacteriaceae in neonatal gut. *BMC Microbiol*; 13: 136.
- Kotwani, A., Joshi, J. & Kaloni, D. (2021). Pharmaceutical effluent: a critical link in the interconnected ecosystem promoting antimicrobial resistance. *Environ Sci Pollut Res* **28**, 32111–32124 <https://doi.org/10.1007/s11356-021-14178-w>
- Kpoda, D. S., Ajayi, A., Somda, M., Traore, O., Guessennd, N., Ouattara, A. S., ... & Dosso, M. (2018). Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. *BMC Research Notes*, 11(1). doi:10.1186/s13104-018-3581-5
- Ku, J. H., Bruxvoort, K. J., Salas, S. B., Varley, C. D., Casey, J. A., Raphael, E., ... & Tartof, S. Y. (2023). Multi-drug resistance of *Escherichia coli* from outpatient uncomplicated urinary

- tract infections in a large US integrated health care organization. In *Open Forum Infectious Diseases* (p. ofad287). Oxford University Press.
- Kubone, P.Z., Mlisana, K.P., Govinden, U., Abia, A.L., & Essack, S.Y. (2020). Antibiotic Susceptibility and Molecular Characterization of Uropathogenic *Escherichia coli* Associated with Community-Acquired Urinary Tract Infections in Urban and Rural Settings in South Africa. *Tropical Medicine and Infectious Disease*, 5.
- Kuehn BM. (2013) IDSA: better, faster diagnostics for infectious diseases needed to curb overtreatment, antibiotic resistance. 310(22):2385–2386. doi:10.1001/jama.2013.283828
- Kuehn BM. (2013). Excessive antibiotic prescribing for sore throat and acute bronchitis remains common. *J Am Med Assoc*. 310(20):2135–6. doi:10.1001/jama. 2013.281452
- Kumawat M, Nabi B, Daswani M, Viqar I, Pal N, Sharma P, Tiwari S, Sarma DK, Shubham S., & Kumar M, (2023). Role of bacterial efflux pump proteins in antibiotic resistance across microbial species. *Microbial Pathogenesis*. doi: <https://doi.org/10.1016/j.micpath.2023.106182>.
- Kondratyeva K.; Salmon-Divon, M.; Navon-Venezia, S. (2020). Meta-Analysis of Pandemic *Escherichia coli* ST131 Plasmidome Proves Restricted Plasmid-Clade Associations. *Sci. Rep.* 10, 36.
- Kresken, M., Pfeifer, Y., Hafner, D., Wresch, R., Körber-Irrgang, B., & Working Party ‘Antimicrobial Resistance’ of the Paul-Ehrlich-Society for Chemotherapy. (2014). Occurrence of multidrug resistance to oral antibiotics among *Escherichia coli* urine isolates from outpatient departments in Germany: extended-spectrum β -lactamases and the role of fosfomycin. *International journal of antimicrobial agents*, 44(4), 295-300.
- Krishna S, Pushpalatha H, Srihari N, Nagabhushan S, & Divya P. (2013). Increasing resistance patterns of pathogenic bacteria causing urinary tract infections at a tertiary care hospital. *Int J Pharm Biomed Res.*;4:105–7.
- Laxminarayan R, Duse A, Wattal C, et al., (2013). Antibiotic resistance-the need for global solutions. *Lancet Infect Dis*; 13: 1057–98.
- Li H., Jun, W., Lijuan, W., Jun L., Xia L., Bin, X. & Yuanfang, Z. (2018). The impacts of delivery mode on infant’s oral microflora
- Li S., Ondon, B. S., Ho S.H., & Fengxiang Li (2023). Emerging soil contamination of antibiotics resistance bacteria (ARB) carrying genes (ARGs): New challenges for soil remediation and conservation. *Environmental Research* 219.
- Liao C., et al., (2017). Integrated Biosensor Assay for Rapid Uropathogen Identification and Phenotypic Antimicrobial Susceptibility Testing. *European Urology Focus*. Volume 3, Issues 2–3, Pages 293-299
- Lima, R., Del Fiol, F. S., & Balcão, V. M. (2019). Prospects for the Use of New Technologies to Combat Multidrug-Resistant Bacteria. *Frontiers in Pharmacology*, 10. doi:10.3389/fphar.2019.00692

