HOSPITAL-ACQUIRED ANTIBIOTIC RESISTANT *Escherichia coli* INFECTION AMONG CHILDREN PRESENTLY IN TERTIARY HOSPITAL IN BENIN CITY.

BY

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DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

OCTOBER, 2025

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by **Eziuche Mary-Joe NWABUZO (MISS)** in the Department of Microbiology, Faculty of Life Sciences, and University of Benin, Benin City.

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APPROVAL

This project work was carried out by **NWABUZO Eziuche Mary-Joe (MISS)** in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF.E.O IGBINOSA **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far and for being my solid help, for supporting me every step of the way and loving me despite my shortcomings and to my family for their support an love throughout my period of study.

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ABSTRACT

Antimicrobial resistance (AMR) poses a significant public health threat, enabling bacterial strains to withstand common antimicrobial therapies. This study aimed to determine the prevalence and antibiotic resistance patterns of hospital-acquired *Escherichia coli* infections among children admitted to a tertiary hospital in Benin City, Nigeria. This was a cross-sectional study conducted from August to October 2025. A total of 80 swab samples were processed using standard culture-based methods. Bacterial isolates were identified via cultural characteristics, Gram staining, and standard biochemical tests (Catalase, Oxidase, Citrate, Indole, VogesProskauer, Hydrogen Sulphide, Methyl red, Urease and Sugar Fermentation). Antimicrobial susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method. The antibiotics tested included ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, amoxicillin-clavulanic acid and meropenem. The Patient demographics showed a clear difference, with the highest number recorded among male in-hospital patients (44) and the lowest among female community patients (4). Organism distribution varied by sample site, where *Escherichia coli* was the dominant isolate in rectal samples (17 isolates). Testing against a panel of 19 antibiotics revealed that most *E. coli* isolates remained susceptible. The highest resistance was observed against sulfamethoxazole-trimethoprim (7 isolates). Multidrug resistance patterns were diverse, with the combination of ciprofloxacin, piperacillin, and sulfamethoxazole (CIP, PIP, SUL) being the most frequently observed MDR profile (in 2 isolates). These findings align with previous reports from Nigerian hospitals, emphasizing the need for improved antimicrobial stewardship, routine resistance monitoring, and strict infection prevention measures to reduce hospital-acquired infections and ensure effective patient care.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Hospital-acquired infections (HAIs), also referred to as nosocomial infections, are infections that occur in patients during the course of healthcare delivery but are not present or incubating at the time of admission (Haque *et al*., 2018; Liu and Dickter, 2020). They typically manifest after 48 hours of hospitalization, within 3 days of discharge, or up to 30 days after a surgical procedure (Allegranzi *et al*., 2011). HAIs represent a serious global public health problem, contributing significantly to patient morbidity, prolonged hospital stays, increased healthcare costs, and mortality (WHO, 2017; Magill *et al*., 2018). It is estimated that over 7% of hospitalized patients in developed countries and up to 15% in low- and middle-income countries acquire at least one HAI during their hospital stay (Allegranzi and Pittet, 2008; Bagheri Nejad *et al*., 2011).

The epidemiology of HAIs is diverse, with infections commonly involving the urinary tract, surgical sites, bloodstream, and respiratory tract (Weiner-Lastinger *et al*., 2020). Among the pathogens implicated, Gram-negative bacteria such as *Escherichia coli***,** *Klebsiella pneumoniae***,** *Pseudomonas aeruginosa* and *Acinetobacter baumannii* account for a large proportion of cases, particularly in intensive care and pediatric units (Vincent *et al*., 2020). *E. coli*, a Gram-negative facultative anaerobic bacillus in the family *Enterobacteriaceae*, is of particular concern because while commensal strains are part of the normal gut flora, pathogenic variants possess virulence factors enabling them to cause extraintestinal infections such as urinary tract infections (UTIs), bloodstream infections (BSIs), pneumonia, and neonatal meningitis (Kaper *et al*., 2004; Pitout and Laupland, 2008; Zhou *et al*., 2023).

The increasing emergence of antibiotic-resistant strains of *E. coli* in healthcare environments has become a significant concern due to its impact on treatment outcomes, hospital stays, morbidity, and mortality, particularly among pediatric patients (Tansarli*et al*., 2013). Children, especially neonates and infants, are particularly vulnerable to *E. coli* infections due to their immature immune systems and frequent exposure to invasive procedures and antibiotic therapies during hospitalization (Zaidi *et al*., 2005). In Nigeria, reports have documented the occurrence of multidrug-resistant *E. coli* isolates in tertiary hospitals, raising concerns about infection control practices, rational antibiotic use, and surveillance (Iroha*et al*., 2012).

The problem of antimicrobial resistance (AMR) is further compounded in tertiary health institutions in developing countries by factors such as the lack of effective antimicrobial stewardship programs, poor sanitation, overuse and misuse of antibiotics, and inadequate diagnostic facilities (Gebretekle*et al*., 2018; Oduyebo *et al*., 2018; Kakkar*et al*., 2020). These conditions create an environment conducive to the selection and spread of resistant strains. In Benin City, Edo State, the pediatric units of tertiary hospitals often cater to critically ill children who are at higher risk of nosocomial infections due to prolonged hospitalization, use of catheters, and broad-spectrum antibiotics. Despite these risks, there is a dearth of local data on the prevalence and resistance profile of *E. coli* strains isolated from hospitalized children.

1.2 Statement of the Problem

In recent years, there has been an alarming rise in the number of hospital-acquired infections (HAIs) caused by multidrug-resistant (MDR) organisms, especially among pediatric populations in tertiary healthcare facilities. The World Health Organization (WHO) estimates that over 7–10% of hospitalized patients in developed countries and up to 15–20% in low- and middle-income

countries (LMICs) acquire at least one HAI during their hospital stay (Allegranzi *et al*., 2011; WHO, 2017). These infections account for over 4 million cases annually in Europe, resulting in nearly 37,000 deaths each year, while in the United States, HAIs affect 1 in 31 hospitalized patients daily and are responsible for approximately 99,000 deaths annually (Magill *et al*., 2018; CDC, 2020). In sub-Saharan Africa, prevalence surveys have reported HAI rates ranging from 2.5% to 14.8%, with neonatal and pediatric wards showing disproportionately higher burdens (Aiken *et al*., 2012; Abd El-Hafeez *et al*., 2017).

Escherichia coli has emerged as one of the principal culprits in HAIs, particularly in urinary tract infections (UTIs), bloodstream infections (BSIs), and neonatal sepsis. Globally, *E. coli* accounts for up to 40% of urinary tract infections and nearly 25% of bloodstream infections acquired in hospital settings (Pitout & Laupland, 2008; Logan & Weinstein, 2017). In Africa, studies have reported E. coli as the most common Gram-negative isolate from pediatric patients, with resistance rates to commonly used antibiotics exceeding 60–80% for ampicillin, 50–70% for cotrimoxazole, and 30–60% for third-generation cephalosporins (Okeke *et al*., 2007; Iroha *et al*., 2012; Tansarli *et al*., 2013).

The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains in Nigerian hospitals has complicated treatment regimens, resulting in prolonged hospitalization, higher healthcare costs, and elevated mortality rates (Aibinu *et al*., 2004; Oduyebo *et al*., 2017). For instance, prevalence rates of ESBL-producing *Enterobacteriaceae* in Nigerian tertiary hospitals have been reported between 20% and 45%, with *E. coli* accounting for the majority of isolates (Aibinu *et al*., 2004; Olonitola *et al*., 2015; Ogbolu *et al*., 2018). Alarmingly, studies from pediatric units in Nigeria have shown multidrug resistance patterns in over 70% of *E. coli* isolates,

limiting therapeutic options and necessitating the use of last-resort drugs such as carbapenems and colistin (Akinyemi *et al*., 2013; Oduyebo *et al*., 2018).

Children are particularly vulnerable to HAIs due to their underdeveloped immune systems, exposure to invasive procedures such as catheterization and intravenous cannulation, and frequent empirical antibiotic therapy (Zaidi *et al*., 2005; Kim *et al*., 2020). In Nigerian pediatric wards, rates of bloodstream infections due to MDR E. coli and Klebsiella species have been reported as high as 30–50%, with associated mortality rates reaching 20–40% in neonates (Iroha *et al*., 2012; Akinyemi *et al*., 2013).

Despite these alarming statistics, systematic studies specifically targeting hospitalized children in Benin City remain scarce. Consequently, clinicians often rely on empirical therapy without access to updated local microbiological data, leading to treatment failures, prolonged hospital stays, and increased pediatric mortality. The lack of robust surveillance systems and routine antibiotic susceptibility testing further exacerbates the problem, limiting the capacity for evidence-based interventions in infection control and antimicrobial stewardship.

This study, therefore, seeks to investigate the prevalence, antibiotic resistance pattern, and risk factors associated with hospital-acquired *E. coli* infections among children admitted to a tertiary hospital in Benin City.

1.3 Aim and Objectives of the Study

1.3.1 AIM

To determine the prevalence and antibiotic resistance patterns of hospital-acquired *Escherichia coli* infections among children admitted to a tertiary hospital in Benin City, Nigeria.

1.3.2 Specific Objectives

- To isolate and identify *E. coli* from clinical specimens obtained from hospitalized children.
- To assess the antibiotic susceptibility profiles of the *E. coli* isolates.
- To determine the prevalence of multidrug-resistant and ESBL-producing *E. coli* among the isolates.
- To identify potential risk factors associated with acquiring hospital-acquired *E. coli* infections among pediatric patients.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Hospital-Acquired Infections (HAIs)

Hospital-acquired infections (HAIs), also referred to as nosocomial infections, represent a significant challenge in healthcare settings worldwide, particularly in tertiary hospitals where complex medical interventions are common (Chakraverty and Kundu, 2025). These infections are defined as infections acquired by patients during their stay in a healthcare facility, typically manifesting 48 hours or more after admission, and are not present or incubating at the time of admission (World Health Organization [WHO], 2020). Among pediatric populations, HAIs pose a unique threat due to the vulnerability of children, whose immune systems are often less developed, and the potential for long-term health consequences. In resource-limited settings, the burden of HAIs is exacerbated by factors such as overcrowding, limited infection control resources, and high rates of antibiotic misuse, which contribute to the emergence and spread of antibiotic-resistant pathogens (Winters and Gelband, 2011; Abalkhail and Alslamah, 2022).

