PREVALENCE OF METHICILLIN-RESISTANT *Staphylococcus aureus* AND OTHER BACTERIA IN URINE SAMPLES OF SELECTED STUDENTS IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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BENIN CITY.

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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 REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc. Hons) IN MICROBIOLOGY

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CERTIFICATION

This is to certify that this project work was carried out by **Imudiase Kalota BRIGHT-IGBINIGIE (Miss)** with matriculation number **LSC2106284** of the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

…… ……..

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(Project Supervisor)

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(Head of Department)

DEDICATION

This project work is dedicated to God and my family members for their unwavering support.

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I express my wholehearted gratitude to God who equipped me with the strength and grace throughout my life and academic journey.

I want to sincerely acknowledge my supervisor **DR (MRS) J.Z. SAIDU** whose scholarly guidance saw to the beginning and conclusion of this research project.

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ABSTRACT

Urinary tract infections (UTIs) are among the most frequently encountered bacterial infections globally, particularly prevalent among young adults such as university students. Risk factors including poor hygiene practices, sexual activity, and limited access to timely healthcare contribute significantly to the occurrence of these infections. This study was designed to investigate the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and other uropathogenic bacteria in urine samples of selected students from the University of Benin. A total of 60 midstream urine samples were aseptically collected from consenting students and subjected to comprehensive laboratory analysis. Urinalysis was performed to detect markers such as leukocytes, nitrites, and protein. The identified microorganisms were subjected to antibiotic susceptibility testing using the standard disc diffusion method. The results showed that 24% of urine samples tested positive for leukocytes, 17% for nitrites, and 15% for protein, indicating possible UTIs. Bacterial counts were generally higher in female students, within 21–25 age group showing the highest mean TVC. Six distinct bacterial species were isolated, with *Staphylococcus aureus* (33%) being the most prevalent, followed by *Escherichia coli* (21%), *Pseudomonas* spp. (11%), and MRSA (16.7%). MRSA occurrence was higher in females compared to males and also peaked in the 21–25 age group. Antibiotic susceptibility testing revealed that *Staphylococcus aureus* showed resistance to cloxacillin and oxacillin, while *Proteus* sp. and *Klebsiella* sp. were susceptible to ofloxacin and ceftriaxone. The highest MAR index of 0.44 was observed in *Staphylococcus aureus*, indicating significant multidrug resistance. The findings indicate the presence of multidrug-resistant uropathogens in the student population. This shows a potential reservoir for transmission, necessitating improved sanitation in hostels and public health campaigns to raise awareness about antibiotic resistance. Further research should focus on molecular characterization of resistance genes and explore alternative strategies such as targeted antimicrobial therapies and hygiene interventions.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Urinary tract infections (UTIs) represent one of the most common bacterial infections globally, affecting millions of individuals annually (Foxman, 2014). They are particularly prevalent among young adults, including university students, due to factors such as poor hygiene practices, sexual activity, and limited access to adequate healthcare facilities in some settings (Foxman, 2014; Saidu and Ologbosere, 2022). Urinary tract infections (UTIs) are caused by a variety of bacterial pathogens, with *Escherichia coli* being the most common, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and others (Flores-Mireles *et al*., 2015). Among these, *Staphylococcus aureus*, particularly its methicillin-resistant strains (MRSA), has emerged as a significant public health concern due to its resistance to multiple antibiotics, complicating treatment and increasing morbidity (Turner *et al*., 2019).

Staphylococcus aureus is a gram-positive, coagulase-positive bacterium that is a common commensal of the human skin and nasal passages but can become pathogenic, causing infections ranging from mild skin infections to severe systemic conditions such as bacteremia, endocarditis, and UTIs (Tong *et al*., 2015). Methicillin-resistant *Staphylococcus aureus* (MRSA) is characterized by resistance to β-lactam antibiotics, mediated by the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics (Peacock and Paterson, 2015). The prevalence of MRSA in clinical settings has increased globally, with significant regional variations. In Nigeria, studies have reported a rising prevalence of MRSA, ranging from 18.3% in 2009 to 42.3% in 2013, with notable resistance

to commonly used antibiotics such as ampicillin, tetracycline, and co-trimoxazole (Nwadike *et al*., 2014; Olalekan *et al*., 2020).

The University of Benin, located in Benin City, Nigeria, is a tertiary institution with a large student population living in close quarters, often in hostels with suboptimal sanitary conditions. These conditions, coupled with the widespread misuse of antibiotics, create an environment conducive to the spread of resistant bacterial strains, including MRSA. Urine samples are a critical medium for studying bacterial pathogens associated with UTIs, as they provide insights into the microbial ecology of the urinary tract and the antimicrobial resistance profiles of isolates (Iregbu and Nwajiobi-Princewill, 2013). Previous studies in Nigeria have reported *S. aureus* as a significant uropathogen, with 33.6% prevalence in some settings and 100% methicillin resistance among isolates from UTI patients (Ekwealor *et al*., 2016). Additionally, other bacteria such as *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. are frequently isolated from urine samples, often exhibiting multidrug resistance (MDR) (Odetoyin *et al*., 2019).

