RELATIONSHIP BETWEEN MULTIPLE ANTIBIOTIC RESISTANCE INDEX AND EXTENDED SPECTRUM BETA–LACTAMASE AMONG CLINICAL ISOLATES OF *Escherichia coli*

BY

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UNIVERSITY OF BENIN, NIGERIA

OCTOBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE IN MEDICAL LABORATORY SCIENCE

CERTIFICATION

This is to certify that this project work was satisfactoryly carried out by Kosisochukwu Victor NNAMA with matriculation number: BMS2001183 in the University of Benin, under the supervision of DR. RICHARD OMOREGIE and approved as a partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) Degree.

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DEDICATION

I dedicate this research to God Almighty, whose mercies and grace have guided me through this academic journey. I also dedicate this work to my beloved mother, Mrs. Ogechukwu Nnama, whose unconditional love, prayers, and support have been my greatest motivation. Her sacrifices and encouragement have played a vital role in shaping my academic and personal growth. My heartfelt dedication also goes to my elder sister and the Husband Mr. and Mrs. Nwosu Delight and Chiemerie for their unwavering support ever since I entered school , and also to my elder brother, Master Nnama Prosper, for being a financial backup all through my academic journey, and finally I want to specially dedicate this work to my Spiritual Father Pastor Olive Chijioke for his fatherly love and support all through my stay in school.

ACKNOWLEDGEMENTS

I render my gratitude to God Almighty for His wisdom, strength, and guidance throughout the course of this research.

My special appreciation goes to my supervisor, **Dr. Richard Omoregie** for his invaluable guidance, diligent support, and constructive feedback, which greatly contributed to the development of this research.

I extend my gratitude to my Head of Department, **Dr. (Mrs.) Z. Omoruyi**, and all my lecturers for their guide, teachings, leadership, encouragement, and continuous support, all through my undergraduate programmer, and also specially my gratitude goes to my external supervisor **Prof. Omoruyi Pius Omosigho,** for his dillgent guidance and tecnocracy during my project defense.

I am deeply grateful to my father in the Lord, **Pastor Olive Chijioke** for his unbending support all through my research both financially and otherwise.

I also want to extend my profound gratitude to my elder brother, **Sir Prosper Nnama** for his financial support throughout my research work, and also to my elder sister **Mrs. Chiemerie Nwosu**, her encouragement and support was really substantial.

Finally, I would like to thank my beloved mother, **Mrs. Ogechukwu A. Nnama** for her prayers, and support throughout my academic journey.

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ABSTRACT

The rise of antimicrobial resistance (AMR) poses a critical threat to effective infection management, with *Escherichia coli* recognized as a key contributor due to its role in both community and hospital-acquired infections of particular concern are extended-spectrum β-lactamase (ESBL)-producing strains, which hydrolyze third-generation cephalosporins and are frequently associated with multidrug resistance. This study investigated the relationship between the Multiple Antibiotic Resistance (MAR) index and ESBL production among *E. coli* isolates obtained from clinical specimens at the University of Benin Teaching Hospital (UBTH). A total of 51 isolates were analyzed using standard microbiological and susceptibility techniques. ESBL production was confirmed in 9 isolates (17.6%), all from urine samples, consistent with previous reports that highlight urinary tract infections as the leading source of ESBL-producing *E. coli*. The MAR index values ranged from 0.1 to 1.0, with 31.4% of isolates exhibiting MAR = 1.0, underscoring the high antibiotic selection pressure in hospital environments. Although ESBL production was more frequent at higher MAR values, statistical analysis showed no significant correlation ($p = 0.1677$). This contrasts with findings from other regions where ESBL producers consistently demonstrate elevated MAR indices. The presence of multidrug-resistant ESBL-producing *E. coli* in UBTH underscores the urgent need for robust antimicrobial stewardship and infection control strategies to mitigate treatment failures and resistance dissemination in Nigeria.

CHAPTER ONE

INTRODUCTION

1.1Background of the Study

The global rise of antimicrobial resistance (AMR) has gotten to an alarming level, with *Escherichia coli being* a key pathogen of concern due to its dual role as a commensal bacterium and a leading cause of severe infections (WHO, 2021). Recent studies highlight that multidrug-resistant (MDR) *Escherichia coli* strains, particularly those producing extended-spectrum β-lactamases (ESBLs), now account for over 60% of clinical isolates in some regions, thereby limiting treatment options (Kazemian *et al.,* 2023). ESBL enzymes, which hydrolyze penicillins, third-generation cephalosporins, and aztreonam, are increasingly encoded on highly mobile genetic elements that facilitate rapid interspecies spread (Rawat *et al.,* 2022). In Nigeria, recent hospital-based surveillance reports ESBL prevalence rates of 42-68% among clinical *Escherichia coli* isolates (Oluwasanmi *et al.,* 2023), with resistance to fluoroquinolones (55%) and aminoglycosides (38%) (Egbule *et al.,* 2021). This resistance profile correlates strongly with poor clinical outcomes, including 2.5-fold higher mortality rates in bloodstream infections caused by ESBL producers (Okeke *et al.,* 2022). The Multiple Antibiotic Resistance (MAR) index has gained renewed importance as a metric for assessing resistance burden, with studies demonstrating that ESBL-positive isolates consistently show MAR indices >0.4 compared to <0.2 in susceptible strains (Ajayi *et al.,* 2023). The COVID-19 pandemic exacerbated AMR trends through increased antibiotic misuse, with a 35% rise in carbapenem consumption observed in Nigerian hospitals between 2020-2022 (Fowotade *et al.,* 2023). This underscores the urgent need for current, facility-specific resistance data to

guide stewardship programs. Recent genomic studies reveal the predominance of blaCTX-M-15 (82%) and blaTEM (64%) genes among Nigerian ESBL isolates (Oluwafemi *et al.,* 2024), highlighting the evolving molecular epidemiology of resistance. This study will generate critical 2024 resistance profiles using standardized EUCAST guidelines (EUCAST, 2023), addressing a key knowledge gap in understanding how MAR indices correlate with molecular resistance markers in contemporary Nigerian *Escherichia coli* isolates.

1.2 Statement of the Problem

The increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* has become a critical public health concern in Nigeria, with recent studies reporting resistance rates as high as 68% in clinical isolates (Oluwasanmi *et al.,* 2023). This alarming trend has significantly compromised the effectiveness of first-line antibiotics, leading to treatment failures and poor clinical outcomes. Recent data indicate that infections caused by ESBL-producing *Escherichia coli* are associated with 2-3 times higher mortality rates compared to non-ESBL infections (Okeke *et al.,* 2022), yet many healthcare facilities continue to rely on empirical antibiotic therapy without current local resistance data. A particularly concerning aspect is the potential relationship between ESBL production and broader multidrug resistance patterns. Studies from 2023 demonstrate that ESBL-producing *Escherichia coli* isolates frequently exhibit high Multiple Antibiotic Resistance (MAR) indices (>0.4), suggesting extensive resistance profiles (Ajayi *et al.,* 2023). However, there remains a significant knowledge gap regarding how consistently these markers correlate in Nigerian clinical settings, particularly in the post-COVID era where antibiotic misuse has surged (Fowotade *et al.,* 2023)..

The current challenges include:

1. Inadequate surveillance systems for tracking resistance patterns in most healthcare facilities

2. Limited understanding of how MAR indices correlate with molecular resistance markers

3. Frequent mismatch between empirical antibiotic choices and actual resistance profiles

4. Lack of standardized protocols for antimicrobial resistance monitoring

1.3 Justification of the Study

This study addresses the critical challenge of antimicrobial resistance in Nigeria by investigating ESBL-producing *Escherichia coli* strains, which recent studies show now account for 60-68% of clinical isolates (Oluwasanmi *et al.,* 2023). The findings will provide essential data to guide antibiotic prescriptions and combat rising treatment failures, particularly important given the 27% increase in carbapenem use during COVID-19 (Fowotade *et al.,* 2023). The research supports national antimicrobial stewardship efforts by identifying high-risk resistance patterns, while the MAR index offers a practical tool for resistance surveillance in resource-limited settings. By correlating phenotypic and genotypic resistance markers, the study will inform both clinical practice and infection control policies. Ultimately, these evidence-based insights will help preserve antibiotic effectiveness and improve patient outcomes across Nigerian healthcare facilities.

1.4 Aim Of Study

The aim of this study is to investigate the relationship between the Multiple Antibiotic Resistance (MAR) index and Extended-Spectrum Beta-Lactamase (ESBL) production among clinical isolates of *Escherichia col.i*

1.5 Specific Objectives

The specific objectives of this study were;

1. To determine the prevalence of ESBL-producing *Escherichia coli* among clinical isolates from University of Benin Teaching Hospital (UBTH)

2. To determine the MAR index of *E coli* isolated in UBTH

3. To evaluate the correlation between high MAR index (≥0.2) and ESBL production in *Escherichia coli* isolates.

1.6 Research Questions

1. What is the antibiotic susceptibility pattern of *Escherichia coli* isolates to commonly used antibiotics?

2. What is the Multiple Antibiotic Resistance (MAR) index of the *Escherichia coli* isolates?

3. What proportion of the *Escherichia coli* isolates produce Extended Spectrum Beta-Lactamase (ESBL)?

4. Is there a significant relationship between the MAR index and ESBL production among *Escherichia coli* isolates?

5. What are the possible clinical and epidemiological implications of high MAR index and ESBL-producing *Escherichia coli* in UBTH?

1.7 Research Hypotheses

1.7.1Null Hypotheses (H_0)

1. There is no significant relationship between high Multiple Antibiotic Resistance (MAR) index (≥0.2) and ESBL production among *Escherichia coli* isolates from the University of Benin Teaching Hospital (UBTH).

1.7.2Alternative Hypotheses (H_1)

1. There is significant positive correlation between high MAR index (≥0.2) and ESBL production among *Escherichia coli* isolates from UBTH.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Escherichia coli* as a Clinical Pathogen

Escherichia coli is a family-based, Gram-negative, facultative anaerobic microorganism that is normally colonizing the gastrointestinal tract of warm-blooded animals (Martinez-Medina, 2021; Geurtsen *et al.,* 2022). Although *Escherichia coli* is normally a component within the normal intestinal flora, some strains result in serious human diseases such as intestinal infections such as diarrhea and dysentery and extra intestinal infections such as urinary tract infections, respiratory tract infections, meningitis and sepsis (Pokharel *et al.,* 2023; Mohsen, 2025). There are at least eleven types of pathotypes of the species, which are broadly divided into intestinal pathogenic *Escherichia coli* (InPEC) and extraintestinal pathogenic *Escherichia coli* (ExPEC) (Geurtsen *et al.,* 2022). Pathogenic *Escherichia coli* is one of the leading public health challenges, as well as enormous economic burdens globally (Pokharel *et al.,* 2023). The bacterium can thrive and be pathogenic depending on diverse environmental conditions such as the availability of nutrients, temperature, pH, and competition among microbes (Mohsen, 2025). An increasing rate of antibiotic resistance has led to more interest in the development of prophylactic vaccines (Pokharel *et al.,* 2023).

Escherichia coli has always been found to be among the most commonly isolated bacterial pathogens in the healthcare facilities in the sub-Saharan Africa. *Escherichia coli* was found as the most common bacteria in Zambian health facilities, and most of them were identified as part of the urine samples that were used (Mubita *et al.,* 2021). Equally, in a tertiary hospital in Nigeria, 75% of *Escherichia coli* isolates were

obtained in the urine, and other isolates were obtained in blood cultures (21%), cerebral spinal fluid, and endocervical swabs (Medugu *et al.,* 2022). The virulence determinants of *Escherichia coli* include adhesins, toxins and iron uptake systems, which make the pathogen be able to colonize, evade the immune system and induce host tissue damage. *Escherichia coli* pathogenic variants are generalized into intestinal and extra-intestinal pathogenic strains. ExPEC are of particular interest in the hospital environment where immunocompromised patients will be prevalent (Kaper *et al.,* 2024).