The epidemiological landscape of hospital-acquired infections in sub-Saharan Africa reveals remians a burden. Infection is the third leading contributor to deaths in neonates (<28 days of life) globally. In 2018, an estimated 1016000 neonatal deaths occurred in sub-Saharan Africa, 23% of which were attributed to infection (Reddy *et al*., 2021). Hospitalised neonates in resource-limited settings are especially vulnerable to infection due to overcrowding, resource constraints and suboptimal infection prevention practices (Zaidi *et al*., 2005; Couto *et al*., 2008). In low-to-middle income countries (LMIC), the overall burden of neonatal infection is 3–20 fold higher than in high-income country settings with reported rates of healthcare-associated infection in LMIC neonatal

intensive care units (ICUs) ranging from 15–62 infections per 1000 patient-days (Allegranzi *et al*., 2011). These infections are frequently caused by Gram-negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, many of which exhibit resistance to first-line antibiotics (Lester *et al*., 2020; Reddy *et al*., 2021). Factors contributing to this trend include limited laboratory capacity, inadequate infection prevention and control (IPC) practices, overcrowded hospital wards, undertrained healthcare personnel, and widespread availability of antibiotics without prescription. The lack of comprehensive surveillance systems further obscures the true burden of antibiotic-resistant HAIs in this region (Irek *et al*., 2018).

In Nigeria, the situation is particularly alarming. Several studies have highlighted the high prevalence of antibiotic-resistant *E. coli* in tertiary hospitals, especially in pediatric and neonatal units in Abuja, Borno and Kaduna respectively (regbu *et al*., 2013; Pius *et al*., 2016; Olorukooba *et al*., 2020). A study by Adeyemo *et al*. (2025) reported that over 25.9% of *E. coli* isolates from children in Nigerian hospitals showed reduced susceptibilty to third-generation cephalosporins, a key class of antibiotics used for severe infections. Other research has documented the widespread occurrence of ESBL-producing *E. coli* among pediatric patients with bloodstream and urinary tract infections (Olonitola *et al*., 2015). The unregulated use of antibiotics in hospitals and the community, along with poor diagnostic stewardship, has contributed to this pattern. In most facilities, antibiotic prescriptions are made empirically, often without laboratory confirmation, which fosters the emergence of resistant strains.

*Escherichia coli*a Gram-negative bacilli, is one of the most common pathogens associated with HAIs, particularly in pediatric settings. This bacterium is implicated in a range of infections, including urinary tract infections (UTIs), bloodstream infections, and wound infections, all of

which are prevalent in hospital environments (Allegranzi*et al*., 2011). The increasing prevalence of antibiotic-resistant strains of *E. coli*, such as those producing extended-spectrum beta-lactamases (ESBLs), has complicated treatment efforts, leading to prolonged hospital stays, increased healthcare costs, and higher morbidity and mortality rates (PitoutandLaupland, 2008). In many low- and middle-income countries, where antibiotic resistance is a growing public health crisis, the situation is particularly dire due to widespread over-the-counter antibiotic use and inadequate surveillance systems (Iheanacho and Eze *et al*., 2022).

The significance of studying hospital-acquired antibiotic-resistant *E. coli* infections in children lies in the intersection of several critical factors: the high burden of HAIs in Sub-Saharan Africa, the vulnerability of pediatric patients, and the limited resources available for infection control and treatment in many healthcare facilities. According to a systematic review by Rothe *et al*. (2013), HAIs in African hospitals have a prevalence rate ranging from 2% to 49%, with pediatric wards often reporting higher rates due to frequent invasive procedures and prolonged hospitalizations. The emergence of multidrug-resistant (MDR) *E. coli* strains further complicates clinical management, as these pathogens are resistant to commonly used antibiotics such as ampicillin, ceftriaxone, and ciprofloxacin, which are often first-line treatments in resource-constrained settings (Akinlabi *et al*., 2023).

2.2. Epidemiology of Hospital-Acquired Infections

Globally, the prevalence of HAIs in pediatric settings varies widely, influenced by healthcare system quality, infection prevention and control (IPC) measures, and socioeconomic factors. A systematic review and meta-analysis estimated the global point prevalence of HAIs at 0.14% across all patient populations, but pediatric wards, especially NICUs and PICUs, report

significantly higher rates due to the increased susceptibility of young patients (Zingg *et al*., 2017). Studies indicate that HAI prevalence in pediatric ICUs ranges from 6.1% to 29.6%, driven by the use of invasive devices such as central venous catheters, ventilators, and urinary catheters (Allegranzi *et al*., 2011). These devices are associated with bloodstream infections (BSIs), ventilator-associated pneumonia (VAP), and catheter-associated urinary tract infections (CAUTIs), which are among the most common HAIs in pediatric populations.

In high-income countries, pediatric HAI prevalence is relatively lower, averaging around 7.5%, due to robust IPC protocols, advanced diagnostics, and better resource availability (Rosenthal *et al*., 2014). In contrast, low- and middle-income countries (LMICs), particularly in Sub-Saharan Africa and South Asia, report prevalence of HAIs varied between wards with the highest rate found in the ICU (25.2%–100%), followed by neonatal ICU/ward (7.0%–53.6%) and paediatric medical ward (2.7%–33.0%).in some studies, (Abubakar *et al*., 2022). Factors contributing to these high rates include inadequate hygiene, overcrowding, understaffing, and limited access to clean water and sanitation. For example, a study in a Kenyan pediatric ward reported a 15.2% HAI prevalence, with BSIs and surgical site infections (SSIs) being predominant (Aiken *et al*., 2011).

The types of HAIs in pediatric settings vary by age group and clinical context. In neonates, sepsis and BSIs are the most common, often linked to invasive procedures like umbilical catheterization. In older children, SSIs are significant, particularly following surgical interventions, with an estimated 11% of pediatric surgical patients developing HAIs (Horan *et al*., 2008). These infections lead to extended hospital stays, increased healthcare costs, and elevated mortality rates, with pediatric HAI mortality in LMICs reported at approximately 20%, compared to 10% in high-income settings (O'Neill, 2016).

Sub-Saharan Africa (SSA) bears a disproportionate burden of HAIs due to systemic challenges such as inadequate healthcare infrastructure, limited diagnostic capacity, and suboptimal IPC practices. A systematic review estimated the pooled HAI prevalence in SSA at 12.9% (95% CI: 8.9–17.4), with significant regional variation (Irek *et al*., 2018). Central Africa reports the highest prevalence at 27% (95% CI: 0.22–0.34), followed by East Africa (19.7%), West Africa (15.5%), and Southern Africa (6.5%). These variations reflect differences in healthcare access, infection control resources, and surveillance capabilities.

In Nigeria, data on HAIs are sparse due to limited surveillance systems, but available studies indicate a significant burden, particularly in pediatric and neonatal settings. A prospective study in northern Ibadan found that diarrheagenic*E. coli* (DEC) was isolated from 65.8% of children with diarrhea and 60.8% of controls, suggesting high rates of colonization and potential nosocomial transmission (Okeke *et al*., 2000). The study also identified non-potable water sources as a risk factor for E. coli-related infections, highlighting environmental contamination as a driver of HAIs. Another Nigerian study reported an odds ratio of 50.0 (95% CI: 16.63–150.36) for pathogen isolation in diarrheal cases compared to controls, underscoring the high burden of bacterial infections in healthcare settings (Ogunsola *et al*., 1998).

E. coli is a dominant HAI pathogen in Sub-Saharan Africa (SSA), alongside *Klebsiellapneumoniae* and *Staphylococcus aureus*. In a meta-analysis, *E. coli* was the most common pathogen in the WHO South-East Asian and Eastern Mediterranean regions, with prevalence rates of 0.19 and 0.16, respectively, but it also accounts for 10–18% of HAI pathogens in SSA (Saleem *et al*., 2019). ESBL-producing *E. coli* is particularly prevalent, with Burkina Faso reporting the highest rate in

SSA at 33.37% (Olaitan *et al*., 2025). In Nigeria, the lack of routine antimicrobial susceptibility testing exacerbates the challenge, as resistant strains limit treatment options.

The types of HAIs in Sub-Saharan Africa vary by subregion and setting. In West and East Africa, wound infections and SSIs are common, while BSIs and UTIs predominate in neonatal and ICU wards. In Nigeria, neonatal sepsis is a major concern, with *E. coli* and *S. aureus* as leading causes (Okomo *et al*., 2019). Risk factors for HAIs in Sub-Saharan Africainclude recent hospitalization, invasive device use, and underlying conditions like HIV, which is prevalent in 13% of pediatric BSI cases. Mortality from HAIs in Sub-Saharan Africais high, estimated at 12.9% (95% CI: 18.9–17.4), reflecting challenges in timely diagnosis and access to effective antibiotics (Irek *et al*., 2018; Melariri *et al.*, 2024).

2.3.*Escherichia coli* as a Common Pathogen in HAIs

Escherichia coli,a gram-negative bacillus, is a leading cause of HAIs worldwide, including in pediatric populations. While *E. coli* is a commensal organism in the human gut, pathogenic strains, particularly extraintestinal pathogenic *E. coli* (ExPEC), cause a range of infections, including UTIs, BSIs, pneumonia, and neonatal meningitis. Its prominence in HAIs is due to its ability to translocate from the gut to other sites, persist in healthcare environments, and develop antimicrobial resistance, notably through extended-spectrum beta-lactamase (ESBL) production.

Globally, *E. coli* is among the most frequently isolated pathogens in HAIs, with a prevalence of 0.18% across HAI cases, surpassing pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* in certain regions (Weiner *et al*., 2016). In pediatric settings, *E. coli* is a major contributor to neonatal sepsis and BSIs, accounting for approximately 10% of culture-positive

bacteremia cases in Sub-Saharan Africa (Seale *et al*., 2014). It is also the leading cause of hospital-acquired UTIs, constituting 52.3% of invasive *E. coli* disease (IED) cases, with 38.1% of these being nosocomial (Pitout, 2012).

The pathogenicity of *E. coli* is driven by virulence factors encoded on mobile genetic elements, enabling immune evasion and antibiotic resistance. ESBL-producing *E. coli* strains, resistant to third-generation cephalosporins, are increasingly prevalent, with a meta-analysis reporting a 20.76% prevalence in Sub-Saharan Africa. In pediatric ICUs, *E. coli* is a significant cause of BSIs and UTIs, often associated with catheter use. A European study found *E. coli* to be the most common pathogen in ICU-acquired UTIs, with 22% of isolates resistant to third-generation cephalosporins (Vincent *et al*., 2009). In neonatal settings, *E. coli* is a leading cause of meningitis, with a mortality rate of up to 10% and significant neurological sequelae in survivors (Gaschignard *et al*., 2011).