The emergence of antimicrobial resistance (AMR) in Nigeria is exacerbated by factors such as over-the-counter availability of antibiotics, inadequate infection control measures, and limited surveillance systems (O'Neill, 2016). These factors contribute to the dissemination of resistant strains within communities and healthcare settings. Among university students, who represent a young, active, and mobile population, the prevalence of MRSA and other resistant bacteria in urine samples is understudied, yet critical for informing targeted interventions. This study focuses on the University of Benin to assess the prevalence of MRSA and other bacterial isolates in urine samples, aiming to contribute to the understanding of AMR patterns in a community setting.

This study seeks to address these gaps by determining the prevalence of MRSA and other bacterial isolates in urine samples from selected students at the University of Benin, Nigeria, and evaluating their antimicrobial susceptibility profiles. Understanding the burden of resistant bacteria in this population will provide critical insights into the epidemiology of AMR in community settings and guide public health interventions.

1.2. Aim and Objectives of the Study

To determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and other bacterial isolates in urine samples of selected students of the University of Benin, Edo State, Nigeria

The specific objectives of this research were to;

1. To enumerate and isolate *Staphylococcus aureus* and other bacterial isolates in the urine samples.
2. determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolated *S. aureus* strains using phenotypic and molecular methods.
3. To evaluate the antimicrobial susceptibility profiles of MRSA and other bacterial isolates to commonly used antibiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1. The Urinary System and Its Infections

The urinary system, also referred to as the renal system, is an intricate arrangement of organs, ducts, and structures tasked with the formation, storage, and elimination of urine (Khan *et al*., 2018). This system plays a crucial role in sustaining internal equilibrium by regulating fluid volume, solute concentration, and pH levels in the body (Peate, 2021). It comprises multiple components working synergistically to filter blood and remove metabolic waste. The kidneys serve as the principal organs of the system, extracting waste and surplus substances from the bloodstream, which are then expelled as urine. Beyond excretion, the kidneys help maintain electrolyte stability, acid-base balance, and blood pressure regulation (Imenez Silva and Mohebbi, 2022).

Once urine is formed in the kidneys, it travels through the ureters—two muscular conduits that direct it into the urinary bladder, where it is temporarily stored. The bladder later expels urine through the urethra, a tubular structure that serves as the passageway for elimination. This coordinated mechanism ensures homeostatic regulation of body fluids and electrolytes, which is essential for overall well-being (Tortora and Derrickson, 2007).

Urinary Tract Infection (UTI) is a general term for infections arising from microbial colonization within the urinary tract. The condition varies in severity, ranging from asymptomatic bacteriuria or funguria (the presence of bacteria or fungi in urine without symptoms) to more severe infections such as pyelonephritis (kidney infection) and cystitis (bladder infection), which can progress to life-threatening sepsis if untreated (Tanagho and Mcaninch, 2004). UTIs primarily affect the lower urinary tract—specifically the urethra and bladder—but may extend to the upper tract, involving the kidneys and ureters.

Diagnosis of UTIs relies on clinical symptoms and laboratory tests. One of the key diagnostic indicators is the presence of more than 10,000 colony-forming units (CFU) per milliliter of urine in a microbiological culture. Additionally, urinalysis and dipstick tests that detect leukocytes or nitrites serve as confirmatory tools (Zorc and Levine, 2005). The National Institute for Health and Clinical Excellence (NIHCE) (2007) provides comprehensive guidelines for diagnosing and managing UTIs, emphasizing the need for combining clinical manifestations such as painful urination, frequent urination, and abdominal discomfort with laboratory findings. UTIs can be categorized as uncomplicated or complicated based on factors like age, immune function, and underlying health conditions such as diabetes, kidney disease, or anatomical abnormalities. While uncomplicated UTIs typically involve the lower tract, complicated UTIs often extend to the upper tract and pose a greater risk of severe complications such as renal impairment and systemic infection.

Although bacterial pathogens—most notably *Escherichia coli*—are the predominant causative agents of UTIs, fungal infections, primarily due to *Candida* species, also contribute, particularly in immunocompromised individuals, patients with indwelling catheters, or those with diabetes mellitus. Fungal UTIs, though less prevalent, have gained recognition in recent years, particularly among individuals with predisposing conditions (Schlievert and Peterson, 2009). Given the crucial role of the urinary system in maintaining physiological balance, timely identification and appropriate management of UTIs are vital in preventing adverse outcomes. A thorough understanding of the infection's pathophysiology, microbial causes, and clinical interventions is essential for optimizing patient care and treatment.

2.2. Microorganisms Present in Urine and Their Etiology

Bacterial infections constitute the majority of UTIs, accounting for approximately 80–85% of symptomatic cases in women. Several factors contribute to the susceptibility of individuals to

these infections, including age, gender, hospitalization, and urinary tract obstructions. Women, in particular, are more prone to UTIs due to anatomical differences such as a shorter urethra and its proximity to the vaginal and anal regions, facilitating bacterial entry. Pregnancy and childbirth further elevate the risk by altering the urinary tract's anatomy and function. While bacteria cause nearly 95% of UTIs, fungal and viral infections also contribute to a smaller percentage of cases (Amdekar *et al*., 2011).

2.2.1. Bacteria

Bacteria, especially those within the *Enterobacteriaceae* family, are the primary pathogens in uncomplicated UTIs. Among these, *Escherichia coli* is the most frequently implicated species, responsible for approximately 80–85% of cases due to its strong adherence to and colonization of urinary tract epithelial cells (Takahashi *et al*., 2006).