One of the most important aspects of *Escherichia coli* infections that make their treatment challenging is the impressive ability of the organism to acquire and spread antimicrobial resistance determinants. Chromosomal mutations, mobile genetic elements like plasmids and transposons and horizontal gene transfer facilitate resistance. *Escherichia coli* exhibits great genetic plasticity which allows the development of resistance to various antibiotic classes by a variety of mechanisms. The bacterium possesses a wide range of antibiotic resistance genes (ARGs) against the major drug classes and includes b-lactams, aminoglycosides, fluoroquinolones, and sulfonamides, most of which are found on mobile genetic elements, which can be transmitted horizontally (Kerek *et al.,* 2025). The predominant resistance is carried out by extended-spectrum b-lactamases and carbapenemases in b-lactam resistance, efflux pumps and fluoroquinolone and aminoglycoside porin mutations, and adaptive resistance mechanisms, such as biofilm formation.

Nigerian studies have always proved *Escherichia coli* to be a leading hospital-associated pathogen in all regions. *Escherichia coli* represented the most commonly encountering type of pathogen with 28.8 percent of all bacterial isolates of clinical specimen in a tertiary hospital in Edo State (Tobin *et al.,* 2021). This conclusion is

backed by a study carried out in Ondo State, where 13.3% of children urine samples contained *Escherichia coli*, which is highly clinically significant in infection of the urinary tract (Wilkie *et al.,* 2024). *Escherichia coli* has been found to cause urinary tract infections and septicemia in inpatients and outpatients within the University of Benin Teaching Hospital (UBTH) just like in other tertiary hospitals. Unregulated use of antibiotics, poor choice of infection control approaches, and insufficient diagnostic facilities are the added factors that contribute to the burden of antibiotic-resistant *Escherichia coli* in Nigeria (Okeke *et al.,* 2020).

2.2 Global and Local Burden of Antibiotic Resistance

Antibiotic resistance is a serious health crisis affecting the world in terms of its power to cure treatment of bacterial infections. The new health, agricultural, and environmental aspects that cause the emergence of multidrug-resistant organisms due to excessive and erroneous use and misuse of antibiotics have complicated the treatment of common infections (Rani, 2024; Lonare, 2024). Resistant bacterial infections cause almost 5 million deaths each year worldwide, and *Escherichia coli* is always among the topmost pathogens that contribute to the death rate (Murray *et al.,* 2022).

The acquisition of antibiotic resistance in bacterial pathogens is very rapid due to the genetic plasticity that allows them to acquire antibiotic resistance via a horizontal gene transfer process by the use of the plasmids and mobile genetic elements. Antibiotic resistance plasmids families are highly mobile, have extensive host ranges, and are more likely to engage in homologous recombination than other plasmids, facilitating the transfer of resistance among widely separated taxa (Coluzzi and Rocha, 2024). Phage-plasmids are a new route of resistance dissemination, as multiple b-lactamic, carbapenamic, aminoglycosidic, fluoroquinolonic, and colistin resistance

genes are frequently located in integrons and can be transmitted without cell-to-cell contact (Pfeifer *et al.,* 2022). Mobile genetic elements in ESKAPE pathogens contain a variety of antibiotic resistance genes, where IncF and IncH plasmids accommodate b-lactam and fluoroquinolone resistance genes, and prophagens possess aminoglycoside resistance genes (Das *et al.,* 2022). Resistance gene inter-plasmid transfer has been extensively studied, and 87 percent of antibiotic resistance gene may be transferred among plasmids, most commonly by the use of insertion sequence such as IS26 (Wang *et al.,* 2024).

Antimicrobial resistance (AMR) is a severe healthcare epidemic in low- and middle-income countries (LMICs), and Nigeria is not an exception. Several systemic conditions cause AMR in such environments, such as unregulated sale of antibiotics, lax pharmaceutical policies, and lax policies on prescription-only medicines (Olajide J. Olagunju *et al.,* 2025; Ehsan, 2025). Causes that are limited to healthcare infrastructure include poor practices in infection control, the absence of adequate diagnostic capacity, and inefficient surveillance systems that increase the spread of resistance (Iheanacho & Eze, 2022; Rony *et al.,* 2023). Socioeconomic obstacles encourage the misuse of antibiotics in self-medication, empirical treatment without culture sensitivity testing, and underdose because of the financial limitation (Olajide J. Olagunju *et al.,* 2025; Ehsan, 2025). Nigeria is among these countries with antibiotic use in hospitalized patients being 65-79 percent, and four out of five patients being prescribed antibiotics every day with no national guidelines (Iheanacho & Eze, 2022). The risks of environmental contamination and healthcare-associated infections are 2-20 times more in LMICs, which provide perfect conditions to develop resistant organisms (Iheanacho *et al.,* 2022; Rony *et al.,* 2023).

2.3 Antibiotic Resistance Profiles and MAR Index

Antibiotic resistance in *Escherichia coli* represents a critical global health threat requiring comprehensive understanding of resistance mechanisms and reliable testing methods. The primary resistance mechanism involves horizontal gene transfer through mobile genetic elements like plasmids and transposons, facilitating acquisition of resistance genes (Nasrollahian *et al.,* 2024). Key mechanisms include extended-spectrum β-lactamases and carbapenemases for β-lactam resistance, efflux pumps and porin mutations affecting fluoroquinolones and aminoglycosides, and adaptive strategies such as biofilm formation (Nasrollahian *et al.,* 2024). Antimicrobial resistance (AMR) represents a critical public health challenge in Nigeria, with widespread resistance to first-line agents consistently documented across multiple pathogens. In cholera cases, previously effective antimicrobials including tetracycline, trimethoprim/sulfamethoxazole, and ampicillin are now reported ineffective due to emerging resistant strains of Vibrio cholerae (Abdulrakib Abdulrahim & Adesola, 2022). Similarly, Salmonella isolates demonstrate extensive resistance, with over 60% developing resistance to two or more antibiotics, most frequently ampicillin, cotrimoxazole, tetracycline, and amoxicillin (Akinyemi *et al.,* 2021).

The Multiple Antibiotic Resistance (MAR) index provides a quantitative measure to summarize resistance data. It is calculated as:

MAR index = Number of antibiotics to which the isolate is resistant/Total Number of Antibiotics tested.

This simple yet powerful metric allows for epidemiological comparisons across isolates, populations, and geographical settings. A MAR index of ≥0.2 is generally considered significant, indicating that the isolate likely originated from an

environment with high antibiotic use or misuse, such as hospitals or intensive animal farming systems. In contrast, MAR values <0.2 suggest exposure to fewer antibiotics and are typical of isolates from environments with limited antibiotic selective pressure. Antibiotic resistance is a major health crisis in the world that endangers the efficacy of the treatment of bacterial infection in the world. The challenge of identifying common infections has become harder because of the emergence of multidrug-resistant organisms which has been caused by excessive and improper use and abuse of antibiotics in healthcare, agriculture, and within the environment (Rani, 2024; Lonare, 2024). Almost 5 million deaths are attributed to resistant bacterial infections globally, and *Escherichia coli* has always been among the most popular factors in this list (Murray *et al.,* 2022).

Genetic plasticity among bacterial pathogens enables quick acquisition of an antibiotic resistance by the horizontal gene transfer pathways involving plasmids and mobile genetic elements. Antibiotic resistance plasmid families are highly mobile, have broad host ranges, and have increased homologous recombination compared to other plasmids, which allows them to transfer resistance to taxonomically distant hosts (Coluzzi & Rocha, 2024). Phage-plasmids are a new mechanism of resistance dissemination, and they contain several resistance genes to b-lactams, carbapenems, aminoglycosides, fluoroquinolones, and colistin that are usually located in integrons and can be transmitted without cell-to-cell contact (Pfeifer *et al.,* 2022). Mobile genetic elements have been identified to contain various antibiotic resistance genes in ESKAPE pathogens, b-lactam and fluoroquinolone resistance genes were found in IncF and IncH plasmids, and aminoglycoside resistance genes were found in prophages (Das *et al.,* 2022). Resistance gene inter-plasmid transfer is also common,

and 87 percent of antibiotic resistance genes may be transferred among plasmids, mostly with the help of an insertion sequence, such as IS26 (Wang *et al.,* 2024).

MAR index used in hospital-based surveillance studies is of special interest in low-resource settings where molecular typing might be not so possible. Indicatively, research in Nigeria has found that *Escherichia coli* isolates with elevated MAR indices usually have a tertiary hospital as their source of origin, highlighting the importance of health institutions as a source of multidrug-resistant strains (Olowe *et al.,* 2021). World literature shows high associations between various antibiotic resistance (MAR) indices and patterns of environmental antibiotic exposure. Multidrug-resistant bacteria carry high burdens with a MAR index of 0.4 to 0.9 in the environmental water sources, and this means that there is a considerable amount of pressure on antibiotics (Hashmi & Jamil, 2023).

The resistance profiles and MAR indices not only have an academic implication but also practical clinical implications. The assessment of resistance patterns will help clinicians to optimize empiric therapy regimes, eliminating those agents with high resistance rates. Abnormally high MAR indices can also be used as one of the early warning signs of multidrug resistance, which would then lead to further diagnostic investigations including ESBL confirmatory studies. This qualifies MAR index as a possible surrogate outcome in the management of infection control and antimicrobial stewardship interventions (Paul *et al.,* 2020).

2.4. Correlation Between MAR Index and ESBL Production

Extended-spectrum beta-lactamase (ESBL) production and high multiple antibiotic resistance (MAR) indices are two interrelated phenomena that amplify the threat posed by *Escherichia coli* in clinical settings. ESBL-producing *Escherichia coli*

demonstrate extensive multidrug resistance patterns beyond β-lactam antibiotics. Studies consistently show these isolates harbor resistance genes to multiple antibiotic classes. In Greece, all 19 ESBL-producing *Escherichia coli* isolates from livestock carried aminoglycoside resistance genes, with additional resistance to sulfonamides (17/19), trimethoprim (14/19), and quinolones (6/19) (Athanasakopoulou *et al.,* 2021). Similarly, clinical isolates from China showed co-resistance to fluoroquinolones, aminoglycosides, and sulfonamides alongside β-lactam resistance (Wang *et al.,* 2025). In Tanzania, 68% of ESBL producers were also quinolone-resistant, with significantly higher resistance rates across all tested antibiotic classes compared to non-ESBL producers (Kimera *et al.,* 2021). UK clinical isolates demonstrated resistance to 4-16 antibiotics from seven classes, with significant correlations between ESBL genes and ciprofloxacin resistance, and integron carriage with trimethoprim/sulfamethoxazole resistance (Ibrahim *et al.,* 2023). These findings confirm that ESBL-producing *Escherichia coli* frequently exhibit multidrug resistance phenotypes encompassing non-β-lactam antibiotics.

Research demonstrates a strong correlation between ESBL production and high multiple antibiotic resistance (MAR) indices, primarily driven by the genetic basis of resistance. Studies consistently show that ESBL-producing *Escherichia coli* exhibit high rates of multidrug resistance, with 73% of isolates being MDR in Egyptian clinical specimens (Masoud *et al.,* 2021) and 93.8% in Malaysian broiler chickens (Lemlem *et al.,* 2023). The predominant ESBL genes identified include blaCTX-M (62.9% prevalence) and blaTEM (45.4% prevalence) (Lemlem *et al.,* 2023), with blaTEM reaching 80% prevalence in clinical isolates (Masoud *et al.,* 2021). These resistance genes are frequently carried on plasmids, particularly IncI1-I(alpha) replicon types, which harbor multiple resistance determinants including ESBL genes

(blaCTX-M-1, blaCTX-M-32, blaTEM-52C) alongside other resistance elements (De Koster *et al.,* 2023). The high MAR indices (>0.2) observed across studies indicate isolates from high-risk contamination sources, with blaCTX-M being the most commonly detected genetic determinant across African surveillance studies (Richter *et al.,* 2023).

2.5. Extended Spectrum Beta-Lactamase (ESBL) in *Escherichia coli*

Extended-spectrum beta-lactamases (ESBLs), which are enzymes secreted by Gram-negative microbes like *Escherichia coli* and Klebsiella pneumonia, mediate resistance to a wide spectrum of beta-lactam antibiotics. These enzymes hydrolyze and inactivate extended-spectrum cephalosporins (such as cefotaxime, ceftriaxone, and ceftazidine) and monobactam aztreonam, but are resistant to clavulanic acid, sulbactam, and tazobactam (Paterson and Bonomo, 2021). Introduction of ESBL-producing *Escherichia coli* is a major change in the epidemiology of antimicrobial resistance, as it has now become a common not just a hospital-acquired bacteria but also a community one.