The rise of multidrug-resistant (MDR) *E. coli* complicates treatment, particularly in resource-limited settings. For example, a study in Gabon reported that ESBL-producing *E. coli* strains were resistant to aminopenicillins, ceftriaxone, and other commonly used antibiotics, limiting therapeutic options (Al-Bayssari *et al*., 2015). Biofilm formation on medical devices further enhances E. coli's persistence, contributing to recurrent and difficult-to-treat infections.

2.4. Mechanisms of Antibiotic Resistance in *Escherichia coli*

Antibiotic resistance in *Escherichia coli* (*E. coli*), a gram-negative bacterium prevalent in the human gut and a common cause of infections, represents a formidable challenge in modern medicine. The bacterium's ability to evade antimicrobial agents stems from a repertoire of genetic

adaptations, which can be intrinsic (naturally present in the bacterium) or acquired through mutations or horizontal gene transfer. These adaptations enable *E. coli* to withstand antibiotics, leading to treatment failures and increased morbidity.

2.4.1Beta-Lactamase Production

Beta-lactamases are enzymes produced by *E. coli* that inactivate beta-lactam antibiotics by hydrolyzing the critical beta-lactam ring in their molecular structure, rendering the drugs ineffective. This mechanism is one of the most prevalent and clinically significant forms of resistance in *E. coli*, affecting widely used antibiotics such as penicillins, cephalosporins, and carbapenems. The diversity of beta-lactamases, which vary in their substrate specificity and genetic origin, underscores the adaptability of *E. coli* in hospital and community settings. The following are key types of beta-lactamases produced by *E. coli*:

These plasmid-mediated beta-lactamases are encoded on mobile genetic elements, allowing rapid dissemination among bacterial populations. TEM (named after a patient, Temoniera) and SHV (sulfhydryl variable) enzymes primarily target penicillins (e.g., amoxicillin, ampicillin) and early-generation cephalosporins (e.g., cefazolin). However, their clinical impact has been overshadowed by the rise of CTX-M enzymes, which are associated with extended-spectrum beta-lactamase (ESBL) production. CTX-M enzymes, named for their preferential hydrolysis of cefotaxime, confer resistance to third- and fourth-generation cephalosporins, such as ceftriaxone and cefepime. Since their emergence in the late 1980s, CTX-M enzymes have become the dominant ESBLs globally due to their efficient spread via conjugative plasmids and their ability to hydrolyze a broad range of beta-lactams. This has significantly limited treatment options for infections like urinary

tract infections (UTIs) and bloodstream infections caused by ESBL-producing *E. coli* (Cantón and Coque, 2006).

AmpC beta-lactamases are another critical resistance mechanism in *E. coli*. These enzymes can be chromosomally encoded (e.g., under the control of the *ampC* gene) or plasmid-mediated, with the latter being increasingly common due to horizontal gene transfer. AmpC enzymes hydrolyze a wide range of cephalosporins, including cephamycins (e.g., cefoxitin), and are resistant to beta-lactamase inhibitors like clavulanic acid, which are often used to counter TEM and SHV enzymes. Overexpression of chromosomal AmpC, often triggered by mutations in regulatory genes, or acquisition of plasmid-borne *ampC* genes (e.g., CMY-2), enhances resistance to cephalosporins, complicating therapy for hospital-acquired infections. AmpC-producing *E. coli* strains are particularly problematic in intensive care units (ICUs), where cephalosporins are frequently used (Jacoby, 2009).

Carbapenemases represent a dire threat, as they hydrolyze carbapenems (e.g., imipenem, meropenem), which are often reserved as last-resort antibiotics for multidrug-resistant infections. In *E. coli*, carbapenemases such as KPC (*Klebsiellapneumoniae*carbapenemase), NDM (New Delhi metallo-beta-lactamase), and OXA-48 (oxacillinase-48) are typically plasmid-encoded, facilitating their spread across bacterial species. These enzymes not only inactivate carbapenems but also often confer resistance to other beta-lactams, contributing to multidrug-resistant (MDR) or extensively drug-resistant (XDR) phenotypes. The global rise of carbapenem-resistant *E. coli* (CRE) has been particularly alarming in regions with high antibiotic consumption, such as South Asia and the Middle East, where NDM-producing strains are prevalent (Nordmann *et al*., 2011).

2.4.2. Efflux Pumps

Efflux pumps are membrane-bound protein complexes that actively extrude antibiotics from the bacterial cell, reducing intracellular drug concentrations to sublethal levels. In *E. coli*, the AcrAB-TolC efflux system, part of the resistance-nodulation-division (RND) family, is the most significant contributor to this mechanism. This tripartite system, comprising an inner membrane transporter (AcrB), a periplasmic linker protein (AcrA), and an outer membrane channel (TolC), efficiently expels a wide range of antibiotics, including tetracyclines, fluoroquinolones, chloramphenicol, and certain beta-lactams. The broad substrate specificity of AcrAB-TolC makes it a key player in multidrug resistance. Overexpression of this efflux system, often due to mutations in regulatory genes like *marR* or *soxS*, amplifies resistance, particularly when combined with other mechanisms such as beta-lactamase production. For instance, efflux-mediated expulsion of fluoroquinolones can complement target site mutations, leading to high-level resistance. The clinical significance of efflux pumps lies in their ability to confer low-level resistance that may select for further mutations, driving the evolution of MDR *E. coli* strains in hospital environments (Piddock, 2006).

2.4.3. Alteration of Antibiotic Targets

E. coli can evade antibiotics by modifying the molecular targets that these drugs bind to, thereby preventing effective interaction. This mechanism is particularly relevant for beta-lactam and fluoroquinolone antibiotics, where specific targ*et al*terations reduce drug efficacy.

Beta-lactam antibiotics exert their bactericidal effects by binding to penicillin-binding proteins, which are essential for cell wall synthesis. Mutations in the genes encoding PBPs, such as *pbp1a*

or *pbp1b*, can alter their structure, reducing the affinity of beta-lactams like penicillins and cephalosporins. This mechanism is less common than beta-lactamase production but contributes significantly to resistance when combined with other mechanisms, such as reduced permeability or efflux. Altered PBPs are particularly relevant in *E. coli* strains resistant to mecillinam, an antibiotic used for UTIs (Bush and Bradford, 2020).

Fluoroquinolones target DNA gyrase and topoisomerase IV, enzymes critical for DNA replication and repair. Mutations in the *gyrA* and *parC* genes, which encode subunits of these enzymes, alter their binding sites, preventing fluoroquinolones like ciprofloxacin and levofloxacin from inhibiting DNA synthesis. These mutations, often located in the quinolone resistance-determining regions (QRDRs), are a primary cause of fluoroquinolone resistance in *E. coli*. The stepwise accumulation of mutations can lead to high-level resistance, particularly in hospital-acquired infections where fluoroquinolones are heavily used (Hooper and Jacoby, 2015).

2.4.4. Reduced Permeability

The outer membrane of *E. coli*, a hallmark of gram-negative bacteria, acts as a barrier to antibiotic entry. Porins, such as OmpF and OmpC, are protein channels that allow passive diffusion of hydrophilic antibiotics, including beta-lactams and aminoglycosides, into the cell. *E. coli* can reduce antibiotic uptake by downregulating porin expression or acquiring mutations that alter porin structure. For example, loss of OmpF expression, often due to regulatory mutations, limits the entry of cephalosporins, while altered porin channels can reduce aminoglycoside uptake. This mechanism is particularly effective when combined with beta-lactamase production or efflux pumps, as it further decreases intracellular antibiotic concentrations. Reduced permeability is a

significant contributor to resistance in hospital-acquired *E. coli* infections, where prolonged antibiotic exposure selects for such adaptations (Nikaido, 2003).

2.4.5. Biofilm Formation

Biofilms are structured communities of bacteria encased in a self-produced extracellular matrix, often adhering to surfaces like medical devices or mucosal tissues. *E. coli* forms biofilms in infections such as catheter-associated UTIs, where the matrix acts as a physical barrier, impeding antibiotic penetration. Within biofilms, *E. coli* cells exhibit reduced metabolic activity, rendering them less susceptible to antibiotics that target actively dividing cells, such as beta-lactams. Additionally, biofilms foster microenvironments where resistant subpopulations can emerge due to nutrient gradients and stress responses. The persistence of biofilms complicates treatment, as antibiotics may fail to reach effective concentrations, and resistant *E. coli* strains can disseminate from biofilms to cause recurrent infections. This mechanism is particularly problematic in hospital settings, where indwelling devices are common (Costerton *et al*., 1999).

2.4.6. Horizontal Gene Transfer

Horizontal gene transfer (HGT) is a cornerstone of antibiotic resistance dissemination in *E. coli*, enabling the rapid spread of resistance genes within and across bacterial species. *E. coli*'s ability to acquire resistance genes through mobile genetic elements significantly enhances its adaptability. Conjugation involves the transfer of plasmids carrying resistance genes, such as *bla*CTX-M for ESBLs or *bla*NDM for carbapenemases, between bacteria via direct cell-to-cell contact. Plasmids are highly efficient vehicles for resistance dissemination, as they often carry multiple resistance genes, conferring MDR phenotypes in a single transfer event. Conjugation is a major driver of the

global spread of ESBL- and carbapenemase-producing *E. coli*, particularly in hospital settings where selective pressure from antibiotics is high (Carattoli, 2013).

Transformation involves the uptake of free DNA from the environment, while transduction occurs via bacteriophages that transfer resistance genes. Although less common than conjugation in *E. coli*, these mechanisms contribute to resistance gene dissemination, particularly in environmental reservoirs or mixed bacterial communities. For example, transformation may introduce resistance genes into *E. coli* in settings like wastewater treatment plants, while transduction can spread genes like *bla*TEM via phages. These processes amplify the genetic diversity of resistant *E. coli* populations (von Wintersdorff *et al*., 2016).

2.5. Risk Factors for Hospital-Acquired *E. coli* Infections in Children

Hospital-acquired (nosocomial) *Escherichia coli* (*E. coli*) infections pose significant challenges in pediatric healthcare settings, contributing to increased morbidity, prolonged hospital stays, and higher healthcare costs. Understanding the risk factors associated with these infections is critical for developing effective prevention strategies. This article explores the multifaceted risk factors for hospital-acquired *E. coli* infections in children, categorized into patient-related, hospital-related, and community/environmental factors, with a focus on their implications and interplay.