- Little P, Stuart B, Francis N, et al, & the GRACE consortium (2013). Effects of internet-based training on antibiotic prescribing rates for acute respiratory-tract infections: a multinational, cluster, randomised, factorial, controlled trial. *Lancet*; 382: 1175–82
- Lobanovska, M.; Pilla, G. (2017). Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future? *Yale Journal of Biology and Medicine*, 90, 135–145.
- Long, S. W., Linson, S. E., Ojeda Saavedra, M., Cantu, C., Davis, J. J., Bretin, T., & Olsen, R. J. (2017). Whole-genome sequencing of human clinical *Klebsiella pneumoniae* isolates reveals misidentification and misunderstandings of *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*. *MSphere*, 2(4), 10-1128.
- Lubbert C, Baars C, Dayakar A et al., 2017. Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens. *Infection*: 45: 479– 491.
- Lupande-Mwenebitu, D., Baron, S. A., Nabti, L. Z., Lunguya-Metila, O., Lavigne, J.-P., Rolain, J.-M., & Diene, S. M. (2020). Current status of resistance to antibiotics in Democratic Republic of Congo: a Review. *Journal of Global Antimicrobial Resistance*. doi:10.1016/j.jgar.2020.07.008
- Ma, X., Kaw, H. Y., Yu, J., Yang, Q., Zhu, L., & Wang, W. (2024). The intracellular concentrations of fluoroquinolones determined the antibiotic resistance response of *Escherichia coli*. *Journal of Hazardous Materials*, 469, 134057. <https://doi.org/10.1016/j.jhazmat.2024.134057>
- Ma J., et al., (2023). Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy, *Microbiological Research*, Volume 266, ISSN 0944-5013, <https://doi.org/10.1016/j.micres.2022.127249>.
- Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, et al.. (2020). Comparison of Gut Microbiota in Exclusively Breast-Fed and Formula-Fed Babies: A Study of 91 Term Infants. *Sci Rep* 10(1):15792. 10.1038/s41598-020-72635-x
- Magiorakos A-P, Srinivasan A, Carey RB et al. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*;18:268–81
- Mahmud Z. H., Kabir M. H., Ali S., Moniruzzaman M., Imran K. M., Nafiz T. N., et al. (2020). Extended-spectrum beta-lactamase-producing *Escherichia coli* in drinking water samples from a forcibly displaced, densely populated community setting in Bangladesh. *Front. Public Health* 8:228. 10.3389/fpubh.2020.00228
- Majumder M.I, Ahmed T, Hossain D, & Begum S.A. (2014). Bacteriology and antibiotic sensitivity patterns of urinary tract infections in a tertiary hospital in Bangladesh. *Mymensingh Med J*;23:99–104.

- Malik S., Rana J. S., Nehra K. (2021). Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. *Biomed. Biotechnol. Res. J.* 5 80–87.
- Masi, M., Réfregiers, M., Pos, K. *et al.* (2017). Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. *Nat Microbiol* 2, 17001. <https://doi.org/10.1038/nmicrobiol.2017.1>
- Mamman, G. P. (2021). Identification And Antibiotic Susceptibility Profile of Methicillin And Erythromycin Resistant *Staphylococcus Aureus* From Clinical And Environmental Samples In Minna, Nigeria (Doctoral dissertation).
- Manyi-Loh, C., Mamphweli, S., Meyer, E., & Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*, 23(4), 795.
- Mechal, T., Hussen, S. & Desta M. (2021). Bacterial Profile, Antibiotic Susceptibility Pattern and Associated Factors Among Patients Attending Adult OPD at Hawassa University Comprehensive Specialized Hospital, Hawassa, Ethiopia. *Infection and Drug Resistance*. 14 99–110.
- Mahazu, S.; Sato, W.; Ayibieke, A.; Prah, I.; Hayashi, T.; Suzuki, T.; Iwanaga, S.; Ablordey, A. & Saito, R. (2022). Insights and genetic features of extended-spectrum beta-lactamase producing *Escherichia coli* isolates from two hospitals in Ghana. *Sci. Rep.* , 12, 1843
- Malik S., Rana J. S., & Nehra K. (2021). Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. *Biomed. Biotechnol. Res. J.* 5 80–87.
- Mamuye, Y. (2016). Antibiotic resistance patterns of common gram-negative uropathogens in St Paul's Hospital Millennium Medical College. *Ethiop J Health Sci.*; 26:93–99. doi: 10.4314/ejhs.v26i2.2.
- Mathers AJ, Peirano G, & Pitout JD. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin Microbiol Rev.*; 28:565–591. <https://doi.org/10.1128/CMR.00116-14> PMID: 25926236
- Martin MJ, Corey BW, Sannio F, Hall LR, MacDonald U, Jones BT, et al. (2021). Anatomy of an extensively drug-resistant *Klebsiella pneumoniae* outbreak in Tuscany, Italy. *Proc Natl AcadSci USA.* ;118(48):e2110227118. <https://doi.org/10.1073/pnas.2110227118> PMID: 34819373
- Mazzariol, A., Bazaj, A., & Cornaglia, G. (2017). Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. *Journal of Chemotherapy*, 29(sup1), 2-9.
- Michael CA, Dale Dominey-Howes and Maurizio Labbate (2014). The antimicrobial resistance crisis: causes, consequences, and management
- Mileto I., Petazzoni G., Corbella M, Gaiarsa S, Merla C, Kuka A, Ramus M, Terulla C, Brandolini M, Piralla A, Cambieri P, & Baldanti F. (2023). Rapid spread of a novel NDM-producing