The most commonly used antibacterial agents are b-lactam antibiotics, which are used to disrupt peptidoglycan synthesis by mimicking D-Ala-D-Ala in crosslinking pentapeptides by binding to bacterial penicillin-binding proteins (PBPs) (Mora-Ochomogo & Lohans, 2021; Kim *et al.,* 2023). The main cause of b-lactam resistance is the development of extended spectrum b-lactamases (ESBLs), which hydrolyze the b-lactam ring and thus make these antibiotics ineffective (Silago, 2021). Although the b-lactams are covalently bound to both the PBPs and the serine b-lactamases, the latter enzymes are capable of deacylating this complex and act on the antibiotic as a substrate instead of an inhibitor (Mora-Ochomogo and Lohans, 2021).

Another notable characteristic of ESBL mediated resistance is that it is plasmid mediated. The ESBLs genes are often located in plasmids, the extrachromosome DNA elements that can be transferred horizontally among bacteria. This transmission via plasmids enhances the transmission of ESBL characteristics in the population of bacteria. ESBL plasmids often possess many more than beta-lactamase resistance determinants, which validates their contribution in the establishment of multidrug resistance. Research has shown that ESBL producing bacteria often contain resistance genes to a wide-range of antimicrobial classes located in the same plasmids. The plasmids in foodborne *Escherichia coli* isolates carried a group of resistance genes, with one cluster comprising sulphonamide, Macrolide and amino glycoside genes, and the other cluster consisting of lincosamide and amino glycoside genes (Darphorn *et al.,* 2021). This is the reason why ESBL producing *Escherichia coli* usually develop multidrug resistance phenotypes, making treatment harder.

ESBL hydrolysis is more efficient with the different enzyme subtypes. As an example, CTX-M enzymes are selective in hydrolyzing cefotaxime, and TEM and SHV derivatives are more effective in hydrolyzing ceftazidime. Regardless of this difference, all the ESBLs exhibit the characteristic feature of inactivating extended-spectrum cephalosporins but are blocked by clavulanic acid. The laboratory detection is based on this peculiarity: susceptibility tests in many cases demonstrate a lowering of the zones of inhibition with cephalosporins of the third generation, and an even greater increase when using the tests in combination with clavulanic acid - the characteristic of ESBL production (Tamma *et al.,* 2020).

In addition to enzymatic hydrolysis *Escherichia coli* that are ESBL producers use a series of other resistance mechanisms to fight antimicrobial agents. Such bacteria

often show a decrease in the expression of porin channels, especially of the orthologues of OmpC and OmpF, which reduces the entry of antibiotics into bacterial cells due to changes in the diameter of pore channels and electrostatic characteristics (Davin-Regli *et al.,* 2024). The resultant effect of these mechanisms is a set of highly resilient strains that are able to resist more than a single antibiotic class.

The internal clinical issues associated with ESBL-producing *Escherichia coli* infections have high resistance and inconsistent treatment responses. Research indicates that cephalosporin-resistance in 80-90% and ESBL production in 51-59% of clinical *Escherichia coli* isolates are covered by the production of ESBL-producing *Escherichia coli* (Amin, 2024; Kettani Halabi *et al.,* 2021). All ESBL producers are resistant to third-generation cephalosporins and quinolones (Kettani Halabi *et al.,* 2021). The rationale behind this is that the bacterial populations tend to carry subpopulations which are more active in ESBL and thus during treatment an emergence of resistance develops very fast. That is why imipenem, meropenem, and ertapenem are carbapenems that are regarded as the gold standard in the treatment of severe ESBL infections (Tamma *et al.,* 2020). Nevertheless, the rising use of carbapenems has spurred the rising resistance of Enterobacteriaceae to carbapenems (CRE) which has precipitated a new wave of resistance crises.

Studies have shown that ESBL producing *Escherichia coli* infections have a huge clinical burden relative to non-ESBL strains with mixed mortality outcomes. Various studies support the long hospitalization of 2-3 days with ESBL infection (Handal *et al.,* 2023; Chen *et al.,* 2024) and an overall 14 percent longer period of hospitalization (Ling *et al.,* 2023). The overall cost of healthcare is also much greater, and ESBL infections add additional financial strain to the patients amounting to 2,047 dollars

(Chen *et al.,* 2024). Moreover, ESBL-producing strains are no longer limited to hospital settings and they have been found to occur in community-acquired infections, such as urinary tract infections in otherwise healthy people. This transition indicates the risk posed by ESBL characteristics of spreading extensively on human and environmental reservoirs.

2.5.1. Common ESBL Genes (blaCTX-M, blaSHV, blaTEM)

Extended-spectrum beta-lactamase (ESBL) production in *Escherichia coli* is primarily mediated by a set of genes that encode enzymes capable of hydrolyzing advanced-generation cephalosporins and related antibiotics. Multiple studies confirm that blaCTX-M, blaTEM, and blaSHV genes are the dominant contributors to extended-spectrum β-lactamase (ESBL) resistance globally. In Sudan, blaCTX-M genes were identified as the most dominant among ESBL-producing Enterobacteriaceae, with 48% of *Escherichia coli* isolates testing positive for blaCTX-M, while blaTEM was predominant in *Klebsiella pneumoniae* (82%) (Altayb *et al.,* 2021).

2.5.1.1 The blaCTX-M Gene Family

The blaCTX-M genes represent the most prevalent and rapidly expanding ESBL family worldwide. CTX-M β-lactamases represent the most prevalent extended-spectrum β-lactamase (ESBL) family globally, having evolved from earlier TEM- and SHV-type ESBLs to become the dominant ESBL type today (Castanheira *et al.,* 2021). Among CTX-M variants, CTX-M-15 is the most widespread worldwide, followed by CTX-M-14, with CTX-M-27 emerging in certain regions (Castanheira *et al.,* 2021). Unlike TEM and SHV, which originated from narrow-spectrum beta-lactamases, CTX-M enzymes evolved from chromosomal beta-lactamases of *Kluyvera* species, an

environmental bacterium (Cantón *et al.,* 2022). CTX-M β-lactamases represent the most prevalent extended-spectrum β-lactamase (ESBL) family globally, having evolved from earlier TEM- and SHV-type ESBLs to become the dominant ESBL type today (Castanheira *et al.,* 2021). Among CTX-M variants, CTX-M-15 is the most widespread worldwide, followed by CTX-M-14, with CTX-M-27 emerging in certain regions (Castanheira *et al.,* 2021).

Among these, CTX-M-15, a member of the CTX-M-1 group, has emerged as the most dominant variant globally, particularly in *Escherichia coli*. It has been strongly linked with community-acquired urinary tract infections and bloodstream infections (Rodríguez-Baño *et al.,* 2020). Its success is largely due to its association with high-risk clones such as *Escherichia coli* sequence type (ST) 131, which combines virulence, adaptability, and multidrug resistance. tudies from Nigeria reveal varying patterns of ESBL production in *Escherichia coli* isolates. Nsofor et al. (2022) found blaCTX-M to be the most prevalent ESBL gene (97.3%) among clinical *Escherichia coli* and K. pneumoniae isolates in southeast Nigeria, followed by blaTEM (75.7%) and blaSHV (32.4%). Similarly, a systematic review by Ayinla & Mateus (2023) confirmed that blaCTX-M variants, particularly blaCTX-M-15, were predominant in West Africa, while blaCTX-M-1 was more common in North Africa.

2.5.1.2. The blaSHV Gene Family

The blaSHV gene family represents a significant component of extended-spectrum β-lactamase (ESBL) resistance in Enterobacterales. Recent surveillance studies demonstrate the widespread distribution of SHV variants, particularly SHV-12, across different geographic regions and bacterial species. In German poultry production, SHV-12 was the predominant variant found in 96% of SHV-positive *Escherichia coli*

isolates, primarily disseminated through horizontal gene transfer via IncX3 and IncI1 plasmids (Irrgang *et al.,* 2021). Similarly, Swiss broiler surveillance revealed SHV-12 in 92% of ESBL-producing isolates, carried on epidemic IncX3 plasmids alongside qnrS1 fluoroquinolone resistance genes (Nüesch-Inderbinen *et al.,* 2023). Clinical studies show variable SHV prevalence: 50.85% in *Escherichia coli* and 35.19% in K. pneumoniae from diverse clinical sources (Bakr *et al.,* 2022), while Romanian hospital isolates demonstrated SHV-1 in all K. pneumoniae strains but only one *Escherichia coli* strain (Ghenea *et al.,* 2022). Studies from Nigeria demonstrate significant prevalence of ESBL-producing *Escherichia coli* and K. pneumoniae with varying distributions of resistance genes. In southeast Nigeria, Nsofor et al. (2022) found ESBL production in 36.5% of isolates, with blaCTX-M being most prevalent (97.3%), followed by blaTEM (75.7%) and blaSHV (32.4%).

2.5.1.3 The blaTEM Gene Family

The TEM β-lactamase family has evolved extensively from its original variants TEM-1 and TEM-2, which conferred resistance only to penicillins and first-generation cephalosporins (Cheng *et al.,* 2021). Through mutations, this family has generated over 220 enzyme variants, with some exhibiting extended-spectrum β-lactamase (ESBL) activity against third-generation cephalosporins while remaining sensitive to β-lactamase inhibitors like clavulanic acid (Cheng *et al.,* 2021; Yassara *et al.,* 2025). The blaTEM gene is among the most frequent ESBL-encoding genes alongside blaCTX-M and blaSHV, carried on plasmids containing mobile genetic elements that facilitate worldwide dissemination (Yassara *et al.,* 2025). Clinical studies demonstrate TEM's continued prevalence, with one study finding TEM significantly more common than other ESBL gene types in multidrug-resistant *Escherichia coli* isolates

(Al-Tahish *et al.,* 2024). Importantly, synonymous mutations play a crucial role in TEM evolution by potentially destabilizing mRNA structure to facilitate efficient translation, supporting the transition to variants with altered phenotypes (Faheem *et al.,* 2021).

TEM-type ESBLs are particularly notable for their ability to hydrolyze penicillins and early cephalosporins, with extended-spectrum variants gaining activity against third-generation cephalosporins. Although their prevalence has been overshadowed by CTX-M, TEM variants remain clinically relevant, especially in regions where plasmid-mediated spread continues unchecked.

2.5.2. Modes of Transmission of ESBL Genes in *Escherichia coli*

The dissemination of extended-spectrum β-lactamase (ESBL) genes in *Escherichia coli* is primarily driven by plasmid-mediated horizontal gene transfer, enabling rapid spread across bacterial populations. Multiple studies demonstrate the critical role of conjugative plasmids in ESBL transmission, with IncF, IncI, and IncH incompatibility groups being particularly important vectors (Salman *et al.,* 2024; Mahmud *et al.,* 2022). Clinical investigations reveal extensive plasmid diversity in ESBL-producing *Escherichia coli*, with resistance genes located on both large plasmids of known replicon types and underestimated small cryptic plasmids (Neffe *et al.,* 2022).

1. Plasmid-Mediated Transmission

Plasmids serve as crucial vehicles for disseminating extended-spectrum β-lactamase (ESBL) genes in *Escherichia coli*, with specific incompatibility groups playing dominant roles. IncF plasmids are particularly prevalent among ESBL-carrying plasmids, representing the most common type found in travelers to Laos and

separating into distinct lineages responsible for major ESBL dissemination (Snaith *et al.,* 2023; Mahmud *et al.,* 2022). Additional incompatibility groups including IncI and IncH families also facilitate horizontal gene transfer of ESBL genes across human, animal, and environmental sectors (Salman *et al.,* 2024). These plasmids demonstrate remarkable epidemic potential, with specific lineages driving global transmission of ESC resistance genes like blaCTX-M-1, blaCTX-M-15, and blaCTX-M-14 across diverse host species and geographic regions (Zamudio *et al.,* 2024).

Extended-spectrum β-lactamase (ESBL) plasmids frequently harbor insertion sequences that play crucial roles in mobilizing resistance genes and enhancing their dissemination. ISEcp1 is particularly significant, as it facilitates the mobilization of blaCTX-M genes and can promote their chromosomal integration (Shawa *et al.,* 2021; Sultan *et al.,* 2022). Studies have demonstrated that ISEcp1-blaCTX-M-15 complexes can form large chromosomal insertions exceeding 10 kb, carrying multiple antimicrobial resistance genes that correspond to multidrug-resistant phenotypes (Shawa *et al.,* 2021). ISCR1 is another important insertion sequence found in ESBL-producing bacteria, contributing to horizontal gene transfer alongside other mobile genetic elements like transposons and integrons (Shafiq *et al.,* 2021; Sultan *et al.,* 2022).