Other notable bacterial contributors include *Staphylococcus saprophyticus*, which accounts for 5–15% of infections, particularly in sexually active young women (Mandell *et al*., 2005). Additional pathogens such as *Enterococcus faecalis, Enterococcus faecium, Klebsiella* species, *Proteus* species, *Pseudomonas* species, and *Morganella* species are commonly detected in patients with recurrent or complicated UTIs (Alzahrani *et al*., 2020). In approximately 95% of cases, UTIs are caused by a single bacterial species, with *E. coli* leading in prevalence, followed by *Proteus mirabilis* (10%) and *Klebsiella* species (6%) (Tanagho, 2004; Ghedira *et al*., 2004).

2.2.2. Viruses

Although viral infections of the urinary tract are less common, they do occur, particularly in association with systemic viral illnesses such as measles, mumps, and varicella (Walsh and Collyns, 2017). Certain viruses, including adenoviruses, have been linked to hemorrhagic

cystitis in children, causing bladder inflammation and painful urination (Al-Jeburi *et al*., 2015). In some cases, viral inclusions have been observed in urine samples of patients with cytomegalovirus or mumps-related nephritis, indicating a viral etiology in specific urinary conditions (Praga *et al*., 2015).

2.2.3. Fungi

Fungal UTIs, commonly referred to as candiduria, are primarily caused by *Candida* species, especially *Candida albicans* (Nicolle, 2000). These infections are more prevalent among individuals with compromised immunity, diabetes, long-term catheterization, or those undergoing prolonged antibiotic therapy (Fisher *et al*., 2011). While *Candida* is the leading fungal pathogen in UTIs, other rare fungi, such as *Mucor* species, have been reported to cause severe infections like prostatitis, epididymitis, and renal infections in immunocompromised patients (Komaroff, 2000). Fungal colonization is typically harmless in healthy individuals but may lead to infections in those with predisposing conditions, particularly in hospital settings where invasive medical procedures increase susceptibility (Kauffman, 2014).

2.2.4. Protozoa

Protozoan infections of the urinary tract are rare and primarily associated with sexually transmitted infections (STIs). *Trichomonas vaginalis*, a flagellated protozoan, is the most common causative agent, leading to trichomoniasis, a condition that affects both men and women. In women, *T. vaginalis* is a significant cause of urethritis and vaginitis, contributing to pelvic discomfort and painful urination. In men, it can cause urethritis and prostatitis, though infections are often asymptomatic (Patel *et al*., 2018). The prevalence of trichomoniasis highlights the role of sexual transmission in protozoan urinary infections,. emphasizing the importance of preventive measures in controlling its spread.

2.3 *Staphylococcus aureus* as a Uropathogen

2.3.1 Characteristics of *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, coagulase-positive, facultative anaerobic bacterium that appears as spherical cells arranged in grape-like clusters under a microscope (Tong *et al*., 2015). Its microbiological properties include the ability to grow in a wide range of conditions, including high salt concentrations, which is exploited in selective media like Mannitol Salt Agar for its isolation (Lowy, 1998). Biochemically, *S. aureus* is characterized by its production of coagulase, an enzyme that clots plasma, distinguishing it from other staphylococcal species, and catalase, which differentiates it from streptococci (Tong *et al*., 2015). It also ferments mannitol and produces golden-yellow colonies due to the pigment staphyloxanthin, which protects the bacterium from oxidative stress (Liu *et al*., 2005).

As a commensal, *S. aureus* commonly colonizes the anterior nares, skin, and mucous membranes of approximately 20–30% of healthy individuals without causing disease (Wertheim *et al*., 2005). Its role as an opportunistic pathogen, however, is well-documented, as it can cause a wide range of infections when it breaches host barriers, such as through skin breaks or mucosal disruption (Lowy, 1998). In the context of urinary tract infections (UTIs), *S. aureus* is less common than gram-negative bacteria like *Escherichia coli* but is increasingly recognized as a significant uropathogen, particularly in specific populations such as catheterized patients or those with underlying conditions (Flores-Mireles *et al*., 2015). Its ability to transition from a commensal to a pathogen is facilitated by its adaptability to host environments and its arsenal of virulence factors, which enhance its pathogenicity in the urinary tract (Tong *et al*., 2015).

2.3.2 Prevalence of *S. aureus* in UTIs

Globally, *S. aureus* accounts for approximately 5–10% of UTI cases, with higher prevalence in hospital settings where catheter-associated infections are common (Ronald, 2003). Studies indicate that *S. aureus* is more frequently isolated in complicated UTIs, such as those associated with indwelling catheters, urological procedures, or hematogenous spread from other infection sites (Muder *et al*., 2006). In community settings, the prevalence of *S. aureus* as a uropathogen is lower, typically ranging from 2–5%, but its presence is significant due to its potential for severe outcomes, including pyelonephritis and bacteremia (Flores-Mireles *et al*., 2015).

In Nigeria and West Africa, *S. aureus* is a notable uropathogen, with studies reporting prevalence rates in UTIs ranging from 10–33.6% depending on the population and setting (Ekwealor *et al*., 2016; Iregbu and Nwajiobi-Princewill, 2013). For instance, a study in Awka, Nigeria, found *S. aureus* in 33.6% of urine samples from patients with UTI symptoms, highlighting its significance in community-acquired infections (Ekwealor *et al*., 2016). Similarly, a study in a tertiary hospital in Abuja reported *S. aureus* as the second most common uropathogen after *E. coli*, with a prevalence of 15.4% among UTI patients (Iregbu and Nwajiobi-Princewill, 2013). In West Africa, studies from Ghana and Senegal have reported comparable prevalence rates, ranging from 8–20%, often linked to poor hygiene practices and healthcare access (Donkor *et al*., 2019). Among university students, data is limited, but a study in Ogun State, Nigeria, found *S. aureus* in 12% of urine samples from healthy students, suggesting asymptomatic carriage may contribute to its prevalence in community settings (Ogbolu *et al*., 2018).