- clone of *Klebsiella pneumoniae* CC147, Northern Italy,. *Euro Surveill.* 28(42):pii=2300522. <https://doi.org/10.2807/1560-7917.ES.2023.28.42.2300522>
- Mills Richael O, et al., (2024). Genomic diversity and antimicrobial resistance in clinical *Klebsiella pneumoniae* isolates from tertiary hospitals in Southern Ghana. *Journal of Antimicrobial Chemotherapy*, Volume 79, Issue 7, July, Pages 1529–1539, <https://doi.org/10.1093/jac/dkae123>
- Ministry of Health-Ghana National Drugs Programme (GNDP) (2017). Standard Treatment Guidelines; 2017 Ministry of Health: Accra,Ghana,; pp. 401–403. ISBN 9789988257873.
- Mitgang, E. A., Hartley, D. M., Malchione, M. D., Koch, M., & Goodman, J. L. (2018). Review and mapping of carbapenem-resistant Enterobacteriaceae in Africa: Using diverse data to inform surveillance gaps. *International Journal of Antimicrobial Agents*. doi:10.1016/j.ijantimicag.2018.05.019
- Mohiuddin Abdul Kader & M. Nasirullah. (2019). UTI Prevalence Among Population with Chronic Conditions. *Journal of Medical Research and Case Reports* 1(2).
- Morita Y, Tomida J, & Kawamura Y. (2014). Responses of *Pseudomonas aeruginosa* to antimicrobials. *Front Microbiol*; 4: 422.
- Mottaghizadeh, F., Mohajjel shoja, H., Haeili, M., & Darban-Sarokhalil, D. (2019). Molecular epidemiology and nitrofurantoin resistance determinants from nitrofurantoin non-susceptible *Escherichia coli* isolated from urinary tract infections. *Journal of Global Antimicrobial Resistance*. doi:10.1016/j.jgar.2019.10.002.
- Muller-Schulte, E., Tuo M.N, Akoua-Koffi, C., Schaumburg, F., & Becker SL. (2020). High prevalence of ESBL-producing *Klebsiella pneumoniae* in clinical samples from central Côte d'Ivoire. *Int. J. Infect. Dis*; 91:207–209.
- Munita, J. M. & Arias C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiol Spectr.* 4(2): doi:10.1128/microbiolspec.VMBF-0016-2015.
- Munck, C., Albertsen M., Telke A, Ellabaan, M, Nielsen P.H, & Sommer M.O.A. (2015). Limited dissemination of the wastewater treatment plant core resistome. *Nat Commun* 6:8452. <https://doi.org/10.1038/ncomms9452>.
- Muthulakshmi M, & Gopalakrishnan S. (2017). Study on urinary tract infection among females of reproductive age group in a rural area of Kancheepuram district, Tamil Nadu. *Int J Community Med Public Health*;4:3915-21.
- Mutiyar, P.K, & Mittal, A.K. (2014). Risk assessment of antibiotic residues in different water matrices in India: Key issues and challenges. *Environ Sci Pollut Res*; 21: 7723-36.
- Nakwatumbah, S, Kibuule, D, Godman, B., Haakuria, V, Kalemeera, F, Baker, A., & Mwangana, M (2017). Compliance to guidelines for the prescribing of antibiotics in acute infections at Namibia's national referral hospital: a pilot study and the implications. *Expert Review of Anti-infective Therapy*. doi:10.1080/14787210.2017.1320220