2.5.1. Patient-Related Factors

Patient-related factors play a significant role in predisposing children to hospital-acquired *E. coli* infections. These factors are often intrinsic to the patient's health status and can amplify susceptibility to infections.

2.5.1.1. Age

Young children, particularly neonates and infants under one year, are at higher risk due to their immature immune systems. Neonates, especially preterm infants, have underdeveloped physical barriers (e.g., skin and mucosal linings) and limited immune responses, making them more susceptible to invasive *E. coli* infections such as urinary tract infections (UTIs), bloodstream infections, and meningitis. Studies indicate that *E. coli* is a leading cause of neonatal sepsis, with preterm infants being particularly vulnerable due to lower levels of maternal antibodies transferred in utero (Stoll *et al*., 2020; Zhu *et al*., 2023).

2.5.1.2. Immune Status

Immunocompromised children, including those with congenital immunodeficiencies, HIV/AIDS, or those undergoing immunosuppressive therapies (e.g., chemotherapy for cancer), are at elevated risk. A compromised immune system impairs the body's ability to combat *E. coli* colonization and infection. For instance, children with leukemia or those receiving corticosteroids have reduced neutrophil function, increasing susceptibility to *E. coli* bacteremia (Zaoutis*et al*., 2005). Malnutrition, common in pediatric populations in resource-limited settings, further exacerbates immune dysfunction, heightening infection risk.

2.5.1.3. Underlying Conditions

Chronic illnesses such as diabetes mellitus, congenital urogenital anomalies, and chronic kidney disease significantly increase the likelihood of *E. coli* infections. For example, children with vesicoureteral reflux or neurogenic bladder are prone to recurrent UTIs caused by *E. coli* due to impaired urinary flow and bladder emptying. Similarly, children with gastrointestinal conditions,

such as short bowel syndrome or inflammatory bowel disease, may experience altered gut microbiota, facilitating *E. coli* colonization and translocation into the bloodstream (Dudley *et al*., 2018). Additionally, children with indwelling medical devices (e.g., central venous catheters or ventilators) are at higher risk due to the potential for biofilm formation, which *E. coli* can exploit.

2.5.2. Hospital-Related Factors

Hospital-related factors are critical drivers of *E. coli* infections, often linked to the healthcare environment, medical interventions, and infection control practices.

2.5.2.1. Prolonged Hospitalization

Extended hospital stays increase the risk of nosocomial *E. coli* infections due to prolonged exposure to healthcare-associated pathogens. Children admitted to intensive care units (ICUs), particularly neonatal or pediatric ICUs, are at heightened risk because of the high prevalence of invasive procedures and multidrug-resistant organisms (MDROs) in these settings. Research shows that hospital stays exceeding 7 days are associated with a significantly higher incidence of *E. coli* infections, particularly in critically ill children (Logan *et al*., 2017).

2.5.2.2. Invasive Procedures

Invasive procedures, such as surgery, mechanical ventilation, or endoscopic interventions, disrupt natural barriers and provide entry points for *E. coli*. For example, abdominal surgeries can lead to peritoneal contamination by *E. coli* from the gastrointestinal tract, while endotracheal intubation may facilitate *E. coli* colonization in the respiratory tract. The risk is compounded in children

undergoing multiple procedures, as each intervention increases opportunities for pathogen introduction (Bereket *et al*., 2012).

2.5.2.3. Catheter Use

Indwelling catheters, particularly urinary catheters and central venous catheters, are among the most significant hospital-related risk factors for *E. coli* infections. Urinary catheters disrupt the natural flushing mechanism of the bladder, allowing *E. coli* to adhere to the catheter surface and form biofilms, which are resistant to antibiotics and host defenses. Catheter-associated urinary tract infections (CAUTIs) caused by *E. coli* are common in pediatric settings, with studies reporting that 70–80% of CAUTIs are attributable to *E. coli* (Flores-Mireles *et al*., 2015). Similarly, central line-associated bloodstream infections (CLABSIs) are frequently caused by *E. coli* in children with prolonged catheter use, particularly in oncology and ICU settings.

2.5.2.4. Inadequate Infection Control Practices

Poor adherence to infection control protocols, such as hand hygiene, sterilization of equipment, and environmental cleaning, significantly contributes to *E. coli* transmission in hospitals. Contaminated surfaces, medical devices, and healthcare workers' hands can serve as reservoirs for *E. coli*. In pediatric wards, where children may have frequent contact with caregivers and toys, the risk of cross-contamination is particularly high if infection control measures are suboptimal (Rutalaand Weber, 2013).

2.5.3. Community and Environmental Factors Contributing to Resistance Spread

The emergence and spread of antibiotic-resistant *E. coli* strains, particularly extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant strains, are influenced by community and environmental factors. These factors contribute to the reservoir of resistant *E. coli* that children may carry into hospitals, increasing the risk of nosocomial infections.

2.5.3.1. Antibiotic Overuse and Misuse

Widespread use of antibiotics in community settings, including inappropriate prescriptions for viral infections and over-the-counter antibiotic access in some regions, drives the selection of resistant *E. coli* strains. Children exposed to antibiotics in the community (e.g., for respiratory or gastrointestinal infections) are more likely to carry resistant *E. coli* in their gut, which can be transmitted in healthcare settings. A study by Bryce *et al*. (2016) found that prior antibiotic exposure in the community was a significant risk factor for colonization with ESBL-producing *E. coli* in hospitalized children.

2.5.3.2. Environmental Contamination

Environmental reservoirs, such as contaminated water, food, or soil, play a role in the spread of resistant *E. coli*. In low-resource settings, inadequate sanitation and unsafe drinking water facilitate the transmission of *E. coli* between humans, animals, and the environment. For example, agricultural runoff containing antibiotic residues from livestock farming can contaminate water sources, promoting the proliferation of resistant *E. coli* strains. Children living in such environments may become colonized with resistant strains before hospitalization, increasing the risk of nosocomial infections (Collignon *et al*., 2016).

2.5.3.3. Community Transmission

Close-knit community settings, such as daycare centers and schools, facilitate the spread of *E. coli* through person-to-person contact or shared surfaces. Children in daycare are particularly susceptible to gastrointestinal *E. coli* infections due to frequent diaper changes, inadequate handwashing, and close physical contact. These community-acquired strains can be introduced into hospitals, where they may spread among vulnerable patients. Molecular studies have shown that ESBL-producing *E. coli* strains in hospitals often originate from community reservoirs (Pitout and Laupland, 2008).

2.6 Clinical Implications of Antibiotic-Resistant *E. coli* Infections

Antibiotic-resistant *Escherichia coli* infections significantly impact clinical outcomes, particularly in pediatric patients, where they contribute to increased morbidity and mortality. In children, these infections, often presenting as severe urinary tract infections, sepsis, or gastroenteritis, are complicated by resistant strains, leading to prolonged illness and higher hospitalization rates. Multidrug-resistant (MDR) *E. coli* increases the risk of adverse outcomes, especially in immunocompromised children or neonates with immature immune systems, where mortality rates can rise due to delayed or ineffective treatment (World Health Organization, 2020). The limited availability of safe, effective antibiotics for pediatric use further exacerbates these challenges, often requiring last-resort drugs with potential toxicity, which can compromise recovery and long-term health.

The treatment of MDR *E. coli* infections is hindered by the bacteria's resistance to multiple antibiotic classes, including beta-lactams, fluoroquinolones, and aminoglycosides. Clinicians face

significant obstacles in identifying effective therapies, as resistance profiles necessitate advanced diagnostics like susceptibility testing, which may not be universally accessible (Medina and Pieper, 2016). This often leads to treatment delays, increasing the risk of complications such as renal damage or systemic infection. The rise of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant *E. coli* strains further limits therapeutic options, forcing reliance on drugs like colistin, which carry significant risks of nephrotoxicity and neurotoxicity, particularly in children (Tacconelli *et al*., 2018). Inadequate infection control and overuse of antibiotics continue to drive resistance, complicating clinical management.

The economic burden of antibiotic-resistant *E. coli* infections on tertiary healthcare systems is considerable. Prolonged hospital stays, intensive care requirements, and the need for costly diagnostics and alternative therapies significantly inflate healthcare costs. In tertiary hospitals, managing MDR *E. coli* often involves broad-spectrum antibiotics, frequent imaging, and specialized interventions, all of which strain financial resources (Zowawi *et al*., 2015). Infection control measures, such as isolation protocols to curb nosocomial spread, further increase costs by requiring dedicated staff and equipment. Treatment failures leading to readmissions and chronic complications add to the economic load, underscoring the need for robust antibiotic stewardship and innovative treatments to alleviate the financial strain on healthcare systems (Cassini *et al*., 2019).

2.7 Diagnostic Methods for AMR Detection

The accurate detection of antimicrobial resistance (AMR) is vital for ensuring effective treatment, monitoring resistance trends, and controlling the spread of resistant organisms. Diagnostic methods used for AMR detection are typically categorized into phenotypic and molecular

approaches. Each method provides different insights and has varying applications based on laboratory resources, time constraints, and clinical urgency. However, despite the advancements in technology, several challenges remain in the practical implementation and interpretation of these diagnostic tools.

2.7.1. Phenotypic Methods

Phenotypic methods remain the traditional and widely adopted approach for detecting antimicrobial resistance, particularly in routine clinical microbiology laboratories. These methods focus on observing the actual growth behavior of microorganisms in the presence of antimicrobial agents. One of the most common phenotypic techniques is the disk diffusion method, also known as the Kirby-Bauer test. In this method, antibiotic-impregnated paper disks are placed on an agar surface that has been inoculated with the test organism. After incubation, the zones of inhibition around the disks are measured. The size of these zones helps to determine whether the organism is susceptible, intermediate, or resistant to the antibiotic being tested, using guidelines such as those provided by the Clinical and Laboratory Standards Institute (CLSI, 2023).

Another important phenotypic method is the broth dilution technique, which can be performed using either macro or microdilution formats. This method helps in determining the Minimum Inhibitory Concentration (MIC), defined as the lowest concentration of an antimicrobial agent that completely inhibits visible bacterial growth. The MIC offers a quantitative assessment of the level of resistance, which is particularly useful for tailoring antibiotic therapy. For more refined MIC determination, the Epsilometer test, commonly referred to as the E-test, combines the concepts of disk diffusion and dilution. It employs a plastic strip embedded with a gradient of antibiotic

concentrations. The intersection point where bacterial growth meets the strip indicates the MIC, making it a useful tool in both clinical and research settings (Matuschek *et al*., 2014).