2.3.3 Pathogenesis of *S. aureus* in UTIs

The pathogenesis of *S. aureus* in UTIs involves its ability to adhere to uroepithelial cells, evade host immune responses, and cause tissue damage. Unlike gram-negative uropathogens, which typically ascend from the periurethral area, *S. aureus* more commonly causes UTIs through hematogenous spread from skin or soft tissue infections, particularly in hospitalized or immunocompromised patients (Muder *et al*., 2006). However, in community settings, *S. aureus* can colonize the perineum or urethra, facilitating ascending infections, especially in individuals with predisposing factors such as poor hygiene or sexual activity (Flores-Mireles *et al*., 2015).

Key virulence factors associated with *S. aureus* in the urinary tract include adhesins, toxins, and immune evasion molecules. Adhesins, such as fibronectin-binding proteins (FnBPs), enable *S. aureus* to adhere to uroepithelial cells, initiating infection (Gordon and Lowy, 2008). Toxins, including hemolysins and leukocidins, contribute to tissue damage and inflammation, exacerbating UTI symptoms (Tong *et al*., 2015). The polysaccharide capsule and protein A help *S. aureus* evade phagocytosis, allowing it to persist in the urinary tract (Gordon and Lowy, 2008). Additionally, biofilm formation on catheters or mucosal surfaces enhances *S. aureus* survival and resistance to antibiotics, complicating treatment (Flores-Mireles *et al*., 2015). These virulence factors collectively enable *S. aureus* to establish infections in the urinary tract, making it a formidable uropathogen in both clinical and community settings.

2.4 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

2.4.1 Mechanisms of Methicillin Resistance

Methicillin resistance in *Staphylococcus aureus* is primarily mediated by the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), a transpeptidase with low affinity for β-lactam antibiotics, including methicillin, penicillin, and cephalosporins (Peacock and Paterson, 2015). The *mecA* gene is carried on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*), which integrates into the *S. aureus* chromosome and confers resistance to multiple β-lactam antibiotics (Hiramatsu *et al*., 2001). PBP2a allows cell wall synthesis to continue despite the presence of β-lactam antibiotics, rendering them ineffective (Peacock and Paterson, 2015).

In addition to *mecA*, other genetic determinants contribute to methicillin resistance. The *mecC* gene, a homolog of *mecA*, has been identified in some MRSA strains, particularly in Europe, and encodes a variant PBP2a with similar resistance properties (García-Álvarez *et al*., 2011). Other resistance mechanisms, such as modifications in penicillin-binding proteins or efflux pumps, may also contribute to reduced susceptibility to antibiotics, though *mecA* remains the dominant mechanism (Peacock and Paterson, 2015). The presence of these genetic elements complicates treatment and necessitates molecular diagnostic techniques, such as polymerase chain reaction (PCR), to confirm resistance in clinical isolates (Hiramatsu *et al*., 2001).

2.4.2 Global and Regional Prevalence of MRSA

Globally, MRSA is a major public health concern, with prevalence varying significantly by region and setting. In hospital settings, MRSA accounts for 20–50% of *S. aureus* infections in many countries, particularly in North America, Europe, and Asia (Turner *et al*., 2019). In

community settings, the prevalence of MRSA is lower, ranging from 1–10%, but has been increasing due to the emergence of community-acquired MRSA (CA-MRSA) (David and Daum, 2010). In Africa, MRSA prevalence in clinical settings ranges from 15–50%, with higher rates in urban hospitals (Falagas *et al*., 2013). In Nigeria, studies have documented a rising prevalence of MRSA, from 18.3% in 2009 to 42.3% in 2013, with significant resistance to commonly used antibiotics like ampicillin, tetracycline, and co-trimoxazole (Nwadike *et al*., 2014; Olalekan *et al*., 2020).

Hospital-acquired MRSA (HA-MRSA) is typically associated with invasive procedures and prolonged antibiotic exposure, while CA-MRSA is increasingly reported in healthy individuals without healthcare exposure (David and Daum, 2010). In Nigeria, hospital-based studies in Lagos and Abuja have reported MRSA prevalence rates of 25–40% among *S. aureus* isolates from clinical specimens, including urine (Iregbu and Nwajiobi-Princewill, 2013). Community-based studies are less common, but a systematic review by Olalekan *et al*. (2020) noted that CA-MRSA prevalence in Nigeria ranges from 10–20%, with higher rates in urban areas where antibiotic misuse is prevalent.

2.4.3 MRSA in Community Settings

The emergence of community-acquired MRSA (CA-MRSA) has shifted the epidemiology of MRSA, with increasing reports of infections among healthy individuals, including young adults and students (David and Daum, 2010). CA-MRSA strains are distinct from HA-MRSA, often carrying smaller SCC*mec* types (IV or V) and producing toxins like Panton-Valentine leukocidin (PVL), which enhances their virulence (Otto, 2010). These strains are typically associated with skin and soft tissue infections but can also cause UTIs, particularly in populations with risk factors such as crowded living conditions or poor hygiene (Flores-Mireles *et al*., 2015).