- Nayak, A. & Roy, A. (2023). Synergistic Antibacterial Activity of Four Medicinal Plants Collected From Birbhum, West Bengal. *International Journal of Research and Analytical Reviews (IJRAR)*. E-ISSN 2348-1269, P- ISSN 2349-5138
- Navon-Venezia, S., Kira K. & Alessandra C. (2017). *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Reviews*, fux013. 252–275
- Newman, M. J., et al. (2011). "Resistance to antimicrobial drugs in Ghana." *Infection and drug resistance* **4**: 215.
- O'Neill J. 2016. Tackling drug-resistant infections globally: final report and recommendations.
- Obeng-Nkrumah N., Dennis S.H., Georgina A.M., Nana K. B., Niels F.M., Newman M. J., Japheth Opintan, A. & Karen A. K, (2023). High level of colonization with third-generation cephalosporin-resistant Enterobacterales in African community settings, Ghana, *Diagnostic Microbiology and Infectious Disease*, Volume 106, Issue 1, ISSN 0732-8893, <https://doi.org/10.1016/j.diagmicrobio.2023.115918>.
- Ogbolu, D.O, Terry A.O.A, Webber M.A, Oluremi A.S, & Oloyede O.M. (2018). CTX-M-15 is established in most Multidrug-Resistant Uropathogenic Enterobacteriaceae and Pseudomonaceae from Hospitals in Nigeria. *Eur J Microbiol Immunol*; 8{1}: 20–24.
- Ondon, B.S., et al., 2021. Sources of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the soil: a review of the spreading mechanism and human health risks. In: de Voogt, P. (Ed.), *Reviews of Environmental Contamination and Toxicology*. Springer International Publishing, Cham, pp. 121–153.
- Opintan, J. A., Newman, M. J., Arhin, R. E., Donkor, E. S., Gyansa-Lutterodt, M., & Mills-Pappoe, W. (2015). Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana. *Infection and drug resistance*, **8**, 379.
- Opintan, J. A. (2018). Leveraging donor support to develop a national antimicrobial resistance policy and action plan: Ghana's success story. *Afr J Lab Med*, **7**(2), 825. <https://doi.org/10.4102/ajlm.v7i2.825>
- Okomo U, Senghore M, Darboe S, Bojang E, Zaman SMA, Hossain MJ, Nwakanma D, Le Doare K, Holt KE, Hos NJ, Lawn JE, Bentley SD, & Kampmann B. (2020). Investigation of sequential outbreaks of *Burkholderia cepacia* and multidrug-resistant extended spectrum β -lactamase producing *Klebsiella* species in a West African tertiary hospital neonatal unit: a retrospective genomic analysis. *Lancet Microbe* **1**:e119–e129. 10.1016/S2666-5247(20)30061-6.
- Osmanovic Dino, David A. Kessler, Yitzhak Rabin & Yoav Soen (2018). Darwinian selection of host and bacteria supports emergence of Lamarckian-like adaptation of the system as a whole
- Ouchar Mahamat, O., Lounnas, M., Hide, M., Dumont, Y., Tidjani, A., Kamougam, K., ... Godreuil, S. (2019). High prevalence and characterization of extended-spectrum β -lactamase producing Enterobacteriaceae in Chadian hospitals. *BMC Infectious Diseases*, **19**(1). doi:10.1186/s12879-019-3838-1

- Owusu, H., Thekkur, P., Ashubwe-Jalemba, J., Hedidor, G. K., Corquaye, O., Aggor, A., Steele-Dadzie, A., & Ankrah, D. (2022). Compliance to Guidelines in Prescribing Empirical Antibiotics for Individuals with Uncomplicated Urinary Tract Infection in a Primary Health Facility of Ghana, 2019-2021. *International journal of environmental research and public health*, 19(19), 12413. <https://doi.org/10.3390/ijerph191912413>
- Parvathi, A., & Kumar, S.H. (2021). Bacterial Resistance to Antimicrobial Agents. *Antibiotics*, 10, 593. <https://doi.org/10.3390/antibiotics10050593>.
- Pasteran F, Gonzalez L.J, Albornoz E., Bahr G, Vila AJ, & Corso A. (2016). Triton Hodge Test: Improved Protocol for Modified Hodge Test for Enhanced Detection of NDM and Other Carbapenemase Producers. *J Clin Microbiol*. 54(3):640-9. doi: 10.1128/JCM.01298-15.
- Payumo, J., Alocilja, E., Boodoo, C., Luchini-Colbry, K., Ruegg, P., McLamore, E., ... & Watanabe, K. (2021). Next Generation of AMR Network. *Encyclopedia*, 1, 871–892.
- Peirano, G., & Pitout, J. D. D. (2019). Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae: Update on Molecular Epidemiology and Treatment Options. *Drugs*. doi:10.1007/s40265-019-01180-3
- Peñaranda G.E, Suasnabar D.F, Foia E., Finello M, Ellena L. M.F, Panchuk A., Dominella F, Hernandez D, Cometto MA, Vázquez SM, Amuchástegui T, Albertini RA, & Saad EJ. (2020). Urinary tract infections in hospitalized patients. *Rev Fac Cien Med Univ Nac Cordoba*. 77(4):265-271. PMID: 33351394.
- Perera, P. D. V. M., Gamage, S., De Silva, H. S. M., Jayatilleke, S. K., de Silva, N., Aydin, A., Enne, V. I., & Corea, E. M. (2022). Phenotypic and genotypic distribution of ESBL, AmpC β -lactamase and carbapenemase-producing Enterobacteriaceae in community-acquired and hospital-acquired urinary tract infections in Sri Lanka. *Journal of global antimicrobial resistance*, 30, 115–122. <https://doi.org/10.1016/j.jgar.2022.05.024>
- Perlaza-Jiménez L, Wu Q, Torres VVL, Zhang X, Li J, Rocker A, Lithgow T, Zhou T, Vijaykrishna D. (2020). Forensic genomics of a novel *Klebsiella quasipneumoniae* type from a neonatal intensive care unit in China reveals patterns of genetic diversity, evolution and epidemiology. *Microb Genom* 6:mgen000433. 10.1099/mgen.0.000433.
- Perez-Muñoz M.E, Arrieta M.C, Ramer-Tait A.E, & Walter J. (2017). A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome*.;5(1):48. doi: 10.1186/s40168-017-0268-4.
- Perry J.A, & Wright G.D. (2013). The antibiotic resistance “mobilome”: searching for the link between environment and clinic. *Front Microbiol* 4:138. <https://doi.org/10.3389/fmicb.2013.00138>
- Peterson E. & Kaur P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Sec. Antimicrobials, Resistance, and Chemotherapy*. Volume 9 <https://doi.org/10.3389/fmicb.2018.02928>