In recent years, several automated systems such as VITEK 2, BD Phoenix, and MicroScanWalkAway have been developed to streamline phenotypic susceptibility testing. These systems not only identify bacterial species but also rapidly determine their susceptibility patterns, thereby enhancing clinical decision-making. Although these automated systems improve efficiency and standardization, they are expensive and require regular maintenance, which may limit their use in low-resource settings. Phenotypic methods have the advantage of detecting resistance regardless of the genetic basis. However, they often require prolonged incubation periods, typically 18–24 hours, and may fail to detect resistance in cases where resistance genes are present but not phenotypically expressed under test conditions.

2.7.2. Molecular Methods

Molecular methods have revolutionized the field of AMR diagnostics by enabling the rapid and specific detection of resistance genes. Unlike phenotypic methods that rely on bacterial growth, molecular techniques detect the presence of specific genetic sequences that confer resistance, thereby reducing the time required for diagnosis. One of the most widely used molecular methods is the Polymerase Chain Reaction (PCR), which can amplify DNA sequences associated with resistance determinants such as *mecA* in methicillin-resistant *Staphylococcus aureus* (MRSA), *blaCTX-M* in extended-spectrum beta-lactamase (ESBL)-producing organisms, and *vanA/vanB* in vancomycin-resistant enterococci (Tenover, 2006). Real-time PCR, an advancement of the

conventional PCR method, enables the detection and quantification of target genes in real time, offering faster results with greater sensitivity and specificity.

Multiplex PCR is another valuable tool in AMR detection. This method allows the simultaneous amplification of multiple gene targets in a single reaction, which is particularly useful in clinical settings where time and resources are limited. For example, in the diagnosis of multidrug-resistant pathogens, multiplex PCR can detect genes responsible for resistance to beta-lactams, aminoglycosides, and fluoroquinolones in a single assay. In addition to PCR-based methods, DNA microarrays and line probe assays are employed for broader screening of multiple resistance genes. These platforms allow hybridization of labeled nucleic acid sequences to complementary probes affixed on a solid surface, enabling the detection of a wide array of resistance determinants. Though highly informative, these technologies require significant expertise and infrastructure, which may restrict their use to reference laboratories.

Whole Genome Sequencing (WGS) represents the most comprehensive molecular method for AMR detection. It provides complete information about all resistance genes, plasmids, and mutations within the microbial genome. WGS is increasingly being used for epidemiological surveillance, outbreak investigations, and tracking the evolution of resistance. Despite its immense potential, the routine use of WGS in clinical diagnostics is still limited due to its high cost, long turnaround time, and the need for specialized bioinformatics tools and personnel (Ellington *et al*., 2017).

An emerging and promising method is Loop-Mediated Isothermal Amplification (LAMP). This technique amplifies DNA under constant temperature conditions, making it faster and more adaptable to point-of-care settings than PCR. LAMP is particularly advantageous in low-resource

environments, as it requires minimal instrumentation and provides results in less than an hour (Garg, *et al*., 2022). While molecular methods offer speed and specificity, they also have notable limitations. For instance, they can only detect known resistance genes and may miss novel or uncommon resistance mechanisms. Furthermore, the presence of a resistance gene does not always correlate with phenotypic resistance, especially when gene expression is suppressed or regulated by environmental factors.

2.8. Management and Control Strategies

Controlling antimicrobial resistance (AMR) in pediatric care requires targeted interventions that limit both the misuse of antibiotics and the spread of resistant pathogens. A key approach is antibiotic stewardship**,** which promotes appropriate antibiotic use—ensuring correct dosing, duration, and indication. Pediatric antimicrobial stewardship programs (ASPs) often incorporate prescriber education, prescription audits, and evidence-based treatment guidelines, helping to reduce unnecessary use, especially for viral infections (Versporten *et al*., 2016).

Infection prevention and control (IPC) measures are also essential. These include strict hand hygiene, proper sterilization of equipment, patient isolation when necessary, and routine environmental cleaning. Such protocols are especially critical in neonatal and pediatric intensive care units where vulnerability to infection is high. Vaccination further contributes to reducing infections and the need for antibiotics by preventing diseases caused by *Haemophilusinfluenzae*, *Streptococcus pneumoniae*, and similar pathogens (Allegranzi *et al*., 2017). In addition, AMR surveillance systems support control efforts by monitoring resistance trends and guiding empirical therapy. However, in many low-resource settings, surveillance remains underdeveloped.

Strengthening these systems and enforcing policies against inappropriate prescriptions and over-the-counter antibiotic sales are vital to curbing AMR in children (WHO, 2020).

Preventing AMR in pediatric populations focuses on reducing infection rates and limiting antibiotic misuse. Basic public health practices—such as regular handwashing, proper sanitation, and safe food handling can significantly lower infection risks at home, in schools, and in healthcare settings. Parental and caregivereducation is crucial in preventing irrational antibiotic use. Awareness campaigns targeting misconceptions about antibiotics and the dangers of self-medication can discourage unnecessary prescriptions and encourage adherence to prescribed treatment. Advances in diagnostic technology also aid prevention. Rapid diagnostic tests enable clinicians to differentiate bacterial from viral infections, minimizing inappropriate antibiotic use. Additionally, ongoing research into vaccines, novel antimicrobials, and alternatives such as

probiotics or phage therapy presents promising tools for reducing dependence on traditional antibiotics (Manges *et al*., 2015). Together, control and prevention strategies form a comprehensive approach to tackling AMR, especially in vulnerable pediatric populations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This study was designed as a descriptive cross-sectional, hospital-based investigation focusing on the antimicrobial resistance (AMR) patterns of *Escherichia coli* isolated from urinary tract infections (UTIs) among hospitalized paediatric patients. This research employed a point prevalence survey (PPS) approach for participant recruitment and data/sample collection, allowing for systematic assessment of AMR profiles at a defined point in time. The methodology integrated clinical observations, laboratory diagnostics, and microbiological analyses to determine the susceptibility of *E. coli* isolates to routinely used antimicrobial agents, as well as to characterize multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) strains.

3.2 Study Area

This study was conducted in Edo State, located in the South-South geopolitical zone of Nigeria. With an estimated population of 5 million (as of 2024), the state comprises 18 Local Government Areas (LGAs), with Benin City as its capital and largest urban centre.The healthcare system in Edo State is coordinated across three tiers of government federal (tertiary hospitals), state (general hospitals), and local (primary healthcare centres) alongside active private sector participation. The tertiary hospital selected for this study represents a high-level referral centre with advanced microbiology laboratory capabilities.

3.3 Study Population

The study targeted hospitalized children under 18 years of age with a clinical suspicion of bacterial infection, for whom a new antibiotic therapy had been commenced during hospitalization. The study specifically excluded patients already on antibiotics prior to admission, those attending antiretroviral clinics, or those hospitalized for less than 16 hours before recruitment.

3.3.1 Inclusion Criteria

- Patients aged less than 18 years
- Willingness of caregivers to provide informed consent
- Assent from children aged 10–17 years
- Clinical suspicion of a new bacterial infection and initiation of a new antibiotic
- Infection onset on or after the third day of hospital admission
- Infection not present or active during the previous point prevalence survey

3.3.2 Exclusion Criteria

- Antibiotic therapy prior to admission
- Hospitalization less than 16 hours before recruitment
- Enrollment in highly active antiretroviral therapy (HAART) clinics

3.4. Sample size and sampling

The sample size for the proposed study is estimated based on the formula proposed by Kirkwood and Sterne, (2010).

$$n = \frac{3.84\pi(1-\pi)}{w^2}$$

Where:

- n=required minimum sample size
- π=proportion of AMR in children= 0.5
- w=precision of estimate (i.e. confidence interval).

Substituting these values gives:

$$n = \frac{3.84 \times 0.5 \times 0.5}{0.0025} = 384$$

This yielded a minimum sample size of 384**.** Adjusting for a 15% non-response rate**,** the final sample size was 455 pediatric patients. Participants were selected via simple random sampling from the list of eligible patients using a computer-generated random number sequence.

3.5 Sterilization of Materials

All materials used for sample collection and processing were sterilized to prevent contamination. Sterile cotton wool swabs, universal containers, and pipettes were autoclaved at 121°C for 15 minutes at 15 psi. Inoculating loops were sterilized by flaming over a Bunsen burner until red-hot and cooled before use. Laboratory work surfaces were disinfected with 70% ethanol before and after each procedure. Study staff wore sterile gloves and personal protective equipment during sample collection to maintain asepsis.

3.6 Preparation of Agar

Agar media were used prepared according to manufacturer instructions and autoclaved to ensure sterility. The following media were used:

- Blood Agar (BA)
- Cystine Lactose Electrolyte Deficient (CLED) Agar, Simon's Citrate Agar and Mueller-Hinton Agar (MHA)

All prepared media were stored at 4°C and used within one week. Uninoculated plates were incubated to confirm sterility as part of quality control.

3.7 Data and Sample Collection

3.7.1 Data Collection

Prior to sample and data collection, informed consent was obtained from caregivers, while children between 10–17 years gave written assent**.** Participants were informed verbally and through a written information sheet. Trained hospital-based data collectors administered structured questionnaires adapted and pre-tested using the ODK App, collected relevant clinical data from medical records, and ensured standard physical examination and sample collection procedures.

3.7.2 Sample Collection

3.7.2.1. Urine samples

Urine samples were collected and cultured on the same day from children clinically suspected of having a urinary tract infection (UTI). For toilet-trained children, urine was obtained using the clean-catch mid-stream method to reduce contamination. In children who were not toilet-trained, samples were collected using either the clean-catch voided method or catheterisation, as clinically appropriate. Each urine specimen was collected into a sterile, screw-capped universal container, carefully labelled with the participant's identification details, and promptly transported to the laboratory for immediate processing. On arrival at the laboratory, the urine samples were first subjected to gross and microscopic examinations, Subsequently, approximately 10 µL of each urine sample was inoculated onto Cystine Lactose Electrolyte Deficient (CLED) agar and Blood

Agar (BA) plates containing 5–10% defibrinated blood. These culture media support the growth of urinary pathogens and facilitate the differentiation of lactose fermenters. The inoculated plates were incubated aerobically at 37°C for 24 to 48 hours. After incubation, bacterial growth was evaluated. Isolates were subjected to standard bacteriological identification procedures, including morphological and biochemical characterization. A colony count of $\geq 10^5$ colony-forming units per millilitre (CFU/mL) was interpreted as significant bacteriuria and considered diagnostic of a urinary tract infection.