Studies on MRSA carriage among healthy populations, including students, highlight its potential as a silent reservoir for transmission. In the United States, nasal carriage of MRSA among college students has been reported at 1–2%, with higher rates in dormitories (Creech *et al*., 2010). In Nigeria, a study among university students in Ogun State found a 5.6% MRSA carriage rate in nasal and skin samples, with 12% of urine samples positive for *S. aureus*, suggesting possible urinary tract colonization (Ogbolu *et al*., 2018). The implications of CA-MRSA in community settings include increased risk of outbreaks, transmission to healthcare settings, and challenges in treatment due to resistance to multiple antibiotics (David and Daum, 2010). Among university students in Nigeria, factors such as shared sanitary facilities, antibiotic misuse, and limited healthcare access may exacerbate MRSA carriage and transmission, underscoring the need for targeted studies in this population (Adeyemo *et al*., 2021).

2.5 Bacterial Invasion of the Urinary Tract

Urinary tract infections (UTIs) arise predominantly due to bacterial invasion, with *Escherichia coli* being the leading causative agent, responsible for around 80–85% of cases (Ortho Women's Report, 2002, as cited in Azubike *et al*., 1994). The mechanism through which bacteria enter the urinary tract can be classified into two main pathways: ascending and descending infections (El-Deeb *et al*., 2015).

Hooton and Stamm (1997) elaborated that UTIs typically originate when bacteria, primarily from the gastrointestinal system, colonize the urethral opening and proliferate. This colonization triggers an infection that can spread through various routes. The most prevalent route is the ascending pathway, where bacteria travel from the urethra into the bladder and may continue their ascent through the ureters to infect the kidneys. Conversely, the haematogenous route involves the spread of bacteria through the bloodstream, leading to

kidney infection during episodes of bacteraemia. Additionally, bacteria can invade the renal system through the lymphatic pathways from the intestines or by directly infiltrating the urinary tract from nearby tissues. The presence of bacterial reservoirs in the vaginal and rectal regions further emphasizes their proximity to the urinary tract, making bacterial transmission more likely.

2.6 Pathways of Bacterial Infection

Among the different modes of bacterial invasion, the ascending route is the most common, frequently leading to kidney infections. Bacteria can migrate from the periurethral area into the bladder, traverse the ureters, and establish themselves in the renal pelvis (George, 2010). Several factors contribute to an individual's susceptibility to UTIs. Any anatomical or functional anomaly that impairs urine flow increases the likelihood of infection (Foxman and Brown, 2003). Even a small bacterial presence that circumvents urinary defenses can rapidly multiply in the bladder, triggering an infection (Fogazzi *et al*., 2004).

In men, an enlarged prostate can hinder urine flow, fostering bacterial growth by causing urine retention. Likewise, the use of diaphragms as contraceptives in women can exert pressure on the bladder neck, preventing complete emptying and leading to urinary stasis, which creates an ideal environment for bacterial colonization. Prolonged diaphragm use further increases bacterial entry and replication. Another major risk factor is urinary catheterization, where catheters, inserted to facilitate urine drainage during medical procedures or immobility, create a direct pathway for bacterial ascent, significantly raising infection risk.

Pregnancy also predisposes women to UTIs due to hormonal fluctuations and mechanical changes affecting the bladder during gestation and childbirth (Konapala *et al*., 2018).

Additionally, lifestyle habits, including wearing tight undergarments, prolonged cycling, and using scented hygiene products in the genital area, can cause irritation, potentially making the bladder more vulnerable to infections. Insufficient water intake exacerbates this risk, as infrequent urination reduces the natural flushing mechanism that helps eliminate bacteria from the urinary tract (Lotan *et al*., 2013). Though the urinary tract is inherently sterile, infections occur when opportunistic bacteria override the body's immune defenses. Understanding the various modes of bacterial entry and associated risk factors is crucial for implementing preventive and therapeutic measures against UTIs.

2.7 Categories of Urinary Tract Infections

2.7.1 Lower and Upper Urinary Tract Infections

UTIs are generally classified based on the affected anatomical site, distinguishing between lower and upper urinary tract infections. Lower UTIs primarily involve the bladder and urethra, while upper UTIs affect the kidneys and ureters.

Cystitis, the most prevalent form of lower UTI, results from bacterial irritation of the bladder mucosa, leading to inflammation and infection (Roussey *et al*., 2008). Symptoms typically include painful urination (dysuria), frequent urination, urgency, and discomfort in the lower abdomen. Another form of lower UTI, urethritis, affects the urethra and is often linked to sexually transmitted infections (STIs), such as those caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium*.

In contrast, upper UTIs, particularly pyelonephritis, involve kidney infections characterized by inflammation of the renal tissue, calyces, and pelvis (Wagenlehner *et al*., 2009). This condition can be acute or chronic. Acute pyelonephritis manifests with systemic symptoms such as fever, chills, flank pain, and nausea, often necessitating hospitalization. Chronic

pyelonephritis results from recurrent or improperly treated infections, leading to kidney scarring and potential long-term renal impairment.

2.7.2 Uncomplicated and Complicated Urinary Tract Infections

UTIs can also be classified based on the patient's overall health and the presence of underlying structural or functional abnormalities in the urinary tract.