- Potter, R. F., D'Souza, A. W., & Dantas, G. (2016). The rapid spread of carbapenem-resistant Enterobacteriaceae. *Drug Resistance Updates*, 29, 30–46. doi:10.1016/j.drug.2016.09.002
- Prah, J. K., Amoah, S., Ocansey, D. W., Arthur, R., Walker, E., & Obiri-Yeboah, D. (2019). Evaluation of urinalysis parameters and antimicrobial susceptibility of uropathogens among out-patients at University of Cape Coast Hospital. *Ghana medical journal*, 53(1), 44-51.
- Prestinaci F., Pezzotti P., & Pantosti A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathog.Glob. Health* 109 309–318. 10.1179/2047773215Y.0000000030PMCID: PMC9396120.
- Rafalsky V.V. & Dovgan E.V. (2017). Resistance of urinary tract infections and the choice of antimicrobial therapy: deceptive simplicity. DOI: <https://dx.doi.org/10.18565/urol.2017.3.104-110>
- Rakovitsky N, Frenk S, Kon H, Schwartz D, Temkin E, Solter E, Paikin S, Cohen R, Schwaber MJ, Carmeli Y, & Lellouche J. (2020). Fourier transform infrared spectroscopy is a new option for outbreak investigation: a retrospective analysis of an extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae* outbreak in a neonatal intensive care unit. *J Clin Microbiol* 58:e00098-20. <https://doi.org/10.1128/JCM.00098-20>.
- Rather, Irfan A. Byung-Chun Kim, Vivek K. Bajpai, Yong-Ha Park (2017). Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. *Saudi Journal of Biological Sciences* Volume 24, Issue 4, Pages 808-812; <https://doi.org/10.1016/j.sjbs.2017.01.004>
- Raquel Garcia-Fierro, Antoine D., Melody D, Estelle Sa, Carla R, Sylvain B, Jean-Yves M, Marisa H., (2022). Comparative phylogenomics of ESBL-, AmpC- and carbapenemase-producing *Klebsiella pneumoniae* originating from companion animals and humans. *Journal of Antimicrobial Chemotherapy*, Volume 77, Issue 5, Pages 1263–1271, <https://doi.org/10.1093/jac/dkac041>
- Raufu, I. A., Ahmed, O. A., Aremu, A., Ameh, J. A., Timme, R. E., Hendriksen, R. S., & Ambali, A. (2021). Antimicrobial and genomic characterization of salmonella niger from pigs and poultry in Ilorin, North-Central, Nigeria. *Journal of Infection in Developing Countries*. 15(12), 1899-1909.
- Redgrave LS, Sutton SB, Webber MA, & Piddock LJV. (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol*; 22: 438–45.
- Regmi, N., Kafle, S., & Paudyal, R. (2018). Multi-drug resistant uropathogens isolated from mid-stream urine samples. *Journal of Institute of Science and Technology*, 23(1), 39–42. <https://doi.org/10.3126/jist.v23i1.22159>.
- Reygaert Wanda C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3): 482–501. DOI: 10.3934/microbiol.2018.3.482