2. Conjugation and Horizontal Gene Transfer

Recent research provides strong evidence that horizontal gene transfer in *Escherichia coli* occurs primarily through conjugation, with plasmids serving as the main vehicles for DNA transfer. Beltrán et al. (2023) demonstrated that conjugative pili act as direct conduits for single-stranded DNA transfer between bacterial cells, resolving the longstanding debate about whether DNA passes through the pilus lumen or requires

tight mating junctions. Mota-Bravo et al. (2023) confirmed conjugation as a main mechanism facilitating horizontal gene transfer in Gram-negative bacteria, highlighting its biological relevance in spreading antibiotic resistance. Conjugative plasmids encoding ESBL genes can move efficiently between different strains and species of Enterobacteriaceae, making hospital environments with high antibiotic pressure ideal hotspots for transmission.

Beyond plasmids, ESBL genes are also associated with transposons and integrons**,** mobile elements that can integrate resistance genes into new genomic contexts. Class 1 integrons, for instance, are commonly associated with ESBL-producing *Escherichia coli* and serve as genetic platforms for the accumulation and dissemination of multiple resistance determinants (Gillings, 20w4). This modularity ensures that ESBL genes are rarely transmitted in isolation but as part of multidrug resistance cassettes.

3. Environmental and Zoonotic Transmission

ESBL-producing *Escherichia coli* represent a significant One Health challenge, with food-producing animals serving as important reservoirs for antimicrobial resistance. Silva et al. (2023) demonstrated high-to-moderate prevalence of ESBL-producing *Escherichia coli* in livestock including pigs, poultry, cattle, fish, and rabbits, with pandemic clones disseminated across different niches including humans and the environment. Giufrè et al. (2021) found CTX-M as the most frequent ESBL type in both human and animal isolates, with CTX-M-15 predominating in humans (75.0%) and cattle (51.1%), while identifying 19 shared sequence types between human and animal sources, supporting potential gene exchange. Freshwater environments also serve as critical reservoirs, with Cho et al. (2023) highlighting that surface water

contaminated with human and animal waste provides ideal conditions for accumulation and dissemination of ESBL-producing Enterobacteriaceae.

Zoonotic transmission is facilitated by plasmid promiscuity, as many ESBL plasmids can cross bacterial species barriers. This environmental dimension complicates control strategies because resistant *Escherichia coli* strains circulate between human, animal, and ecological reservoirs in a One Health context (Madec & Haenni, 2022).

4. Clonal Expansion and High-Risk Lineages

While plasmid-mediated HGT is the primary driver of ESBL dissemination, clonal expansion of high-risk *Escherichia coli* lineages further amplifies their impact. For example, *Escherichia coli* ST131 is a globally dominant clone strongly associated with blaCTX-M-15 plasmids (Nicolas-Chanoine *et al.,* 2024). This synergy between plasmid-mediated transmission and clonal spread allows resistance to persist and expand even without frequent gene transfer events.

2.5.3 Clinical Impact of ESBL-Producing *Escherichia coli*

The emergence and widespread dissemination of extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* have posed significant challenges to clinical medicine and public health worldwide. One of the most pressing clinical impacts is the limited therapeutic options available for the treatment of infections caused by ESBL-producing strains. These organisms are resistant to third-generation cephalosporins, which are often used as first-line empirical therapy for urinary tract infections (UTIs), bloodstream infections, pneumonia, and intra-abdominal infections. As a result, clinicians are forced to rely on last-resort antibiotics such as carbapenems, colistin, and tigecycline. Carbapenem-resistant Enterobacteriaceae (CRE) have

emerged as a major global public health threat due to inadequate treatment options and the widespread use of carbapenems as last-resort antibiotics (Tilahun *et al.,* 2021). The inappropriate use of antimicrobials in humans and animals, coupled with increased global connectivity, has facilitated the transmission of these drug-resistant pathogens worldwide (Tilahun *et al.,* 2021; Hansen, 2021).

Infections caused by ESBL-producing *Escherichia coli* are associated with increased morbidity and mortality compared to infections caused by non-ESBL-producing strains. Several studies have demonstrated that patients infected with these organisms often experience longer durations of hospitalization, delays in receiving appropriate therapy, and higher rates of complicationsResearch on ESBL-producing *Escherichia coli* bloodstream infections (BSIs) demonstrates significant clinical impact and mortality concerns. A Chinese study found that ESBL-producing *Escherichia coli* accounted for 40.98% of BSIs, with ESBL positivity identified as an independent risk factor for mortality (Zhao *et al.,* 2022). However, a Norwegian study showed no statistically significant difference in 30-day mortality between ESBL-producing and non-ESBL *Escherichia coli* BSIs (10.9% vs 9.0%), despite delayed effective antibiotic treatment in the ESBL group (Handal *et al.,* 2023). Research from Nigeria demonstrates significant prevalence of ESBL-producing Enterobacteriaceae with concerning clinical implications. Egbule & Ejechi (2021) found that 75.1% of *Escherichia coli* and *K. pneumoniae* isolates were multidrug resistant, with 36.8-39.3% producing ESBLs, predominantly carrying blaCTX-M genes. Similarly, Mofolorunsho et al. (2021) reported ESBL production in 69% of *Escherichia coli* and 31% of *K. pneumoniae* isolates, with complete resistance to cefotaxime and high resistance rates to multiple antibiotics including amoxicillin-clavulanic acid and ciprofloxacin.

The clinical burden of ESBL-producing *Escherichia coli* extends beyond individual patient outcomes and has profound implications for healthcare systems. Prolonged hospitalization and the need for more expensive antibiotics impose a considerable financial burden on healthcare institutions, especially in low- and middle-income countries where resources are already constrained. Additionally, the requirement for strict infection control measures, such as patient isolation and enhanced surveillance, further strains healthcare facilities (Pitout & Laupland, 2020). In the Nigerian healthcare context, the rising prevalence of ESBL-producing *Escherichia coli* has created significant challenges for infection prevention and control, as many hospitals lack adequate laboratory capacity to promptly identify these organisms and implement appropriate interventions (Ogefere *et al.,* 2021).

Another critical clinical impact of ESBL-producing *Escherichia coli* is their contribution to community-acquired infections. Traditionally, ESBL-producing Enterobacteriaceae were considered hospital-associated pathogens, but over the past two decades, their presence in the community has increased markedly. ESBL-producing *Escherichia coli* are now frequently implicated in community-acquired urinary tract infections, which represent one of the most common bacterial infections globally. This shift underscores the expanding reservoir of resistance genes in the community, often linked to antibiotic misuse in outpatient settings and the food production industry (Rodríguez-Baño *et al.,* 2024). Research demonstrates that non-prescribed antibiotic dispensing is widespread across sub-Saharan Africa and other low- and middle-income countries (LMICs), contributing significantly to antimicrobial resistance. In Africa, the highest rates of antibiotic purchasing without prescription occur in Eritrea (up to 89.2%), Ethiopia (up to 87.9%), Nigeria (up to 86.5%), Tanzania (up to 92.3%), and Zambia (up to 100% of pharmacies dispensing

without prescription) (Sono *et al.,* 2023). A systematic review found that self-medication with antibiotics has a pooled prevalence of 78% across LMICs, with rates ranging from 50% to 93.8% (Torres *et al.,* 2021). Key drivers include high consultation costs, convenience, patient requests, limited knowledge about antibiotics and antimicrobial resistance, and weak enforcement (Sono *et al.,* 2023). Community pharmacies serve as primary sources for non-prescribed antibiotics, with significant variation between countries based on policy frameworks and cultural norms (Do *et al.,* 2021). Lack of pharmacist knowledge and lax law enforcement are primary determinants of inappropriate dispensing practices (Esteves *et al.,* 2023).

2.5.4 Prevalence Patterns Globally, in Africa, and Nigeria

In the last three decades, ESBL producing *Esherichia coli* has become prevalent all over the world, thus becoming one of the most urgent global public health issues. It has been reported globally that ESBL-producing *Escherichia coli* is quite prevalent in different regions, and it is especially high in Asia, some of Europe, and Latin America. In Asia, e.g., a surveillance study conducted in China has reported prevalence rates of more than 50 percent in clinical isolates, and studies in India have reported prevalence rates between 40 and 70 percent in hospital and community settings (Sharma *et al.,* 2020; Zhang *et al.,* 2021). Likewise, the systematic review reported that the 45.0% case fatality rate has been observed among multidrug-resistant organisms in Latin America and the Caribbean, and patients with the infections had almost twice as high risk of death than those with non-resistant infections (Ciapponi *et al.,* 2023). On the other hand, some high-income areas like Northern Europe have a comparatively lower prevalence, usually between 5-10 percent, because of more stringent antimicrobial stewardship and stronger surveillance (ECDC, 2021).

Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* is a major threat to health worldwide and the prevalence rates in this situation are particularly alarming in Africa. Existing prevalence of ESBL-producing *Escherichia coli* in sub-Saharan Africa on a comprehensive meta-analysis found an overall prevalence of 20.76, with West Africa reporting the highest prevalence of 22.80 and Burkina Faso reporting the highest prevalence of 33.37 (Olaitan *et al.,* 2025). Molecular studies also found high prevalence rates in East, Central, and Southern Africa with most blaCTX-M genes and especially CTX-M-15 (Onduru *et al.,* 2021). The 2018-2019 global surveillance data indicated that the ESBL non-CRE phenotypes had more than 50 percent in some countries such as Kenya and Kuwait (Karlowsky *et al.,* 2022). The intestinal carriage studies revealed higher rates in healthcare settings (21.1) than in community settings (17.6) with both settings experiencing alarming proportions of increase over the years- health care settings increased 3- times and community settings increased 10- times between 2001-2020 (Bezabih *et al.,* 2022).

In Nigeria, literature has always shown a high prevalence rate of ESBL-producing *Escherichia coli* in various regions and clinical environments. In Southwest Nigeria, Odetoyin and Adewole (2021) discovered that 25.9 percent of *Escherichia coli* isolates were ESBL producers in a tertiary hospital, and the isolates were majorly isolated in urine samples. In a similar study, Egbule and Ejechi (2021) have found that 36.8 percent of *Escherichia coli* and *Klebsiella pneumoniae* isolates expressed ESBLs with phenotypic assays in Delta State hospitals. Nwafuluaku et al. (2021) found a very high prevalence in Anambra State where 74.5% of uropathogenic *Escherichia coli* isolates expressed phenotypic production of ESBL with CTX-M as the most common ESBL type (100%). Medugu et al. (2022) also discovered that 95.3 percent of *Escherichia coli* isolates at an Abuja tertiary hospital were multidrug-resistant, with

high resistance to ceftriaxone (80.4) and other beta-lactam antibiotics. These results indicate that ESBL-producing *Escherichia coli* is a major therapeutic challenge in all healthcare facilities in Nigeria.

One of the most striking features of the Nigerian situation is that ESBL production is also seen in the hospital-acquired infections and the community-acquired infections, especially the urinary tract infections of otherwise healthy individuals. This change indicates that ESBL genes have become rampant in society, probably because of the uncontrolled use of antibiotics, inadequate hygiene, and the transmission of resistant strains via food and water in the community (Aibinu *et al.,* 2022). The prevalence of ESBL producing *Escherichia coli* in the hospital and community settings is indicative of a system wide issue in Nigerian healthcare system such as inaccessibility to diagnostic centers, absence of routine antimicrobial susceptibility testing, and a lack of awareness of the population about the harmfulness of antibiotic misuse.

2.6. Antibiotic Resistance Profiles of *Escherichia coli*

Escherichia coli (*Escherichia coli*) is one of the most common Gram-negative bacteria implicated in both community-acquired and hospital-acquired infections, particularly urinary tract infections (UTIs), bloodstream infections, and intra-abdominal infections. Its role as both a commensal organism in the human gut and an opportunistic pathogen makes it an important reservoir of resistance genes. The antibiotic resistance profile of *Escherichia coli* has changed dramatically over the last few decades, driven largely by the misuse and overuse of antibiotics in both clinical and non-clinical settings. Increasingly, *Escherichia coli* isolates show multidrug resistance (MDR), defined as resistance to at least one agent in three or more

antimicrobial categories, which poses significant challenges to treatment (Magiorakos *et al.,* 2022).