Uncomplicated UTIs occur in otherwise healthy individuals with normal urinary anatomy and function. These infections, which are more common in women, are typically caused by *Escherichia coli* and present with symptoms localized to the lower urinary tract, such as dysuria, urgency, and lower abdominal discomfort. They usually resolve with short courses of antibiotics and rarely lead to long-term complications (Masson *et al*., 2009).

Complicated UTIs, on the other hand, arise in individuals with underlying conditions that either predispose them to infections or hinder normal urinary flow and immune response. Factors contributing to complicated UTIs include congenital urinary tract abnormalities, kidney stones, urethral strictures, and prolonged catheterization (Mobley and Warren, 1996). Immunocompromised individuals, such as those with diabetes, cancer, or undergoing chemotherapy, face a heightened risk of infection. Pregnant women are also particularly vulnerable due to hormonal and anatomical changes that can lead to urinary stasis and vesicoureteral reflux.

Catheter-associated urinary tract infections (CAUTIs) represent a significant subset of complicated UTIs. They occur in individuals with indwelling urinary catheters and often involve polymicrobial infections, which are more resistant to conventional antibiotic treatments. CAUTIs account for a substantial proportion of hospital-acquired infections (Gray and Moore, 2009). The presence of multidrug-resistant organisms, such as extended-

spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, further complicates treatment, often requiring prolonged and more intensive antibiotic therapy, sometimes necessitating hospitalization and intravenous medications.

2.8 Clinical Manifestations of UTIs

The clinical presentation of UTIs results from complex interactions between the host and the pathogen, influencing bacterial invasion and persistence. Symptoms vary depending on the site of infection, leading to classifications such as cystitis (bladder infection), urethritis (urethral infection), and pyelonephritis (kidney infection) (Schilling *et al*., 2001).

2.8.1 Cystitis

Cystitis, or bladder inflammation due to infection, manifests with symptoms such as the presence of pus in urine (pyuria), frequent urination, urgency, and discomfort in the lower abdomen (Gunther *et al*., 2001). The urine may appear turbid, have an unpleasant odor, and in roughly 30% of cases, may contain blood (hemorrhagic cystitis). Causes of hemorrhagic cystitis include infections, radiation exposure, chemotherapy, and immunosuppressive drugs (Gupta *et al*., 1999). Some women may experience cystitis despite low bacterial counts (102–104 CFU/ml), making bacterial detection via Gram staining challenging. Physical examination typically reveals tenderness in the suprapubic region.

2.8.2 Urethritis

Urethritis is an inflammatory condition affecting the urethra, which may be infectious or non-infectious. Its symptoms often overlap with those of cystitis. Many cases are associated with sexually transmitted pathogens such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (Braunwald *et al*., 2001). Laboratory findings often reveal the presence of white blood cells

and bacteria in unspun urine samples. Pyuria (≥104 leukocytes/ml) is detected in 96% of symptomatic women but in only 1% of asymptomatic cases. The presence of pyuria without bacteriuria may indicate *Chlamydia* infections or other conditions like vaginal leukorrhea. Among elderly women, especially those in nursing homes, over half exhibit pyuria without classic UTI symptoms. This condition is particularly common among diabetic and aging populations (NIHCE, 2007).

2.8.3 Pyelonephritis

Pyelonephritis occurs when bacteria ascend from the lower urinary tract to the kidneys, potentially presenting as an acute or chronic condition. Acute pyelonephritis is a severe infection that can lead to bloodstream invasion (bacteremia) (Gunther *et al*., 2001). Symptoms, which often develop rapidly within hours or a day, include fever, chills, nausea, vomiting, and diarrhea. While some cases may also exhibit cystitis symptoms, others may not. Clinical examination typically reveals fever, an elevated heart rate, and muscle soreness. Tenderness in one or both costo-vertebral angles upon deep palpation is a common finding. In severe cases, symptoms of Gram-negative sepsis dominate the presentation. Unspun urine samples subjected to Gram staining typically show an abundance of white blood cells and bacteria. Hematuria frequently occurs in the acute phase, and persistent blood in the urine may indicate underlying conditions such as kidney stones, malignancies, or tuberculosis (Braunwald *et al*., 2001).

2.9 Epidemiology of UTIs

Urinary tract infections are among the most commonly diagnosed infections in outpatient care settings (Gales *et al*., 2000). In 1997, UTIs accounted for nearly 7 million visits to physicians' offices and approximately 1 million emergency room visits (Foxman, 2002).

Globally, UTIs affect an estimated 150 million individuals annually, with direct healthcare costs exceeding $6 billion (Gupta, 2011). They represent a significant public health concern, affecting millions of people each year. In the United States alone, more than 8 million cases are reported annually, contributing to an economic burden of roughly $1.6 billion in healthcare expenses (National Institutes of Health, 1999; Foxman, 2002).

In Nigeria, studies report a 24% prevalence of asymptomatic bacteriuria among rural children and 6% among their urban counterparts. In developing countries, UTIs are frequently encountered, with an estimated 250 million new cases annually (Nicolle, 2002; Ronald, 2005). Ethiopia alone reports over 2 million cases per year (Tessema *et al*., 2007).

The increasing emergence of antibiotic-resistant strains has intensified the public health burden of UTIs, particularly in low-resource settings. Contributing factors include poverty, poor sanitation, and the widespread distribution of counterfeit antibiotics (Abubakar, 2009). Understanding the epidemiology of UTIs—including their prevalence, risk factors, and resistance patterns—is crucial for developing targeted interventions and improving treatment strategies.