- Ribeiro TG, Novais A, Peixe L, & Machado E. (2016). Atypical epidemiology of CTX-M-15 among Enterobacteriaceae from a high diversity of non-clinical niches in Angola. *J Antimicrob Chemother*.71: 1169–1173. <https://doi.org/10.1093/jac/dkv489> PMID: 26888909
- Roer L, Overballe-Petersen S, Hansen F, Schønning K, Wang M, Røder BL, et al. (2018). *Escherichia coli* Sequence Type 410 is causing New International High-Risk Clones. *mSphere journal.*; 3(4): e00337–18. <https://doi.org/10.1128/mSphere>.
- Rodrigues C, Passet V, Rakotondrasoa A, & Brisse S. (2018). Identification of Klebsiella pneumoniae, Klebsiella quasipneumoniae, Klebsiella variicola and related phylogroups by MALDI-TOF mass spectrometry. *Front Microbiol* 9:3000. 10.3389/fmicb.2018.03000.
- Rousham, E. K., Unicomb, L., & Islam, M. A. (2018). Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. *Proceedings of the Royal Society B: Biological Sciences*, 285(1876), 20180332.
- Runcharoen C, Raven KE, Reuter S, Kallonen T, Paksanont S, Thammachote J, et al. (2017). Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. *Genome Medicine*; 9: 81. <https://doi.org/10.1186/s13073-017-0471-8>.
- Salimova, G., Ableeva, A., Tatyana L., Zariya Z. & Aidar S., (2020). The Role of Agriculture in Gross Added Value. *Montenegrin Journal of Economics*. Vol. 16, No. 1, 183-191
- Santajit S., & Indrawattana N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *BioMed Research International*. Article ID 2475067, 8 pages <http://dx.doi.org/10.1155/2016/2475067>
- Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi journal of biological sciences*, 22(1), 90-101.
- Sharma P., Netam A. K., & Singh R. (2020). Prevalence and in vitro antibiotic susceptibility pattern of bacterial strains isolated from tribal women suffering from urinary tract infections in District Anuppur, Madhya Pradesh, India. *Biomed. Res. Ther.* 7 3944–3953. DOI; 10.15419/bmrat.v7i8.625
- Shields, R. K., Zhou, Y., Kanakamedala, H., & Cai, B. (2021). Burden of illness in US hospitals due to carbapenem-resistant Gram-negative urinary tract infections in patients with or without bacteraemia. *BMC Infectious Diseases*, 21(1). doi:10.1186/s12879-021-06229-x
- Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016) Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Front. Microbiol.*, 7, 1728.
- Singh, P., Lal, V., & Malik, S. (2021). Bacterial pathogens in urinary tract infection and their antibiotic susceptibility pattern. *American Journal of Microbiological Research*, 9(3), 75-82. Supporting surveillance capacity for antimicrobial resistance: laboratory capacity

- strengthening for drug resistant infections in low- and middle-income countries. *Wellcome Open Res.* 2017;2:91.
- Sirijatuphat, R., Sripanidkulchai, K., Boonyasiri A, Rattanaumpawan P., Supapueng O., Kiratisin P, et al. (2018). Implementation of Global Antimicrobial Resistance Surveillance System (GLASS) in patients with bacteremia. *PLoS One*;13: e0190132.
- Sirijatuphata, R., Thamlikitkula V, Suchada P., Orawan S., & Pattarachai, K. (2020). Implementation of global antimicrobial resistance surveillance system (GLASS) in patients with bacteriuria. *Journal of Global Antimicrobial Resistance* 20 p 60–67.
- Shor E, Schuyler J, Perlin DS. 2019. A novel, drug resistance-independent, fluorescence-based approach to measure mutation rates in microbial pathogens. *mBio* 10:e00120-19. <https://doi.org/10.1128/mBio.00120-19>.
- Soncini, J.G.M, Louise C., Elder S., Vanessa L.K., Ariane, T.T, Zuleica N.T, Gerson N, Renata KTK, Caio, AMA, Nilton L & Eliana C.V. (2022). Genomic insights of high-risk clones of ESBL-producing *Escherichia coli* isolated from community infections and commercial meat in southern Brazil
- Sood S., & Gupta R. (2012). Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. *Indian J. Community Med.* 37 39–44. DOI; 10.4103/0970-0218.94023
- Tacconelli, E., Mazzaferri, F., de Smet, A. M., Bragantini, D., Eggimann, P., Huttner, B. D., ... & Rodríguez-Baño, J. (2019). ESCMID-EUCIC clinical guidelines on decolonization of multidrug-resistant Gram-negative bacteria carriers. *Clinical microbiology and infection*, 25(7), 807-817.
- Taneja Neelam & Sharma Megha (2019). Antimicrobial resistance in the environment: The Indian scenario. *Indian J Med Res* 149, pp 119-128 DOI: 10.4103/ijmr.IJMR_331_18
- Tao Z, Shi A, Zhao J (2015). Epidemiological perspectives of diabetes. *Cell Biochemistry and Biophysics*.73:181-185
- Tetty R., Beverly E., Prudence T., John Arko-Mensah, Samuel O. A., Christian O.N, William B., & Julius, F. (2024). Genomic analysis of multidrug-resistant *Escherichia coli* from Urban Environmental water sources in Accra, Ghana, Provides Insights into public health implications. *PLOS ONE* | <https://doi.org/10.1371/journal.pone.030153>
- Thabit, A. G., Tharwat R. El-Khamissy, Maggie A. Ibrahim and Adel E. & Attia (2011). Detection Of Extended-Spectrum -Lactamase Enzymes (Esbls) Produced By *Escherichia Coli* Urinary Pathogens At Assiut University Hospital. *Bull. Pharm. Sci., Assiut University*, Vol. 34, Part 2,, pp. 93-103.
- Thongpan, L. et al. (2021). Characterization of *Klebsiella pneumoniae* complex isolates from pigs and humans in farms in Thailand: population genomic structure, antibiotic resistance and virulence genes. *J. Antimicrob. Chemother.* **76**, 2012–2016.