A key feature of *Escherichia coli* is a significant pathogen in both hospital and community settings, with increasing resistance to β-lactam antibiotics, particularly third-generation cephalosporins such as cefotaxime and ceftazidime (Nowakowska, 2021; Amin, 2024). Recent studies demonstrate concerning levels of cephalosporin resistance among clinical *Escherichia coli* isolates globally. In a clinical study, 80-90% of *Escherichia coli* isolates showed resistance to cephalosporin drugs, with 51-55% identified as ESBL producers (Amin, 2024). Similarly, Wu et al. (2021) found high resistance rates to third-generation cephalosporins in neonatal *Escherichia coli* isolates, with 65% resistant to cefuroxime and 60% to ceftriaxone, alongside a 55% ESBL detection rate. A Moroccan hospital study reported 12% resistance to third-generation cephalosporins, with ESBL phenotype rates increasing from 3% in 2012 to 11.16% in 2018 (Benaissa *et al.,* 2021). Milano et al. (2021)

Beyond cephalosporins, *Escherichia coli* exhibits significant resistance to fluoroquinolones, a class of antibiotics widely used for treating UTIs and other infections. The resistance mechanisms include mutations in the quinolone resistance-determining regions (QRDR) of target enzymes (DNA gyrase and topoisomerase IV) as well as plasmid-mediated quinolone resistance genes such as qnr. Research from Nigeria demonstrates significant fluoroquinolone resistance in *Escherichia coli* isolates, supporting concerns about therapeutic limitations in this region. Kawa et al. (2023) found overall fluoroquinolone resistance rates of 77% in Period 1, decreasing to 47.8% in Period 2 among clinical isolates from a tertiary hospital in northeastern Nigeria. Multiple plasmid-mediated quinolone resistance (PMQR) genes were

identified, including aac(6′)-Ib-cr, oqxA/oqxB, and various qnr genes, alongside chromosomal mutations in gyrA, parC, and parE genes. Anayo et al. (2023).

Aminoglycosides, including gentamicin and amikacin, are commonly used in combination therapy for severe infections. However, resistance to gentamicin among *Escherichia coli* isolates is increasingly reported, primarily due to aminoglycoside-modifying enzymes (AMEs) and efflux pump mechanisms. While amikacin retains better activity against resistant strains compared to gentamicin, resistance levels are rising, especially in resource-limited countries where surveillance and antimicrobial stewardship are weak (Seni *et al.,* 2020).

Carbapenems, including imipenem and meropenem, are considered the last line of defense against multidrug-resistant *Escherichia coli*. Alarmingly, resistance to carbapenems has emerged globally, often mediated by carbapenemase enzymes such as NDM-1, KPC, and OXA-48-like enzymes. Although carbapenem resistance rates in *Escherichia coli* remain relatively lower than in *Klebsiella pneumoniae*, the increasing reports from Asia and Africa are worrisome. In Nigeria, studies have reported carbapenem resistance rates of between 5% and 15% among clinical *Escherichia coli* isolates, underscoring the growing threat of pan-resistant strains (Ogefere *et al.,* 2020).

Other classes of antibiotics, such as sulfonamides and tetracyclines, also show high levels of resistance among *Escherichia coli* isolates. The resistance to trimethoprim-sulfamethoxazole is particularly notable, with rates exceeding 70% in many regions, including Nigeria (Iroha *et al.,* 2022). This resistance is often linked to the widespread and indiscriminate use of these inexpensive antibiotics in both human and veterinary

medicine. Tetracycline resistance, similarly, is common and associated with efflux pump mechanisms and ribosomal protection proteins encoded by tet genes.

The resistance profiles of *Escherichia coli* are shaped not only by antibiotic misuse in human medicine but also by their use in agriculture and animal husbandry. Antibiotic residues in food and the environment provide selective pressure for resistant strains to thrive and spread. Importantly, resistance genes are often carried on mobile genetic elements such as plasmids and transposons, which facilitate horizontal gene transfer between bacteria. Antimicrobial resistance in *Escherichia coli* represents a critical global public health threat, with resistance mechanisms rapidly spreading among Enterobacteriaceae through horizontal gene transfer (Sundaramoorthy *et al.,* 2022; Lynch *et al.,* 2021). *Escherichia coli* has developed diverse and sophisticated resistance systems, including extended-spectrum β-lactamases, carbapenemases, efflux pumps, and porin mutations that confer resistance to multiple antibiotic classes (Nasrollahian *et al.,* 2024; Sundaramoorthy *et al.,* 2022).

2.6.1 Multiple Antibiotic Resistance (MAR) Index in *Escherichia coli*

The concept of the Multiple Antibiotic Resistance (MAR) index has become a vital epidemiological tool in the assessment of antimicrobial resistance among bacterial pathogens, including *Escherichia coli*. The MAR index is calculated as the ratio of the number of antibiotics to which an isolate is resistant to the total number of antibiotics against which it has been tested (Krumperman, 1983). This simple yet powerful measure provides insights into both the extent of resistance in individual isolates and the potential risk level of the environment from which these isolates originated. A MAR index value of ≥0.2 is generally interpreted as an indication that the bacterial strain has originated from a high-risk source where antibiotics are frequently used or

misused, such as hospitals, intensive farming systems, or communities with unrestricted antibiotic access.

In the case of *Escherichia coli*, MAR index analysis has been instrumental in demonstrating the bacterium's extensive resistance capabilities across multiple antibiotic classes. Studies globally have shown that clinical *Escherichia coli* isolates often present MAR indices well above the 0.2 threshold, underscoring their multidrug-resistant nature. Recent studies from India and Egypt demonstrate alarming rates of multidrug resistance (MDR) among uropathogenic *Escherichia coli* isolates. In the Sonipat region of Haryana, India, 83% of *Escherichia coli* isolates from urinary tract infections exhibited MDR patterns, with maximum resistance observed to cephalosporins and fluoroquinolones (Nehra *et al.,* 2021). In China, ESBL-producing *Escherichia coli* are prevalent across human, animal, and environmental domains, with horizontal gene transfer facilitated by plasmids and mobile genetic elements like IS26 and IS1 (Salman *et al.,* 2024).

Antimicrobial resistance (AMR) represents a critical public health threat across sub-Saharan Africa, driven by widespread inappropriate antibiotic use and inadequate stewardship programs. High rates of antibiotic dispensing without prescription are documented across the region, with rates reaching up to 92.3% in Tanzania, 89.2% in Eritrea, and 100% of pharmacies in Zambia dispensing antibiotics without prescription (Sono *et al.,* 2023). Key drivers include high consultation costs, limited access to healthcare, and weak regulatory enforcement (Sono *et al.,* 2023). The Global Point Prevalence Survey across Ghana, Uganda, Zambia, and Tanzania found 50% antimicrobial use prevalence in hospitals, with most prescriptions from WHO 'Access' and 'Watch' categories (Arcy *et al.,* 2021). Among gram-negative bacteria, Klebsiella

species show the highest resistance rates, while *Escherichia coli* demonstrates significant resistance to carbapenems (Gahimbare *et al.,* 2024). Addressing this crisis requires strengthened antimicrobial stewardship programs, improved surveillance systems, and enhanced diagnostic capabilities (Gulumbe *et al.,* 2022).

Recent studies in Nigeria reveal concerning levels of antimicrobial resistance among bacterial isolates from clinical settings. Research from Port Harcourt demonstrated high multiple antibiotic resistance (MAR) indices in *Escherichia coli* isolates, with 25% showing a MAR index of 0.4 and 14.8% exceeding the critical threshold of 0.2, indicating widespread antibiotic resistance (Isaiah *et al.,* 2025). Similarly, a study in Benin City found *Klebsiella pneumoniae* isolates with MAR indices ranging from 0.42 to 1.00, with inpatients showing higher resistance levels (0.82) compared to outpatients (0.74) (Ogefere & Idoko, 2024). Lagos-based surveillance identified 79.3% multidrug resistance prevalence among clinical isolates, with particularly high resistance to cephalosporins (87.5% to cefotaxime) (Chukwu *et al.,* 2022). These findings reflect broader challenges in Nigeria's healthcare system, including poor antimicrobial stewardship, unrestricted antibiotic access, and inadequate infection control practices, contributing to the emergence and spread of resistant bacterial strains (Iheanacho & Eze, 2022).

The utility of the MAR index extends beyond measuring resistance levels in individual isolates; it also provides a means of tracking the potential sources of resistant bacteria. A high MAR index suggests repeated exposure to antimicrobial agents, which is typical in hospital environments where patients often receive prolonged and broad-spectrum antibiotic therapy. Similarly, isolates from communities with unrestricted access to antibiotics, as is the case in many parts of

Nigeria and Africa, frequently exhibit MAR indices above the 0.2 threshold. Thus, MAR index analysis serves as a proxy for identifying high-risk environments and populations in which resistance is most likely to thrive.

Furthermore, the MAR index can help inform public health policies by highlighting trends in resistance that may not be immediately apparent from standard susceptibility testing. For example, isolates with MAR indices approaching or exceeding 0.5 indicate resistance to at least half of the antibiotics tested, pointing to the presence of high-risk clones that may spread rapidly within hospitals and communities. These findings underscore the need for targeted interventions, such as antimicrobial stewardship programs, improved diagnostic capabilities, and stricter antibiotic regulation.

2.6.2 Correlation Between High MAR Index (≥0.2) and ESBL Production in *Escherichia coli*

The correlation between the Multiple Antibiotic Resistance (MAR) index and extended spectrum beta-lactamase (ESBL) production in *Escherichia coli* has been increasingly recognized as a critical indicator of the multidrug-resistant (MDR) phenotype in clinical isolates. ESBL-producing *Escherichia coli* are characterized by their ability to hydrolyze β-lactam antibiotics, particularly third-generation cephalosporins, and often co-harbor resistance genes against other classes of antibiotics such as aminoglycosides, fluoroquinolones, and sulfonamides. Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) pose a significant global health threat, with co-resistance phenomena largely facilitated by mobile genetic elements (MGEs) including plasmids and transposons carrying ESBL-encoding genes. The most prevalent ESBL genes identified are blaCTX-M, blaTEM,

and blaSHV, with blaCTX-M-15 being the most common variant across human, animal, and environmental reservoirs (Zhang *et al.,* 2025; Shafiq *et al.,* 2023). Multiple plasmid incompatibility groups, including IncFIA, IncI1, IncY, IncFIB, IncN, and IncX4, facilitate horizontal gene transfer of these resistance determinants (Zhang *et al.,* 2025). As a result, ESBL-producing *Escherichia coli* typically exhibit MAR index values ≥0.2, reflecting resistance to multiple antibiotic classes and underlining the strong association between ESBL production and high MAR indices.

Multiple studies across different regions have documented the prevalence and characteristics of ESBL-producing *Escherichia coli* and their association with multidrug resistance. In Egypt, 73% of *Escherichia coli* isolates were multidrug resistant with high ESBL production (89.4%), and isolates showed high MAR indices indicating significant public health concern (Masoud *et al.,* 2021). Similarly, research conducted in China showed that ESBL-producing *Escherichia coli* displayed MAR values ranging from 0.4 to 0.8, compared to much lower values in non-ESBL producers (Zhang *et al.,* 2025). These findings highlight the role of ESBL genes not only in β-lactam resistance but also in promoting co-resistance to other antibiotic classes, likely due to plasmid-mediated linkage of resistance determinants.

A meta-analysis across Africa found widespread ESBL-producing Enterobacterales in water, plants, and soil, with consistently high MAR indices for *Escherichia coli*, *Klebsiella pneumoniae*, and Salmonella spp., underscoring the environmental dissemination of resistance (Richter *et al.,* 2023). In Ghana, 64% of *Escherichia coli* from urinary tract infections were MDR, and 50% were ESBL-positive, with high resistance to commonly used antibiotics (Asamoah *et al.,* 2022). Similarly, in Tanzania, ESBL-producing *Escherichia coli* from various sources showed high

resistance to ciprofloxacin (70.8%) and gentamicin (46.0%), with most isolates harboring multiple ESBL genes (Mwakyoma *et al.,* 2023).