2.10 Risk Factors for Urinary Tract Infections

2.10.1 Anatomical and Physiological Considerations

Various anatomical and physiological attributes contribute to an individual's vulnerability to urinary tract infections (UTIs). Structural irregularities that hinder normal urine flow or bladder emptying increase infection susceptibility. Conditions such as pelvic organ prolapse, multiple sclerosis, bladder tumors, and bladder stones create an environment favorable for bacterial colonization (Scholes *et al*., 2000). Women with congenital or acquired urinary tract anomalies face a higher risk of developing pyelonephritis, which may be unresponsive to oral

antibiotics or complicated by bloodstream infections. Additionally, the shorter length of the female urethra provides a direct route for bacterial entry into the bladder, further increasing infection rates (Neumann *et al*., 2005).

Functional issues like constipation can also predispose individuals to UTIs by causing partial obstruction and residual urine retention after urination. On the other hand, circumcision in males has been shown to significantly lower UTI risk. Studies have consistently reported that uncircumcised boys exhibit a higher prevalence of UTIs compared to their circumcised counterparts (Schoen *et al*., 2000; Singh-Grewal *et al*., 2005).

2.10.2 Influence of Age

The likelihood of developing UTIs varies across different age groups, with the highest occurrence observed in infancy and adolescence. Statistics indicate that about 3% of prepubescent girls and 1% of prepubescent boys experience at least one episode of UTI. Moreover, asymptomatic bacteriuria becomes increasingly prevalent at both ends of the age spectrum (Heffner and Gorelick, 2008). In postmenopausal women, factors such as vaginal prolapse, shifts in vaginal microbial flora, and urinary incontinence contribute to a heightened risk of UTIs (Perrotta *et al*., 2008).

2.10.3 Gender and Contraceptive Use

Biological sex plays a significant role in UTI susceptibility. Women are disproportionately affected by community-acquired UTIs compared to men, except during infancy when structural factors contribute to infection risk (Stamm, 2001; Foxman, 2002). Sexual activity is a well-established risk factor, as it facilitates bacterial entry into the bladder, increasing the likelihood of infection (Lee and Neild, 2007).

A longitudinal cohort study highlighted a high incidence of symptomatic UTIs among sexually active young women, with contributing factors including frequent sexual intercourse, use of diaphragms with spermicides, and a history of recurrent UTIs. Spermicidal diaphragms disrupt the natural vaginal microbiota, making colonization by uropathogenic bacteria more likely, thereby raising the risk of UTIs by 7.4 to 11.5 times (Handley *et al*., 2002). Similarly, case-control studies have linked the use of nonoxynol-9-coated condoms, inadequate personal hygiene, and frequent sexual encounters to an increased probability of developing UTIs (Scholes *et al*., 2000; Handley *et al*., 2002).

2.10.4 Impact of Diabetes Mellitus

Diabetes mellitus is a major predisposing factor for UTIs, with diabetic individuals facing an elevated risk due to compromised immune function, poor glycemic control, and bladder dysfunction stemming from neuropathy. Those with diabetes are more prone to infections of the lower urinary tract, and their risk of progression to upper urinary tract infections is higher due to the impaired local immune defenses (Geerlings *et al*., 2002; Meiland *et al*., 2002).

Research indicates that diabetic women are particularly susceptible to recurrent and severe UTIs, which, if untreated, can lead to kidney and perirenal complications. These outcomes arise not only from bacterial overgrowth but also from diabetes-related vascular damage and poor wound healing. As a result, UTIs in diabetic patients tend to be more severe, necessitating early diagnosis and aggressive treatment to prevent long-term kidney impairment (Nitzan *et al*., 2015).

Additionally, asymptomatic bacteriuria is more prevalent in diabetic individuals, posing a clinical challenge as it can evolve into symptomatic infections or pyelonephritis. Regular UTI

screening, along with effective blood sugar management, is crucial in mitigating these risks (Zeng *et al*., 2020).

2.10.5 Role of Urinary Catheterization

Urinary catheterization is a medical procedure used to facilitate bladder drainage in patients unable to urinate naturally (Pomfret and Winder, 2007). Catheters may be inserted either through the urethra (urethral catheterization) or directly into the bladder via the lower abdominal wall (suprapubic catheterization). While urethral catheters are typically preferred for short-term use due to their ease of insertion, suprapubic catheters are often selected for long-term applications to reduce urethral trauma (Barbara, 2001).

Despite their medical necessity, catheters carry a significant risk of complications, the most serious being catheter-associated urinary tract infections (CAUTIs). Bacterial colonization can occur through external migration along the catheter's surface or internally via the catheter lumen. The probability of infection escalates with prolonged catheter use, with studies showing that nearly all patients develop bacteriuria after 30 days of continuous catheterization (Warren, 2001).

Short-term catheterization usually results in asymptomatic bacteriuria, while long-term use is associated with more severe complications such as pyelonephritis, bloodstream infections, bladder stones, and chronic renal infections. Additional issues include catheter blockages, urine leakage, and loss of bladder muscle tone due to extended decompression. Persistent bladder irritation and inflammation over time can contribute to the formation of bladder stones, urethral strictures, and, in rare cases, even bladder cancer (Warren, 2001; John *et al*., 1987; Jackson, 2006).