- Tóth. K., Damjanova, I., Laczkó, L., Buzgó, L., Lesinszki, V., Ungvári, E., ... & Szabó, D. (2024). Genomic Epidemiology of C2/H30Rx and C1-M27 Subclades of *Escherichia coli* ST131 Isolates from Clinical Blood Samples in Hungary. *Antibiotics*, 13(4), 363.
- Tulara, N. K. (2018). Nitrofurantoin and fosfomycin for extended spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of global infectious diseases*, 10(1), 19-21.
- United Nations Population Division (2014). The World at Six Billion. Available from: <http://www.un.org/esa/population/publications/sixbillion/sixbilpart1.pdf>
- UNESCO (2019). World Water Assessment Programme. (Available from: <http://www.unesco.org/new/en/natural-sciences/environment/water/wwap/>)
- Van Duin, D & Paterson, D. (2016). Multidrug Resistant Bacteria in the Community: Trends and Lessons Learned. *Infect. Dis. Clin. N. Am.*, 30, 377–390.
- Van Duin, D. (2017). Carbapenem-resistant Enterobacteriaceae: What we know and what we need to know. *Virulence*, 8(4), 379–382. doi:10.1080/21505594.2017.1306621
- Varela, M. F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L. M., ... & Kumar, S. H. (2021). Bacterial resistance to antimicrobial agents. *Antibiotics*, 10(5), 593.
- Valenzuela-Valderrama, M., Bustamante, V., Carrasco, N., González, I. A., Dreyse, P., & Palavecino, C. E. (2020). Photodynamic treatment with cationic Ir(III) complexes induces a synergistic antimicrobial effect with imipenem over carbapenem-resistant *Klebsiella pneumoniae*. *Photodiagnosis and Photodynamic Therapy*, 30, 101662. doi:10.1016/j.pdpdt.2020.101662
- Venkitapathi, S., Sharon, B. M., Ratna, T. A., Arute, A. P., Zimmern, P. E., & De Nisco, N. J. (2021). Complete genome sequences of three uropathogenic *Klebsiella quasipneumoniae* strains isolated from postmenopausal women with recurrent urinary tract infection. *Microbiology Resource Announcements*, 10(11), 10-1128.
- Wang, H., et al., (2016). Antibiotics in drinking water in shanghai and their contribution to antibiotic exposure of school children. *Environ. Sci. Technol.* 50 (5), 2692–2699
- Waseem, H., Williams, M. R., Stedtfeld, R. D., & Hashsham, S. A. (2017). Antimicrobial Resistance in the Environment. *Water environment research : a research publication of the Water Environment Federation*, 89(10), 921–941. <https://doi.org/10.2175/106143017X15023776270179>
- Wilson, D.N. (2014). Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat Rev Microbiol* ; 12(1):35–48.
- Winterhalter Mathias (2021). Antibiotic uptake through porins located in the outer membrane of Gram-negative bacteria. *Expert opinion on drug discovery*, 18:4, 449-457, DOI: 10.1080/17425247.2021.1847080