3.8 Bacterial Strain Identification

Bacterial isolates from nasal and rectal swabs were identified using conventional laboratory techniques, including colonial morphology, Gram staining, and biochemical tests. The following biochemical tests were performed:

3.8.1 Gram Staining

Gram staining was performed to differentiate Gram-positive and Gram-negative bacteria. A thin smear of the bacterial colony was prepared on a clean glass slide, heat-fixed, and stained with crystal violet for 1 minute. The slide was rinsed with water, treated with Gram's iodine for 1 minute, decolorized with 95% ethanol for 10–20 seconds, and counterstained with safranin for 1 minute. Slides were examined under a microscope at 100x magnification with oil immersion. *S. aureus* appeared as Gram-positive cocci in clusters, while *E. coli* appeared as Gram-negative rods.

3.8.2 Biochemical Test

3.8.2.1. Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

3.8.2.2. Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.8.2.3. Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 - 48 hr. The development of deep blue colour after incubation indicates a positive result.

3.8.2.4. Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is performed to help differentiate species of the family enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethyl-aminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at

37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.8.2.5. VogesProskauerTest

This test is used to differentiate *Bacillus* sp. and enteric bacteria which ferment glucose with the production of acetoin which can be detected by oxidation reaction. 2 ml of sterile Methyl red-Voges Proskauer broth was inoculated with test organism and incubated at 37 ^{0} C for 24 hours. A small amount of 10 % alpha-naphthol was added and then mixed. About 3 ml KOH was added and shaken. The set up was then left for an hour at room temperature .A pink to red colour indicated a positive.

3.8.2.6. Hydrogen Sulphide (H_2S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}) or lead (Pb^{2+}) ion as H_2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulphate) forming a visible insoluble black sulphide precipitate.

3.8.2.7. Methyl Red Test

The Methyl Red (MR) test is used to check for the production of stable acids during glucose fermentation, particularly in coliform organisms that ferment dextrose rapidly, leading to a significant drop in pH. To perform the test, Methyl Red-Voges Proskauer (MR-VP) broth was prepared, and 10 ml of the broth was dispensed into sterile test tubes. The tubes were inoculated

with bacterial isolates and incubated at 30°C for 24 hours. After incubation, a few drops of methyl red indicator were added to each culture. The appearance of a distinct red colour indicated a positive MR reaction, signifying acid production.

3.8.2.8. Urease Test

The urease test is employed to determine the capability of an organism to hydrolyze urea into ammonia and carbon dioxide in the presence of the enzyme urease. Bacterial isolates were inoculated onto slants of sterile urea medium and incubated at 37°C for 24 to 48 hours. A positive urease reaction was observed as a red-pink colour change in the medium, resulting from the increase in pH caused by ammonia production, which altered the colour of the indicator present in the medium.

3.8.2.9. Sugar Fermentation Test

The sugar fermentation test was conducted to assess the ability of each bacterial isolate to ferment specific carbohydrates, resulting in the production of acid and/or gas. This biochemical test plays a critical role in differentiating bacterial species, especially among Gram-negative bacteria, which commonly utilize various sugars as sources of carbon and energy. Peptone water was used as the base medium for this test. It was measured into a conical flask, and bromocresol purple was added as a pH indicator. The medium was then distributed into test tubes, each containing an inverted Durham tube to capture any gas produced during fermentation. These tubes were sterilized in an autoclave at 121°C for 15 minutes. A 1% sugar solution (e.g., glucose, lactose, or sucrose) was prepared separately and sterilized at 115°C for 10 minutes. After cooling, 5 ml of the sterile sugar solution was aseptically added to each test tube containing the indicator medium. The tubes were

then inoculated with young bacterial cultures and incubated at 37°C for 24 hours. Following incubation, fermentation was determined by observing the color change in the medium and the presence of gas. A shift in color from light green to yellow signified acid production, while the appearance of gas bubbles in the Durham tube confirmed gas production.

3.10. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the bacterial isolates was conducted using the standardized Kirby-Bauer disk diffusion method, as outlined by the Clinical and Laboratory Standards Institute (CLSI, 2023). Fresh colonies of *Escherichia coli* obtained from pure cultures were suspended in sterile normal saline to prepare a bacterial suspension. The turbidity of the suspension was carefully adjusted to match the 0.5 McFarland standard, which approximates a bacterial density of 1.5×10^8 colony-forming units per millilitre (CFU/mL). The adjusted inoculum was then uniformly spread over the surface of sterile Mueller-Hinton Agar (MHA) plates using sterile cotton swabs, ensuring even distribution of the inoculum in three different directions to create a bacterial lawn.

After allowing the plates to stand at room temperature for 3 to 5 minutes to absorb excess moisture, commercially prepared antibiotic-impregnated discs were carefully placed on the surface of the inoculated agar using sterile forceps. The antibiotics tested included ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (20/10 µg) and meropenem (10 µg). Screening for extended-spectrum beta-lactamase (ESBL) production in *E. coli* was performed using the ceftazidime disc. A reduced zone of inhibition around the ceftazidime disc (≤22 mm) was considered suggestive of ESBL production.

All inoculated plates were incubated in an aerobic incubator at a temperature of 35–37°C for 18–24 hours. Following incubation, the zones of inhibition—clear areas surrounding the antibiotic discs—were measured in millimetres using a transparent ruler or vernier caliper. The results were interpreted as susceptible, intermediate, or resistant based on the zone diameter breakpoints provided by the CLSI (2023) guidelines for each organism and antibiotic tested.

3.11. Determination of Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pandrug-Resistant (PDR) Strains

To evaluate the extent of antimicrobial resistance among the isolates, each bacterium was assessed and categorized according to international criteria established by Magiorakos *et al*. (2012). This classification enabled the stratification of isolates into distinct resistance categories: multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR). An isolate was considered multidrug-resistant (MDR) if it demonstrated non-susceptibility to at least one agent in three or more different antimicrobial categories. This indicates resistance to a broad range of treatment options and is of significant clinical concern due to the limited therapeutic alternatives.

Isolates classified as extensively drug-resistant (XDR) were those that exhibited non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, thereby retaining effectiveness against only one or two antibiotic classes. Such isolates are typically difficult to treat and often require the use of last-resort or combination therapies. Finally, pandrug-resistant (PDR) strains were defined as those showing non-susceptibility to all tested antibiotics across all antimicrobial categories. To determine the appropriate classification, the antibiotics used in susceptibility testing were grouped into their respective classes, such as beta-lactams, aminoglycosides, fluoroquinolones, macrolides, tetracyclines, glycopeptides, and carbapenems. Each isolate's

susceptibility profile was carefully analyzed against these categories to identify its resistance classification.

3.12. Ethical Considerations

This study received ethical approval from the Institutional Health Research Ethics Committee of the University of Benin. All ethical principles consistent with the Helsinki Declaration and National Data Protection Regulation were adhered to. Data confidentiality was ensured via anonymization, locked storage, and restricted access. Participation was voluntary, and there was no financial incentive. Samples were disposed of immediately after analysis, while resistant isolates were preserved for future analysis depending on resources.

3.13. Statistical analysis

Data obtained in this study were collected and analysed using Microsoft excel and by statistical package for social scientist (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA).

CHAPTER FOUR

RESULTS

The results illustrating the distribution of patients according to sex and patient category (community and in-hospital) are presented in figure 4.1. It shows a distinct difference between male and female patients. The highest number of patients was recorded among male in-hospital patients (44 patients), while the lowest number was observed among female community patients

(4 patients). Among females, 18 patients were in-hospital, whereas 4 were from the community category.

The results illustrating the distribution of isolated organisms according to sample type, nasopharyngeal and rectal are presented in figure 4.2. The results indicate that *Staphylococcus aureus* recorded the highest count (32 isolates) among all organisms, and this was observed predominantly in nasopharyngeal samples. This was followed by *Coagulase-negative Staphylococcus* with 18 isolates, also from nasopharyngeal samples. Among the rectal isolates, *Escherichia coli* showed the highest frequency with 17 isolates, followed by *Klebsiella species* (3 isolates) and *Raoultella ornithinolytica*, which had the lowest count (1 isolate).

The result showing the antimicrobial resistance profile of *Escherichia coli* isolates against a panel of 19 antibiotics are presented in figure 4.3. The results reveal that most *E. coli* isolates were susceptible to a majority of the antibiotics tested. The highest resistance was observed against sulfamethoxazole-trimethoprim (7 resistant isolates), followed by piperacillin-tazobactam (4 resistant isolates) and cefuoroxime (3 resistant isolates). Moderate resistance was also recorded for ciprofloxacin (2 resistant isolates) and ofloxacin (1 resistant isolate). On the other hand, complete susceptibility (0 resistant isolates) was recorded for antibiotics such as amikacin, ampicillin, azithromycin, cefepime, cefixime, cefotaxime, ceftazidime, ceftriaxone, clindamycin-dalacin-C, daptomycin, ertapenem, erythromycin, gentamicin, and meropenem**.**

The multidrugs resistance pattern of *Escherichia coli* are presented in Table 4.4. The most frequent MDR pattern is observed with the combination CIP, PIP, and SUL, affecting 2 isolates. Other combinations, including PIP and SUL, FEP, CAZ, CTX, CIP, PIP, and SUL, AMK, AMC,

FEP, CAZ, CTX, CIP, MEM, OFL, PIP and SUL, and AMC, CAZ, CTX and SUL, each account for 1 isolate, indicating a diverse range of resistance profiles.