Recent studies from Nigeria demonstrate significant multidrug resistance and ESBL production among Enterobacterales isolates. Ogefere & Idoko (2024) found *Klebsiella pneumoniae* isolates with MAR indices ranging from 0.42 to 1.00 (mean 0.7994), with inpatients showing higher indices (0.82) than outpatients (0.74). Egbule & Ejechi (2021) reported 75.1% of *Escherichia coli* and K. pneumoniae isolates were multidrug resistant, with 36.8% producing ESBLs phenotypically and 39.3% by PCR, predominantly carrying blaCTX-M genes (17.2%). Medugu et al. (2023) characterized 49 beta-lactam resistant isolates showing 85.5% resistance to third-generation cephalosporins and 65.3% to carbapenems, with blaCTX-M-15 (77.6%) and blaNDM-1 (51.5%) being prevalent. Adekanmbi et al. (2021) found 49% of uropathogenic *Escherichia coli* were ESBL-positive, with 100% resistance to ciprofloxacin and cephalosporins, and all isolates carrying blaTEM, blaCTX-M-1, and blaCTX-M-9 genes. These findings highlight the critical antimicrobial resistance crisis in Nigerian healthcare settings.

Mechanistically, the correlation between high MAR index and ESBL production can be explained by the genetic environment of ESBL genes. These genes are often located on conjugative plasmids that carry multiple resistance determinants. Multiresistance plasmids carrying blaCTX-M genes frequently harbor additional resistance genes, creating genetic linkages that facilitate co-selection for multiple antibiotic classes. Studies demonstrate that blaCTX-M-15-carrying plasmids commonly co-transfer resistance to aminoglycosides, fluoroquinolones, sulfonamides, and tetracyclines (Minja *et al.,* 2021; Shawa *et al.,* 2021). Specifically, ciprofloxacin,

tetracycline, and sulphamethoxazole-trimethoprim resistance co-transferred in 85.3% of conjugation events involving blaCTX-M-15 plasmids, while gentamicin resistance transferred in 50% of cases (Minja *et al.,* 2021).

The clinical implications of this correlation are profound. High MAR index values in ESBL-producing *Escherichia coli* mean that treatment options are severely restricted, often leaving carbapenems as the only reliable class of antibiotics. However, the overuse of carbapenems in response to rising ESBL prevalence is now leading to the emergence of carbapenem-resistant *Escherichia coli*, further exacerbating the antimicrobial resistance crisis (Logan & Weinstein, 2021). The correlation between MAR and ESBL therefore serves as an early warning system for clinicians and policymakers, signaling the potential for treatment failures, increased healthcare costs, and higher morbidity and mortality rates.

Previous Studies

Igbinosa et al. (2023) have analyzed the occurrence of multidrug-resistant extended-spectrum b-lactamase (ESBL)-producing *Escherichia coli*, which are isolated in farm products and farm environment in Edo State, Nigeria. The paper was designed to assess the level of multidrug resistance of the isolates, the primary objective being to understand how the level of risk can be described by the use of the MAR index values. The researchers used the conventional microbiological methods such as culture, antibiotic susceptibility test by the Kirby-Bauer disk diffusion method, and phenotypic identification of ESBL production. The results showed that ESBL-producing isolates had a significantly higher index of MAR with a range of 0.27 to and 0.62 compared to those of non-ESBL isolates. These findings underscored that the environments analyzed in the farms were high-risk sources of resistant bacteria

because of the high level of exposure to antibiotics. The authors concluded that MAR index is a good epidemiological tool to help in the identification of the environmental hotspots where ESBL-producing bacteria thrive and they suggested the need to enhance monitoring systems in the agricultural environment.

Beshiru et al. (2024) explored the prevalence of ESBL-producing and non-ESBL-producing *Escherichia coli* in surface water systems in Edo State, Nigeria, aiming to measure the ability of aquatic environments to serve as the reservoirs of the multidrug-resistant organisms. The research design was a cross-sectional study design, water sampling, culture techniques, antibiotic resistance profiling, and phenotypic ESBL detection methods. Resistance pressure was assessed by computation of values of MAR index to compare data with analysis. The result showed that ESBL positive isolates had indices of MAR between 0.18 and 0.46, which was a significantly high value compared to non-ESBL. The authors concluded that aquatic ecosystems are important in the persistence and reproduction of ESBL-producing organisms that have high MAR indices. The article highlighted the severe necessity of more stringent waste management regulations and antibiotic release into surface water as these two areas contribute to the antibiotic gene transfer and development of multidrug resistance.

Lemlem et al. (2024) determined the prevalence and molecular profiling of ESBL producing *Escherichia coli* in broiler chicken and poultry farm settings in Malaysia. The primary aim of the research was to establish the possibility of the association of MAR index values with ESBL production in livestock-associated isolates. The methodology entailed systematic gathering of fecal and environmental samples, isolation of *Escherichia coli*, antibiotic susceptibility evaluation and PCR-based molecular verification of ESBL genes. The study indicated that most of the isolates

presented a MAR index value above 0.2 with ESBL-producing strains having much higher indexes with most of the indices between 0.4 and 0.9. It was also found through its results that ESBL producers often had several resistance genes, which added to their high MAR values. The researchers came to the conclusion that the ESBL production is closely related to the high indices of MAR in poultry environments and food animals are the reservoirs of resistant pathogen which can be transferred to the human food chain.

Saeed et al. (2023) conducted a cross-sectional study of *Escherichia coli* that causes avian disease in backyard chicken in Pakistan and targeted the frequencies of the ESBL production and how it correlates with multidrug resistance. The objective of the study was to determine whether ESBL-positive isolates had a higher value of MAR index than non-ESBL strains, and to prove the epidemiological significance of MAR as a means of resistance and monitoring. The study design was random sampling of poultry feces, microbiological isolation, antimicrobial susceptibility test and confirmatory tests on the production of ESBL. The findings revealed that the geometric average score of MAR index of ESBL-positive isolates was 0.25 which is highly significant compared to the 0.17 which was the ESBL-negative isolates. The researchers determined that ESBL production was highly linked with greater multidrug resistance as indicated by the values of MAR index and that there was a need to improve antibiotic stewardship in backyard poultry systems to reduce the spread of resistance.

Mandujan et al. (2023), investigated the incidence of ESBL-producing *Escherichia coli* in food-producing animals in Mexico with the purpose of examining the resistance patterns in relation to the value of MAR index. The researchers employed phenotypic susceptibility testing and diagnosis of ESBL genes (blaCTX-M and

blaTEM) using PCR. Their results revealed that ESBL-producing isolates were always very high in terms of the MAR indices, which means that they were resistant to various antibiotic classes, among them b-lactams, aminoglycosides, and tetracyclines. Most of the MAR values were above the 0.2 mark indicating that the isolates were obtained in environments with strong pressure of antibiotic use. The results of the study were that the presence of ESBL production along with high MAR indices is a sign of an plasmid-mediated co-resistance. These authors pointed out that this is a significant burden on veterinary and human health, since foodborne diseases caused by such pathogens can be a risk to the entire antimicrobial resistance control campaign.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

This investigation was carried out at the University of Benin Teaching Hospital's (UBTH) Medical Microbiology Laboratory in Benin City Edo State, from July 21, 2025, to September 4, 2025. UBTH is a multi-speciality healthcare facility in Nigeria with around 910 bed space. The hospital is situated between latitudes 6.3903°N and 5.6118°E in Ugbowo, Benin City, Edo State, Nigeria.

3.2 Study Design

The study design for this research was cross-sectional

3.3 Ethical Approval

The protocol for this study was approved by the Ethical Committee of the College of Basic Medical Sciences, University of Benin, Benin City, Edo State.

3.4 *Escherichia coli* isolates

Isolates of *E .coli* were collected from some processed samples consisting of urine, wound swab, high vaginal swab, endocervical swab, throat swab, ear swab and eye swab and sub-cultured, to obtain a pure colony of the *E. coli.* An isolate was identified as *E. coli* if it was a gram negative baccilli, oxidase negative, lactose fermenting, motile, indole positive, citrate and urea negative

3.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the *Escherichia coli* isolates was executed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, consistent with CLSI guidelines (2024). Briefly,

3.5.1. Antibiotics Used

- Amoxicillin-clavulanate
- Ceftazidime
- Cefotaxime
- Imipenem
- Meropenem
- Cefepim
- Ciprofloxacin
- Ofloxacin
- Gentamicin

3.6 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined as described by Isaiah *et al*. (2025). Briefly, MAR index was calculated using the formula

$$\text{MAR Index} = \frac{\text{Number of antibiotics resisted}}{\text{Total antibiotics tested}}$$

3.7 Detection of ESBL

The production of Extended Spectrum Beta-Lactamase (ESBL) among Escherichia coli isolates was assessed following the procedure described by Ogefere et al. (2015). In this method, bacterial isolates were suspended in sterile distilled water, and the turbidity was adjusted to match the 0.5 McFarland standard. A sterile cotton swab was then immersed in the standardized suspension, and excess fluid was removed by gently rotating the swab against the inner wall of the test tube. The entire surface of a Mueller–Hinton agar plate was evenly inoculated by swabbing in three directions to ensure uniform distribution of the test organism. A 30 µg amoxicillin–clavulanate disc (Oxoid, England) was positioned at the center of the plate, while 30 µg ceftazidime and 30 µg cefotaxime discs (Oxoid, England) were placed 25 mm away on opposite sides of the central disc. The inoculated plates were incubated at 37°C for 18–24 hours, and isolates were considered ESBL-positive when an enhanced inhibition zone was observed between the amoxicillin–clavulanate disc and either the ceftazidime or cefotaxime disc, or both.

3.8 Statistical Analysis

The data obtained were analyzed with Chi square (X2) test using the statistical sofware INSTAT® (Graph Pad Inc., USA). Level of significance was set at $p<0.05$.

CHAPTER FOUR

RESULT

The result obtained in this study as shown in Table 4.1-4.4 and Fig. 4.1-4.2. Out of the 51 *Escherichia coli* isolates 9(17.6%) produced Extended Spectrum Beta

Lactamase(ESBL). All the 9 ESBL *E.coli* were recovered from urine specimen (Table 4.1).

Although the prevalence of ESBL producing *Escherichia coli* were higher in males than females (males vs females: 22.2% vs 12.5%), gender did not significantly (p=0.5884) affect the prevalence of ESBL producing *E.coli* (Table 4.2).

Fig. 4.1 showed the prevalence of ESBL producing *E.coli* among the various age groups. The prevalence of ESBL producing E.coli rose from 0% in the age of <1-15 years to 33.3% in the age group of 46-60, and dropped to 18.2% in the age group of 61-75 years. Then it rose again to 25% in the age group of ≥76 years. However age did not significantly (p= 0.6323) affect the prevalence of ESBL-producing *E.coli.*

The wards/clinics the E.coli were recovered from did not significantly (p=0.8372) affect the prevalence of ESBL production. (Table 4.3)

The MAR(Multiple Antibiotic Resistance) Index of all *E.coli* isolate range from 0.1–1.0, with the isolate having MAR index (1.0) being the most prevalent (31.4%). *E.coli* isolate with MAR index 0.6 were the highest producers of ESBL, however there was no significant relationship between the MAR index and ESBL production (Table 4.4).

Prevalence of ESBL production, did not differ significantly (p=0.8921) between Multiple Drug Resistant and Non-Multiple Drug Resistant E.coli (Fig 4.2)

Table 4.1: Prevalence of Extended Spectrum Beta Lactamase(ESBL) Among *Escherichia coli* Isolates from Various Clinical Specimen

Specimen	No. of *Escherichia coli*	No. positive for ESBL (%)

Wound Swab	9	0 (0.0)*
Throat Swab	2	0(0.0)*
High Vaginal swab	9	0(0.0)*
Endocervical swab	4	0(0.0)*
Urine	24	9(37.5)
Ear swab	2	0(0.0)*
Eye swab	1	0(0.)*
Total	51	9(17.6)

ESBL(extended spectrum β-lactamase); *= not used in statistical analysis

Table 4.2: Distribution of ESBL(Extended Spectrum Beta Lactamase) producing *Eschericia coli* Among Gender of Patients

Gender	No. Of *Escherichia coli*	No. ESBL(%)
Male	27	6(22.2)
Female	24	3(12.5)
Total	51	9(17.6)

p= 0.5884

Fig 4.1: Distribution of Extended Spectrum Beta Lactamase Among Age Group of Patients *Escherichia coli* were recovered from.