- Wistrand-Yuen E, Knopp M, Hjort K et al., (2018). Evolution of high-level resistance during low-level antibiotic exposure. *Nat Commun*: 9: 1599.
- Woodford N, Turton J, F, & Livermore D.M (2011). Multiresistant Gramnegative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev*; 35:736–55.
- Worku, S., Derby, A., Sinishaw, M. A., Adem, Y. & Biadlegne, F. (2017). Prevalence of bacteriuria and antimicrobial susceptibility patterns among diabetic and nondiabetic patients attending at Debre Tabor Hospital, Northwest Ethiopia. *International Journal of Microbiology*.
- World Health Organization (WHO). Antimicrobial Resistance: Global Report on Surveillance (2014). Available from: <http://www.who.int/drugresistance/documents/surveillancereport/en/>
- World Health Organization. Global Antimicrobial Resistance Surveillance System (GLASS) report: Early Implementation 2017-2018. Available from: <https://www.who.int/glass/resources/publications/early-implementation-report-2017-2018/en/>
- World Health Organization. The Global Antimicrobial Resistance Surveillance System (GLASS): Manual for Early Implementation in Humans 2015. Available from: <https://www.who.int/antimicrobial-resistance/publications/surveillance-system-manual/en/>.
- World Health Organization [Internet]. Antimicrobial resistance: global report on surveillance, WHO 2014 [Updated June 2014; cited 20 October 2017]. Available from: http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf
- World Health Organization. (2014). Antimicrobial resistance: global report on surveillance. Geneva.
- World Health Organization (2019). Global Antimicrobial Resistance Surveillance System (GLASS): Molecular Methods for Antimicrobial Resistance (AMR) Diagnostics to Enhance the Global Antimicrobial Resistance Surveillance System; World Health Organization: Geneva, Switzerland.
- Wright G. D. (2010). The antibiotic resistome. *Expert opinion on drug discovery*, 5(8), 779–788. <https://doi.org/10.1517/17460441.2010.497535>
- Wyres K.L., Lam M.M.C., & Holt K.E. (2020). Population genomics of *Klebsiella pneumoniae*. *Nat. Rev.Microbiol.*18:344–359.doi: 10.1038/s41579-019-0315-1.
- Yang Z, Guo Z, Qiu C, Li Y, Feng X, Liu Y, Zhang Y, Pang P, Wang P, Zhou Q, Han L, & Dai W. (2016). Preliminary analysis showed country-specific gut resistome based on 1267 feces samples. *Gene* 581:178–182. <https://doi.org/10.1016/j.gene.2016.01.043>.
- Yasen R. L., (2023). Antimicrobial Resistance pattern in Gram-Negative Uropathogens in Duhok City, Northern Iraq. doi: <https://doi.org/10.1101/2023.02.23.23285568>

- Zhang et al. (2020). Global trends of antimicrobial susceptibility to ceftaroline and ceftazidime–avibactam: a surveillance study from the ATLAS program (2012–2016) *Antimicrob Resist Infect Control*. 9:166 <https://doi.org/10.1186/s13756-020-00829-z>
- Zhang, YL., Huang, FY., Gan, L.L. *et al.* (2021). High prevalence of *bla*_{CTX-M} and *bla*_{SHV} among ESBL producing *E. coli* isolates from beef cattle in China's Sichuan-Chongqing Circle. *Sci Rep* 11, 13725. <https://doi.org/10.1038/s41598-021-93201-z>
- Zhiyi, Z., Gorden P. J., Xiaoqin, X., Zheng, Y. & Li G. (2021). Whole-genome analysis of *Klebsiella pneumoniae* from bovine mastitis milk in the U.S. *Environ. Microbiol.* 24, 1183–1199.
- Zhou Y., Xiaocui W., Chunyang W., Peiyao Z., Yang Y., Bingjie W., Yanlei X., Huilin Z., Yinjuan G., Jingyi Y., Fangyou Y., David S. & Perlin, (2023). Emergence of KPC-2 and NDM-5-coproducing hypervirulent carbapenem-resistant *Klebsiella pneumoniae* with high-risk sequence types ST11 and ST15, *mSphere journal*, Volume 9, Issue 1, ISSN 2379-5042, <https://doi.org/10.1128/msphere.00612-23>.



Appendix1:



Institutional Review Board
37 Military Hospital
Neghelli Barracks
ACCRA

Tel: 059 1759506
Email: irbmilhosp@gmail.com

02 March 2021

ETHICAL CLEARANCE

37MH-IRB/NF/PPN/456/2021

On 02 March 2021 the 37 Military Hospital (37MH) Institutional Review Board (IRB) approved your protocol.

TITLE OF PROTOCOL: Antimicrobial Resistance in Global Antimicrobial Resistance Surveillance System (GLASS) Priority Pathogens: A Retrospective Study at a Ghanaian Tertiary Hospital

PRINCIPAL INVESTIGATOR: Francis Kwame Morgan Tetteh

Please note that a final review report must be submitted to the Board at the completion of the study.

Please report all serious adverse events related to this study to 37MH-IRB within seven (7) days verbally and fourteen (14) days in writing.

This certificate is valid till 01 March 2022.


DR EDWARD ASUMANU
(37MH-IRB, Vice Chairman)