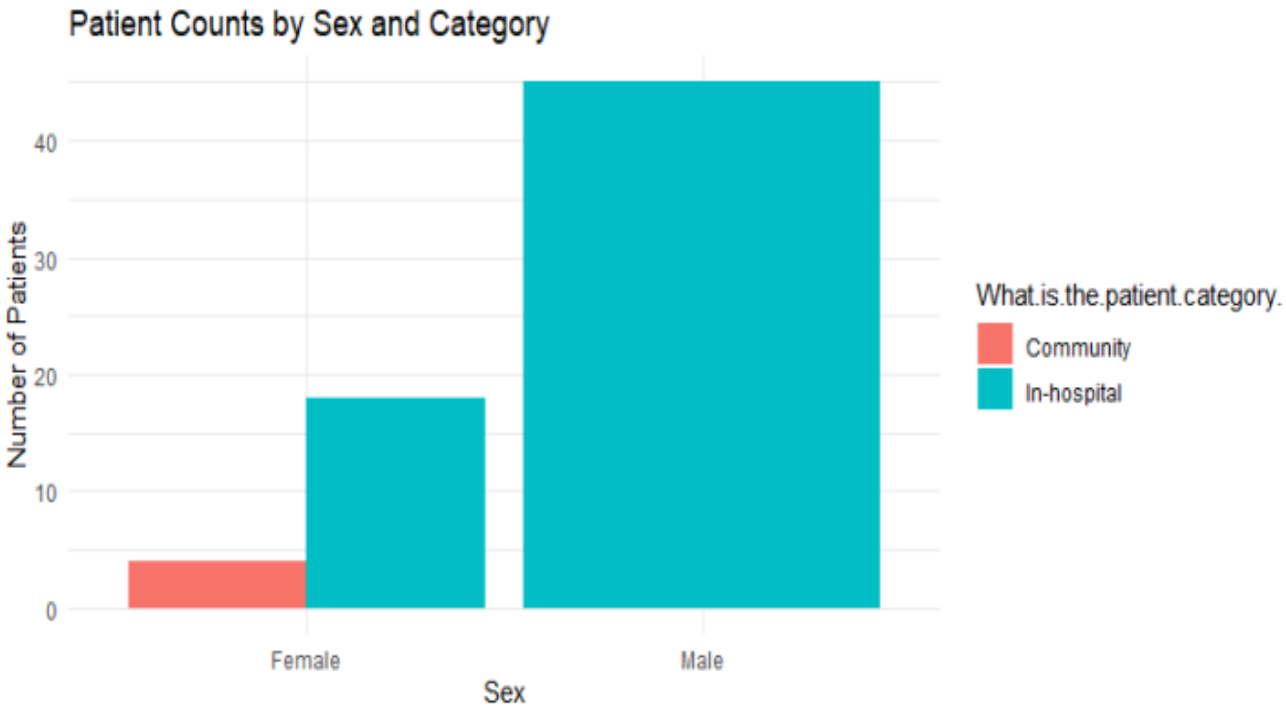

Figure 4.1: Patient Counts by Sex and Category

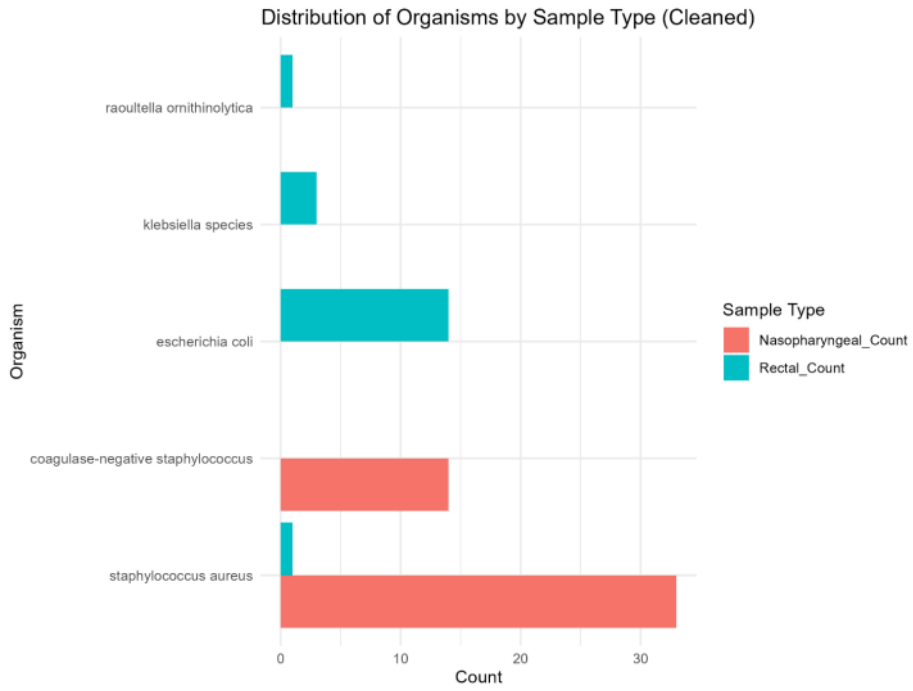

Figure 4.2: Distribution of Organisms by Sample Type (Cleaned)

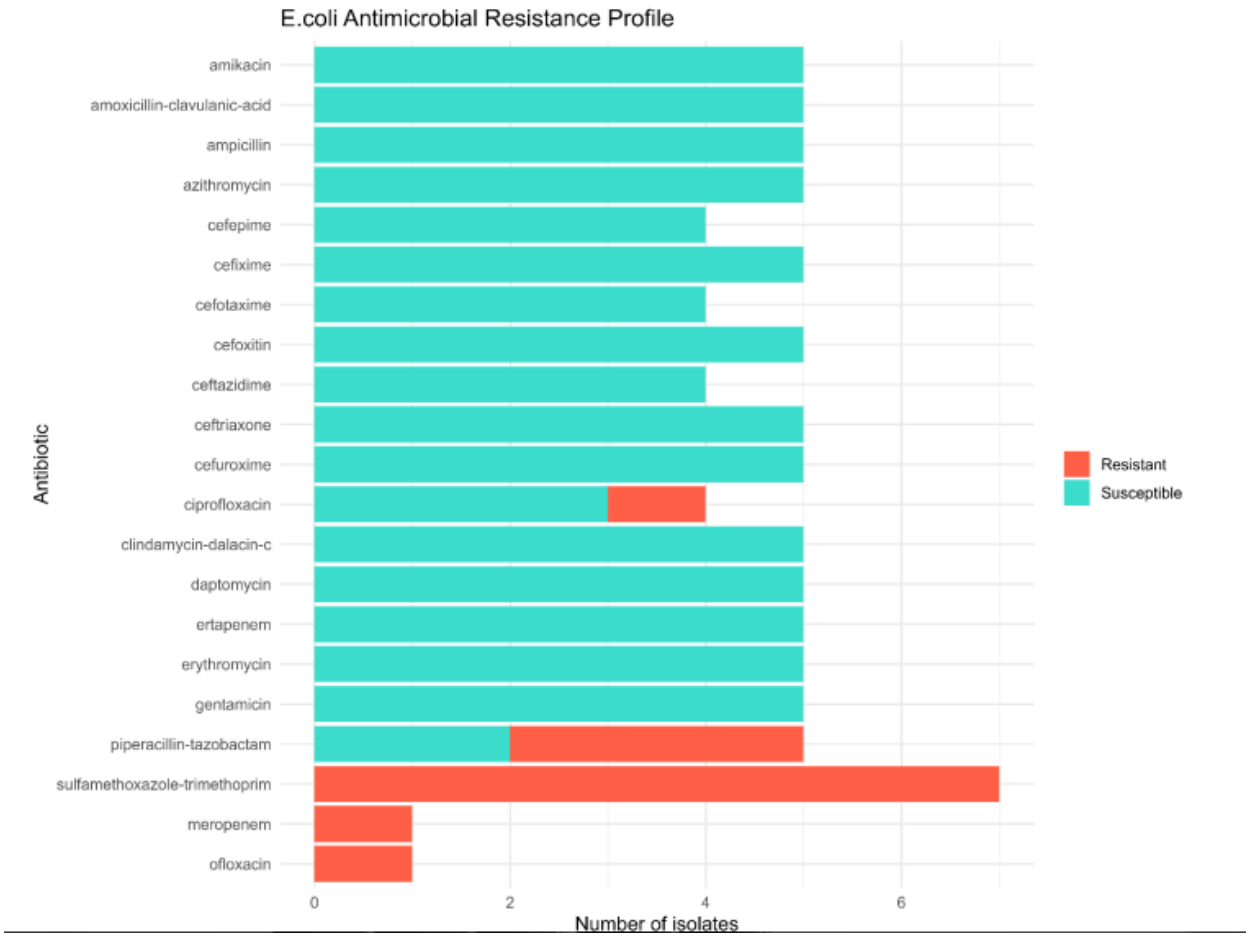

Figure 4.3: Antimicrobial Resistance Profile of *E. coli*

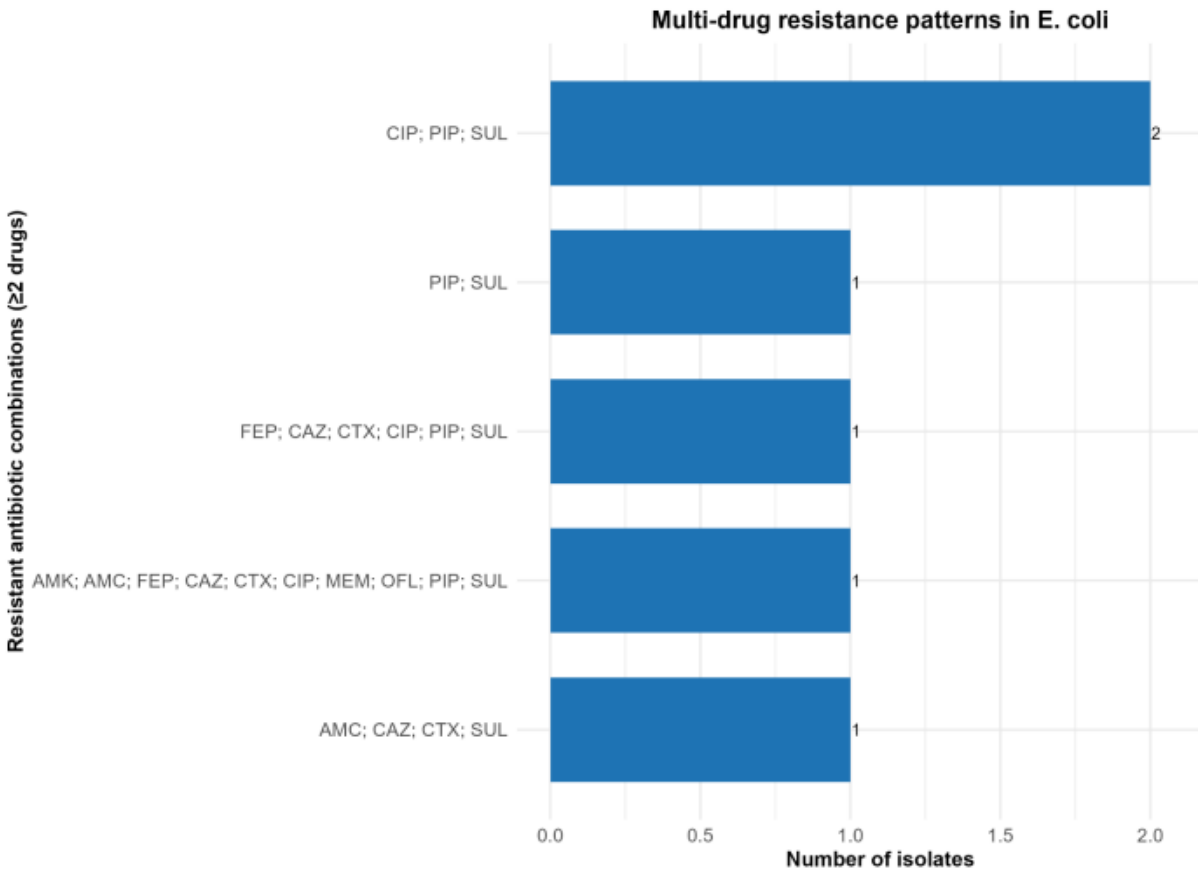

Figure 4.4. Multi-Drug Resistance Patterns in *E. coli*

CHAPTER FIVE

DISCUSSION

Hospital-acquired antibiotic-resistant *Escherichia coli* infections are among the most significant challenges confronting modern healthcare systems, particularly in pediatric settings. *E. coli*, a Gram-negative facultative bacillus, is a common commensal of the intestinal tract but can cause severe opportunistic infections such as urinary tract infections, sepsis, meningitis, and wound infections when it acquires virulence or resistance determinants. In hospitals, where antibiotic pressure and cross-transmission are high, multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains frequently emerge, leading to increased morbidity, mortality, and treatment costs (Giami *et al*., 2025; Wilkie *et al*., 2024).