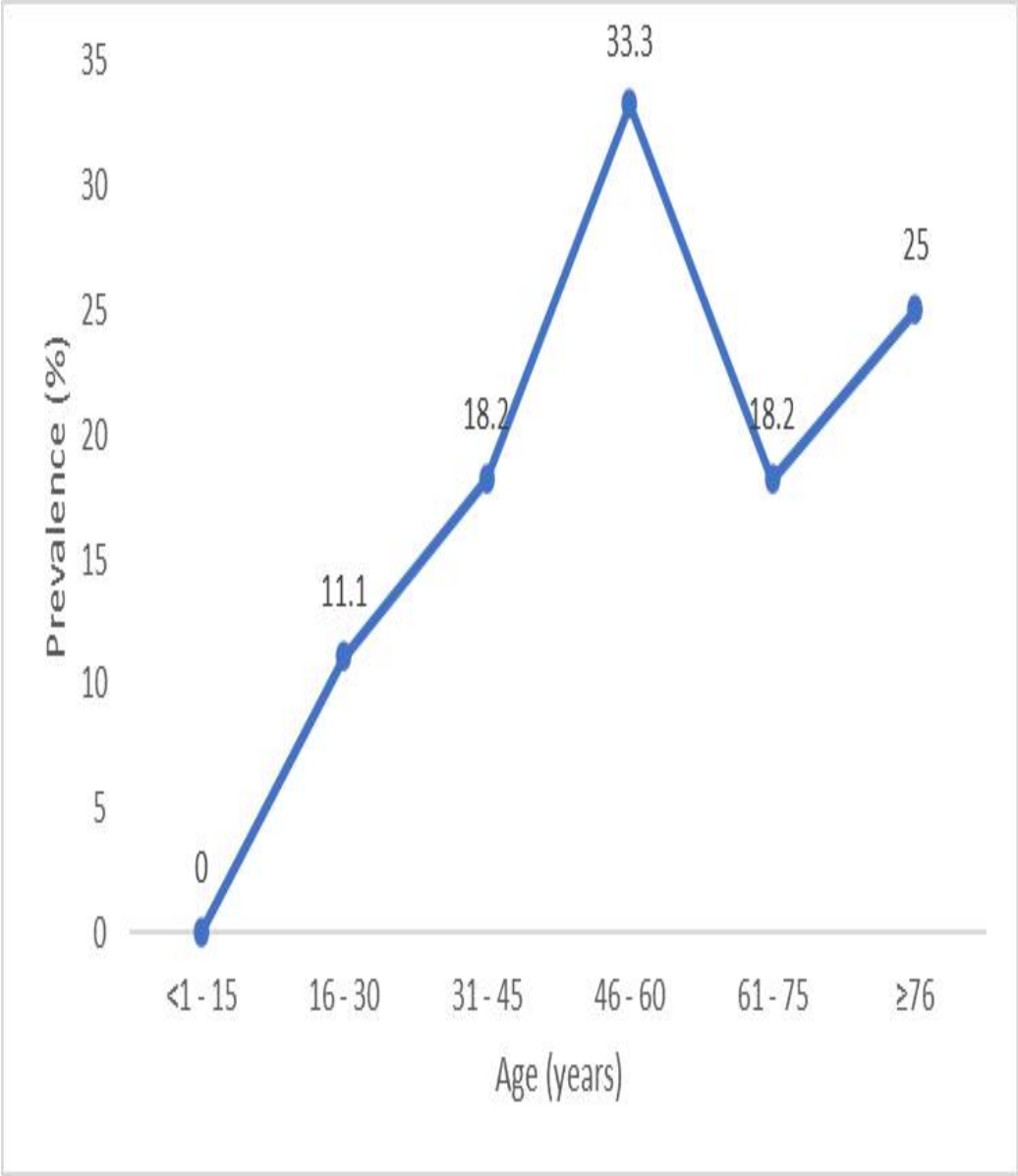


Table 4.3: Distribution of Extended Spectrum Beta Lactamase(ESBL) Producing *Escherichia coli* from Various Wards/Clinics

Wards/Clinics	No. of *Escherichia coli*	No. positive for ESBL (%)
Emergency ward	6	2(33.3)
Geriatrics Ward	5	1(20.0)
Surgical Ward	5	1(20.0)
Intensive Care Unit	1	0(0.0)*
General Practice Centre	12	1(8.3)
Ear Nose and Throat	1	0(0.0)*
Obstetrics and Gynaecology Ward	4	1(25.0)
Out Patients Ward	5	2(40.0)
Special Care Baby Unit	1	0(0.0)*
Medical Ward	2	0(0.0)*
Paediatric Ward	4	0(0.0)*
Maternity Ward	1	0(0.0)*
Oncology Ward	4	1(25.0)
Total	51	9(17.6)

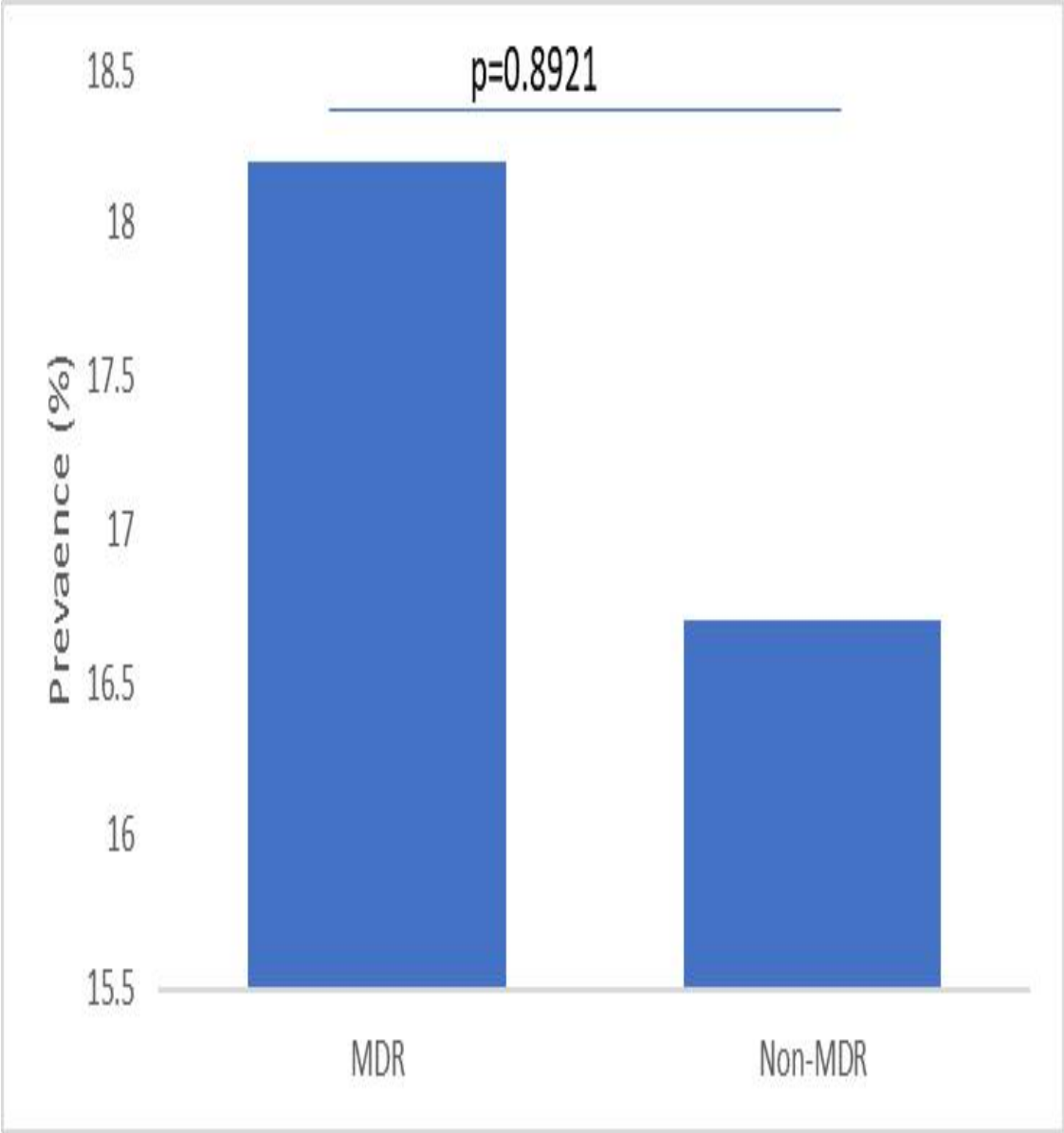
p= 0.8372; *= not used in statistical analysis

Table 4.4: Multiple Antibiotics Resistance Index of ESBL(Extended Spectrum Beta Lactamase)

MAR Index	No. Of *Escherichia coli*	No. Positive for ESBL(%)
0.0	0	0(0.0)*
0.1	1	0(0.0)*
0.2	3	0(0.0)*
0.3	3	0(0.0)*
0.4	6	0(0.0)*
0.5	0	0(0.0)*
0.6	6	3(50.0)
0.7	4	1(25.0)
0.8	7	3(42.9)
0.9	5	1(20.0)
1.0	16	1(6.3)
Total	51	9(17.6)

P=0.1677; *= not used in statistical analysis

Fig 4.2: Distribution of ESBL Producing *Escherichia coli* Among MDR and Non-MDR Isolates



MDR- Multiple Drug Resistant

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigated the relationship between MAR index and ESBL production among clinical isolates of *Escherichia coli* from the University of Benin Teaching Hospital (UBTH). Out of 51 *E. coli* isolates, 9 (17.6%) were confirmed as ESBL

producers, all of which were recovered from urine specimens. This finding corroborates earlier studies that identified *E. coli* as the most common cause of urinary tract infections and one of the leading hospital-associated pathogens in Nigeria (Tobin *et al.*, 2021; Medugu *et al.,* 2022).

The prevalence rate of 17.6% observed in this study is lower than previously reported rates from Nigerian tertiary hospitals, where ESBL prevalence has ranged from 36–68% (Oluwasanmi *et al.,* 2023; Egbule and Ejechi, 2021). The variation may be attributed to differences in sample size, geographical distribution, and temporal changes in antimicrobial use.

With respect to demographic factors, the study revealed that ESBL production was more common in male patients (22.2%) compared to females (12.5%), although the difference was not statistically significant (p=0.5884). Age group distribution also showed no significant association (p=0.6323), despite relatively higher ESBL prevalence among patients aged 46–60 years. These findings suggest that ESBL occurrence is not confined to a specific demographic but may be influenced more by antibiotic exposure and healthcare practices (Pitout & Laupland, 2020).

The MAR index values of the isolates ranged from 0.1 to 1.0, with the most frequent MAR value being 1.0 (31.4%). A high MAR index (≥0.2) generally indicates that isolates originated from high-risk environments with intense antibiotic pressure (Krumperman, 1983; Paul *et al.,* 2020). Consistent with this, Nigerian surveillance studies have documented MAR indices as high as 0.82 among hospital isolates, reflecting widespread antibiotic misuse and weak stewardship systems (Ogefere & Idoko, 2024; Chukwu *et al.,* 2022).

Although ESBL-positive isolates in this study exhibited relatively higher MAR values (e.g., MAR 0.6 with 50% ESBL positivity), no statistically significant relationship was established between ESBL production and MAR index (p=0.1677). This finding contrasts with reports from other regions, such as Pakistan and Mexico, where ESBL-producing *E. coli* consistently demonstrated significantly higher MAR indices compared to non-ESBL strains (Saeed *et al.,* 2023; Mandujan *et al.,* 2023). It is possible that the limited sample size in the present study reduced statistical power, or that local antibiotic usage patterns result in resistance development through non-ESBL mechanisms such as efflux pumps, porin mutations, or biofilm formation (Nasrollahian *et al.,* 2024).

Nonetheless, the high frequency of isolates with MAR ≥0.6 indicates a serious multidrug resistance burden at UBTH, consistent with national and global concerns. Studies from Egypt, Malaysia, and sub-Saharan Africa have similarly shown strong associations between ESBL genes (particularly blaCTX-M and blaTEM) and elevated MAR values (Masoud *et al.,* 2021; Lemlem *et al.,* 2023; Richter *et al.,* 2023). The lack of a clear statistical correlation in this study does not diminish the clinical significance of MAR index as an epidemiological tool for detecting high-risk resistant clones (Beshiru *et al.,* 2024).

The findings underscore the urgent need for antibiotic stewardship, improved diagnostic capabilities, and stricter infection control practices. Inappropriate antibiotic prescribing, self-medication, and weak regulation of antimicrobial sales in Nigeria have been major drivers of resistance (Iheanacho & Eze, 2022; Olajide Olagunju *et al.,* 2025).

5.2. Conclusion

This study established a 17.6% prevalence of ESBL-producing Escherichia coli among clinical isolates at UBTH, with all cases recovered from urine samples. Although ESBL producers exhibited higher MAR indices compared to non-producers, the relationship between MAR index and ESBL production was not statistically significant.

The high MAR values (up to 1.0) observed indicate substantial multidrug resistance, reflecting heavy antibiotic pressure in the hospital environment. While demographic factors (age, sex, and ward distribution) showed no significant association with ESBL production, the persistence of multidrug resistance remains a pressing clinical challenge.

In line with global trends, the findings highlight that ESBL-producing *E. coli* are important reservoirs of resistance genes, with potential for rapid dissemination within both hospital and community settings. MAR index analysis remains a valuable surveillance tool, capable of identifying high-risk environments for resistance emergence.

5.3. Recommendations:

1. Strengthening antimicrobial stewardship programs at UBTH to limit indiscriminate antibiotic use.

2. Routine surveillance of MAR index and ESBL production in clinical isolates to inform empirical therapy.

3. Incorporation of molecular typing (e.g., detection of blaCTX-M, blaTEM, blaSHV) to better understand resistance epidemiology.

4. Implementation of infection control measures, including patient isolation and improved hygiene practices, to limit cross-transmission.

5. Public health education to discourage self-medication and over-the-counter antibiotic misuse.

By adopting these measures, it may be possible to reduce the spread of multidrug-resistant *E. coli* strains and preserve the efficacy of available antibiotics.

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APPENDIX I

IDENTIFICATION TEST

To identify Escherichia coli and differentiate it from other Enterobacteriaceae, a series of standard microbiological tests were performed. These included Gram staining, motility test, citrate utilization test, urease test, and indole test.

GRAM STAINING

Principle: Gram staining differentiates bacteria into Gram-positive (purple) and Gram-negative (pink/red) based on differences in cell wall composition (Gram, 1884). Gram-negative organisms have a thinner peptidoglycan layer and a higher lipid content, which allows the crystal violet-iodine complex to be washed out by alcohol.

Procedure: A thin smear of the bacterial culture was heat-fixed, stained with crystal violet, treated with iodine, decolorized with alcohol, and counterstained with neutral red.

Interpretation: *E. coli* appeared as Gram-negative short rods stained pink under the microscope.

MOTILITY TEST

Principle: Bacterial motility is due to the presence of flagella. Motile organisms move away from the line of inoculation, producing diffuse turbidity, while non-motile organisms grow only along the stab line.

Procedure: Motility was assessed using semi-solid agar medium. A straight stab inoculation was made with a sterile needle, and the tubes were incubated at 35–37 °C for 18–24 h.

Interpretation: Diffuse, hazy growth radiating from the line of inoculation indicated motility. *E. coli* is motile.

CITRATE UTILIZATION TEST

Principle: This test determines the ability of an organism to utilize citrate as the sole carbon source and ammonium salts as the nitrogen source. Utilization leads to alkaline by-products, changing the bromothymol blue indicator from green to blue (Simmons citrate agar).

Procedure: The bacterial isolate was inoculated onto Simmons citrate agar slants and incubated at 35–37 °C for 24–48 h.

Interpretation: A positive result is shown by growth with blue coloration of the medium. *E. coli* is typically citrate negative (no growth, medium remains green).

UREASE TEST

Principle: This test detects the production of urease enzyme, which hydrolyses urea into ammonia and carbon dioxide. Ammonia raises the pH, causing phenol red indicator to change from yellow to pink.

Procedure: The isolate was inoculated into urea broth or onto urea agar slants and incubated at 35–37 °C.

Interpretation: A positive result is indicated by a pink-red color. *E. coli* is urease negative (no color change, medium remains yellowish).

INDOLE TEST

Principle: This test detects the ability of an organism to produce indole from the amino acid tryptophan using the enzyme tryptophanase. Indole reacts with Kovac's reagent (p-dimethylaminobenzaldehyde) to form a red compound.

Procedure: The isolate was inoculated into tryptone broth and incubated at 35–37 °C for 24–48 h. After incubation, 0.5 mL of Kovac's reagent was added.

Interpretation: A red ring at the surface of the broth indicates a positive result. *E. coli* is indole positive.

APPENDIX II

1. Mueller-Hinton Agar (MHA)

Purpose: Used for antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method.

Preparation:

Weighed 38 g of Mueller-Hinton agar powder and dissolved in 1 L of distilled water.

Heated while stirring to completely dissolve.

Sterilized by autoclaving at 121 °C for 15 minutes.

Allowed to cool to 45–50 °C and poured into sterile Petri dishes (~20 mL per plate).

Plates were allowed to solidify at room temperature and stored at 4 °C until use.

Nutrient Agar Slant

Purpose: Used for maintenance and sub-culturing of bacterial isolates.

Preparation:

Dissolved 28 g of nutrient agar powder in 1 L of distilled water.

Sterilized by autoclaving at 121 °C for 15 minutes.

Cooled to 45–50 °C and poured into test tubes at an angle to form slants.

SAllowed to solidify and stored at 4 °C until use.

Citrate Medium (Simmons Citrate Agar)

Purpose: To rate ability of bacteria to utilize citrate as the sole carbon source.

Preparation:

Dissolved 13.5 g of Simmons citrate agar powder in 1 L of distilled water.

GSterilized by autoclaving at 121 °C for 15 minutes.

Cooled to 45–50 °C and poured into sterile slant tubes.

Allowed to solidify and stored at 4 °C until use.

Urea Agar Base

Purpose: To detect urease enzyme production, which hydrolyzes urea to ammonia.

Preparation

Dissolved 23.5 g of urea agar base in 500ml of distilled water.

Sterilized by autoclaving at 121 °C for 15 minutes.

Allowed to cool to 45–50 °C and poured into slant tubes.

Stored at 4 °C until use.

Peptone Water

Purpose: Used for indole test and general bacterial growth.

Preparation

Dissolved 10 g of peptone and 5 g of sodium chloride in 500ml of distilled water (or use commercial peptone water powder).

Sterilized by autoclaving at 121 °C for 15 minutes.

Allowed to cool and dispensed into sterile test tubes.

RStored at 4 °C until use.

Media Constituents

Mueller Hinton Agar

· Beef Extract– 2.0gram

· Acid Hydrolysate of Casein– 17.5gramm

· Starch– 1.5

· Agar–17.0gram

Distilled water – 500ml

2. Citrate Medium (Simmons')

· Sodium Citrate-

· Ammonium Dihydrogen Phosphate-

· Dipotassium Phosphate-

· Monopotassium Phosphate- 0.

· Sodium Chloride

· Magnesium Sulfate

· Bromothymol Blue (pH indicator)

· Agar

3. **Urease Medium (Christensen's)**

· Peptone

· Glucose

· Sodium Chloride

· Monopotassium Phosphate

· Phenol Red (pH indicator)

· Urea

· Agar

4**. Nutrient Agar**

· Peptone

· Beef Extract

· Sodium Chloride

· Agar

5. Peptone Water

· Peptone

· Sodium Chloride

Chemical Reagent

All chemicals used in this study were of analytical grade and they include;

Gram stain Reagent

Primary Stain: Crystal Violet

· Crystal Violet

· Ethanol (95%)

· Ammonium oxalate

· Distilled water

Mordant: Gram's Iodine

· Iodine (I_2) crystals:

· Potassium Iodide (KI)

· Distilled water

Decoloriser- Acetone

Safranin

· Safranin O dye:

· Ethanol (95%):

· Distilled water

Materials Used

Wire loop

Petri dish

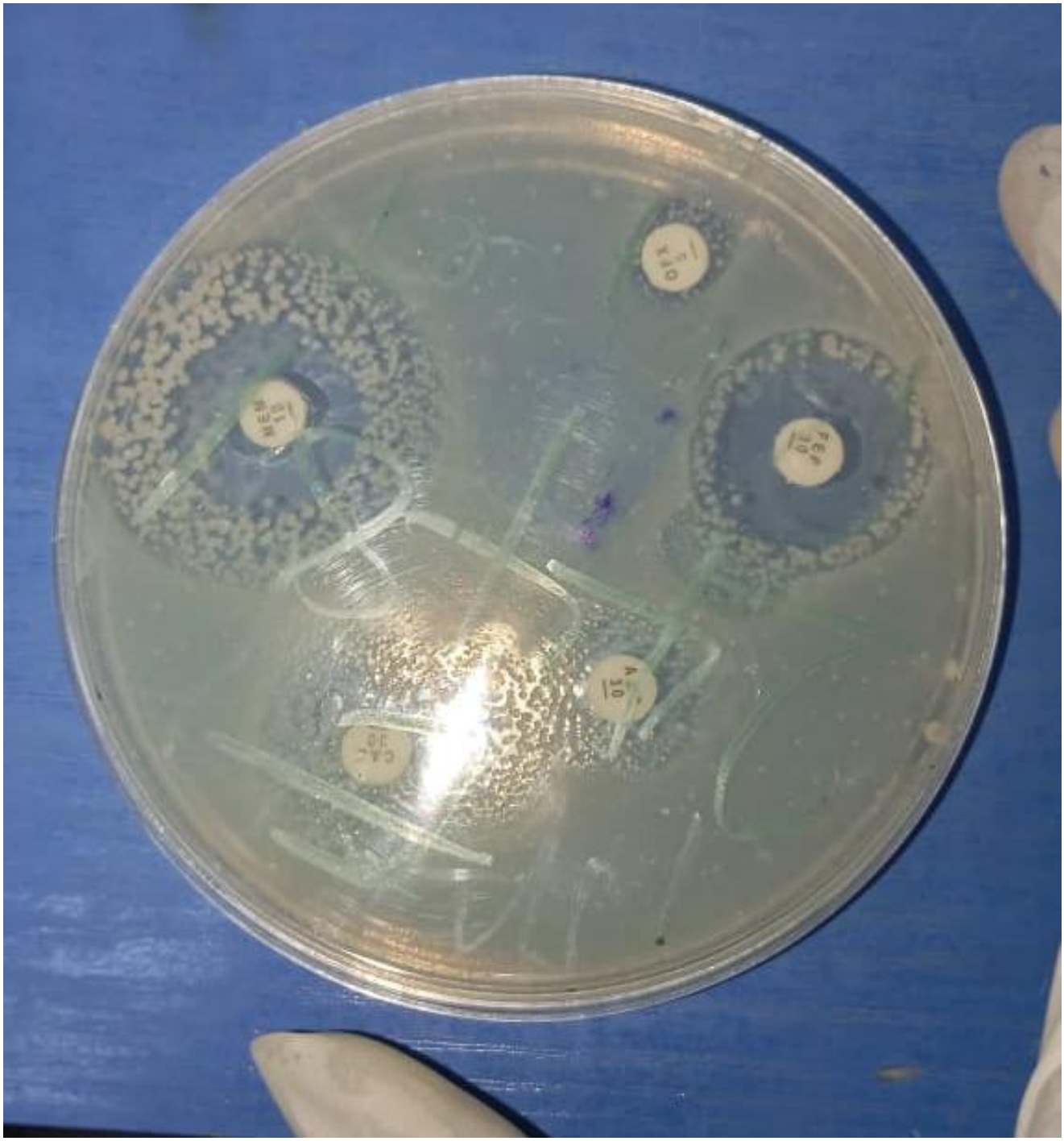
Normal saline

Equipment used

Microscope

Hot air Oven

Refrigerator



Sensitivity testing showing ESBL positive