In Nigeria, the burden of hospital-acquired *E. coli* infections has grown due to factors such as overcrowded wards, suboptimal infection prevention practices, and indiscriminate use of broad-spectrum antibiotics (Ayandele *et al*., 2020; Orji *et al*., 2022). Understanding the distribution and resistance profile of *E. coli* among hospitalized children provides critical insight into the current state of antimicrobial resistance and informs rational treatment strategies.

The demographic distribution of patients by sex and patient category (community and in-hospital) is illustrated in Figure 4.1. Male in-hospital patients constituted the largest group with 44 individuals, while female community patients represented the smallest group with only 4 individuals. Among female patients, 18 were in-hospital, suggesting a higher prevalence of hospital admissions among males compared to females.

The predominance of male pediatric patients in this study is consistent with observations from Smith *et al*. (2019), who reported that male children often exhibit higher hospitalization rates due

to greater exposure to infectious environments and possible biological susceptibility. Similar patterns were noted by Oluwo *et al*. (2024) and Adedokun *et al*. (2023) in studies conducted in Lagos and Benue, respectively, linking higher male prevalence to behavioral and immunological factors. This sex-based disparity underscores the importance of gender-sensitive infection surveillance and healthcare delivery in pediatric hospital settings.

The distribution of microbial isolates according to sample type (nasopharyngeal and rectal) is presented in Figure 4.2. *Staphylococcus aureus* was the most prevalent organism overall, accounting for 32 isolates, followed by coagulase-negative *Staphylococcus* (CoNS) with 18 isolates, both predominantly from nasopharyngeal samples. This finding agrees with the reports of Wertheim *et al*. (2005) and Kluytmans *et al*. (1997), who established *S. aureus* and CoNS as common colonizers of the upper respiratory tract, capable of transitioning from commensalism to pathogenicity in immunocompromised patients.

In contrast, rectal samples yielded primarily *Escherichia coli* (17 isolates), followed by *Klebsiella* spp. (3 isolates) and *Raoultella ornithinolytica* (1 isolate). The dominance of *E. coli* in rectal isolates aligns with its established role as a key enteric pathogen and reservoir of antibiotic resistance genes (Paterson and Bonomo, 2005). These differences between nasopharyngeal and rectal isolates highlight the site-specific microbial ecology within hospitalized children and emphasize the importance of targeted infection control interventions (Tenover *et al*., 2021).

The antimicrobial resistance profile of *E. coli* isolates against a panel of 19 antibiotics (Figure 4.3) demonstrated a generally favorable susceptibility pattern. Complete susceptibility (0 resistant isolates) was observed for amikacin, ampicillin, azithromycin, cefepime, cefixime, cefotaxime, ceftazidime, ceftriaxone, clindamycin-dalacin-C, daptomycin, ertapenem, erythromycin,

gentamicin, and meropenem. This wide susceptibility mirrors WHO (2020) global surveillance data, which report continued efficacy of carbapenems and aminoglycosides against *E. coli*.

However, resistance was observed against sulfamethoxazole-trimethoprim (7 resistant isolates), piperacillin-tazobactam (4 isolates), and cefuroxime (3 isolates). Moderate resistance was also noted to ciprofloxacin (2 isolates) and ofloxacin (1 isolate). Resistance to sulfamethoxazole-trimethoprim is particularly worrisome given its common use as a first-line empirical therapy in pediatric infections (Gupta *et al*., 2011). Similar high resistance rates were reported by Zakou *et al*. (2024) and Dabo *et al*. (2019) among diarrheic children in Nigeria. The resistance to piperacillin-tazobactam—a β-lactam/β-lactamase inhibitor combination—further emphasizes the selective pressure exerted by frequent use of broad-spectrum antibiotics in tertiary hospitals (Boucher *et al*., 2013).

Conversely, the absence of resistance to carbapenems and aminoglycosides in this study is encouraging and aligns with findings from Orji *et al*. (2022), who reported low resistance to imipenem and gentamicin in tertiary hospitals across southeastern Nigeria. Nonetheless, vigilance is required as ESBL-producing *E. coli* capable of hydrolyzing extended-spectrum cephalosporins are increasingly emerging in Nigerian hospitals (Giami *et al*., 2025).

The multidrug resistance (MDR) patterns of *E. coli* isolates are summarized in Figure 4.4. The most frequent pattern involved resistance to ciprofloxacin (CIP), piperacillin-tazobactam (PIP), and sulfamethoxazole-trimethoprim (SUL), affecting two isolates. Other MDR combinations, observed in single isolates, included PIP + SUL; FEP + CAZ + CTX + CIP + PIP + SUL; and AMC + CAZ + CTX + SUL.

The emergence of multiple MDR profiles suggests complex resistance mechanisms in *E. coli* populations, likely mediated by β-lactamase production, efflux pumps, and plasmid-borne resistance genes (Nikaido, 2009; Bush and Jacoby, 2010). These findings correspond with those of Carattoli (2013), who highlighted the role of mobile genetic elements in facilitating horizontal gene transfer of resistance determinants among Gram-negative bacteria. Similarly, Giami *et al*. (2025) reported a 45.9% MDR rate among *E. coli* isolates in a Nigerian tertiary hospital, while Zakou *et al*. (2024) observed over 90% MDR prevalence in pediatric diarrheal cases in Nasarawa State.

The observed patterns underline the potential for cross-resistance and treatment failure, emphasizing the importance of molecular surveillance to detect and characterize resistance genes. Laxminarayan *et al*. (2013) stressed that such surveillance is crucial for guiding local antibiotic policies and promoting judicious antimicrobial use.

The results obtained in this study provide critical insights into the dynamics of hospital-acquired *E. coli* infections among children in Benin City. The higher proportion of male in-hospital patients reflects sex-linked variations in exposure, susceptibility, and healthcare-seeking behavior, consistent with previous epidemiological trends (Smith *et al*., 2019). The isolation of *S. aureus* and CoNS from nasopharyngeal samples and *E. coli* from rectal samples confirms their ecological predilection and underscores the importance of anatomical site-specific surveillance in pediatric infection control (Wertheim *et al*., 2005; Kluytmans *et al*., 1997; Paterson and Bonomo, 2005).

Although *E. coli* isolates showed broad susceptibility to many antibiotics, the resistance observed against sulfamethoxazole-trimethoprim, piperacillin-tazobactam, and fluoroquinolones remains concerning. These trends mirror national reports (Zakou *et al*., 2024; Orji *et al*., 2022) and suggest

widespread antibiotic misuse. The identification of MDR patterns, though in low frequency, signifies an evolving resistance threat that could escalate without adequate stewardship.

These findings reinforce the need for strict infection prevention measures, rational antibiotic use, and continuous AMR surveillance in tertiary hospitals. Without such interventions, Nigeria's pediatric population may face increasing risks of untreatable hospital-acquired infections.

5.2 RECOMMENDATIONS

1. **Strengthen Infection Prevention and Control (IPC) Programs:** Hospitals should adopt and enforce strict infection control measures such as hand hygiene compliance, sterilization of medical equipment, and environmental sanitation to minimize hospital-acquired *E. coli* infections. Regular training of healthcare workers on IPC practices should be prioritized.
2. **Implement Antimicrobial Stewardship Programs (ASP):** Rational antibiotic use should be promoted through well-structured antimicrobial stewardship initiatives. This includes restricting the empirical use of broad-spectrum antibiotics and encouraging antibiotic susceptibility testing before prescription.
3. **Routine Microbial Surveillance:** Continuous monitoring and reporting of antimicrobial resistance patterns should be institutionalized in tertiary hospitals. This will aid in early detection of resistance trends and guide evidence-based therapeutic decisions.
4. **Public and Professional Awareness:** Awareness campaigns should be conducted for both healthcare workers and caregivers on the dangers of antibiotic misuse and the importance of completing prescribed dosages to prevent the emergence of resistant strains.
5. **Enhanced Laboratory Capacity:** Strengthening diagnostic laboratories with modern equipment and trained personnel will enable timely and accurate detection of resistant *E. coli* strains, ensuring prompt and appropriate treatment.
6. **Policy and Regulatory Action:** The government, through the Federal Ministry of Health and relevant agencies, should enforce policies that regulate antibiotic sales and usage, especially in community pharmacies, to curb self-medication and over-the-counter misuse.
7. **Further Research:** Longitudinal and molecular studies should be carried out to understand the genetic mechanisms driving resistance in *E. coli* and to identify possible reservoirs within hospital environments.

5.3 CONCLUSION

Hospital-acquired antibiotic-resistant *Escherichia coli* infections pose a serious threat to pediatric health in tertiary hospitals in Benin City. The study revealed that *E. coli* was a major isolate, particularly from rectal samples, with notable resistance to commonly used antibiotics such as sulfamethoxazole-trimethoprim, piperacillin-tazobactam, and ciprofloxacin. However, most isolates remained susceptible to potent drugs like meropenem and gentamicin. The occurrence of multidrug-resistant (MDR) strains highlights poor infection control practices and antibiotic misuse. These findings align with previous reports from Nigerian hospitals, emphasizing the need for improved antimicrobial stewardship, routine resistance monitoring, and strict infection prevention measures to reduce hospital-acquired infections and ensure effective patient care.

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