To mitigate CAUTI risk, strict adherence to aseptic insertion protocols, regular catheter maintenance, and timely removal are crucial. The use of antimicrobial-coated catheters and closed drainage systems has been shown to lower infection rates. Proper training of healthcare personnel is also essential in reducing catheter-related complications and improving patient outcomes.

2.11 Diagnosis

The definitive approach to diagnosing urinary tract infections (UTIs) involves the identification of the responsible microorganisms in urine samples (Schmiemann *et al*., 2010). A detailed patient history, coupled with an assessment of existing symptoms, significantly aids in making an accurate diagnosis. Typical signs of UTIs include painful urination (dysuria), increased frequency and urgency of urination, as well as discomfort in the lower abdomen. In cases where the infection has progressed to the upper urinary tract, symptoms such as fever, chills, and flank pain may manifest, indicating pyelonephritis.

A quantitative urine culture is indispensable for confirming a UTI. Bacterial loads ranging from 10^3 to 10^5 colony-forming units per milliliter (CFU/mL) serve as indicators of infection. Adopting proper urine collection techniques, such as obtaining midstream "clean catch" samples, is essential to minimize sample contamination. These specimens must be collected in sterile containers and transported promptly to the laboratory for analysis. In laboratory settings, urine is cultured using differential and selective media like MacConkey agar and Blood agar to isolate and identify uropathogens (Akter *et al*., 2014).

Urine culture remains the most robust and widely endorsed diagnostic tool for identifying the bacteria responsible for UTIs and guiding appropriate treatment strategies (NIHCE, 2007). A calibrated inoculating loop is used to streak the urine sample onto culture media, followed by

incubation at 37°C for 18–24 hours. Bacterial colony counts are then assessed, with a threshold of $\geq 10^5$ CFU/mL in a clean-catch specimen considered diagnostic for a UTI (Wilson and Gaido, 2004).

Additionally, microscopic urine examination provides supplementary diagnostic insights, particularly in symptomatic patients (Braunwald *et al*., 2001; Cheesbrough, 2001; Wilson and Gaido, 2004). Gram staining of either centrifuged or uncentrifuged urine specimens enables the visualization of bacteriuria. The presence of bacteria in uncentrifuged urine exceeding 10^4 CFU/mL is strongly indicative of infection (Cheesbrough, 2001). However, infections involving lower bacterial loads (10^2 to 10^4 CFU/mL) might not always be detected microscopically. Therefore, while bacterial presence on microscopy supports a UTI diagnosis, the absence of visible bacteria does not entirely exclude an infection (Braunwald *et al*., 2001).

2.12 Treatment and Prevention

2.12.1 Treatment

Once urine culture results are available, antimicrobial susceptibility testing should guide treatment decisions (Braunwald *et al*., 2001). The primary goal of UTI treatment is to alleviate symptoms, prevent systemic infection, and eradicate pathogenic bacteria from fecal and vaginal reservoirs. Antimicrobial testing helps determine the efficacy of antibiotics such as Amoxicillin, Cephalexin, Gentamicin, and Nalidixic acid against the isolated pathogen. Effective antibiotic therapy ensures the delivery of adequate drug concentrations to the affected site to eliminate the infection.

The treatment of UTIs, whether complicated or uncomplicated, aims to achieve two critical objectives:

1. Prompt resolution of symptoms and prevention of recurrence in affected individuals.
2. Minimizing the emergence of antimicrobial resistance within the microbial ecosystem, or at least limiting its further escalation.

2.12.2 Prevention

Enhancing public awareness of UTI risk factors and causative agents is a key component of prevention. Before resorting to antimicrobial therapy, implementing simple preventive measures can significantly reduce the likelihood of UTIs. These include maintaining adequate hydration to dilute urine, avoiding tight-fitting clothing to discourage bacterial colonization, practicing good personal hygiene, ensuring complete bladder emptying to flush out bacteria, and modifying contraceptive methods to limit spermicide or diaphragm use. Additionally, restricting the use of urinary catheters is an important preventive strategy (Faraz *et al*., 2020).

Certain dietary components, such as citrus juices and berries from the Vaccinium family (e.g., cranberries, bilberries, and lingonberries), have been reported to help prevent symptomatic UTIs and bacteriuria in women with recurrent infections. These fruits contain compounds that reduce bacterial adherence to urinary tract linings, thereby inhibiting bacterial colonization (Ronald, 2005; Jackson, 2006).

Given the global rise in antibiotic-resistant uropathogens, alternative non-antimicrobial strategies for UTI treatment and prevention are being explored. Preliminary studies have examined an anti-*E. coli* vaccine designed to prevent bacterial adhesion to uroepithelial cells, particularly in women prone to recurrent UTIs (Zakri *et al*., 2008). Furthermore, vaginal probiotics containing Lactobacillus species have been investigated for their potential in restoring normal vaginal flora and reducing UTI recurrence (Reid, 2002). Similarly, the

consumption of fermented dairy products enriched with probiotics has been linked to a reduced risk of UTIs (Kontiokari *et al*., 2003).

2.13 Antimicrobial Resistance Patterns

2.13.1 Global Trends in Antimicrobial Resistance

Antimicrobial resistance (AMR) represents one of the most pressing global public health challenges, threatening the effective treatment of bacterial infections, including urinary tract infections (UTIs). The World Health Organization (WHO) estimates that AMR contributes to approximately 1.27 million deaths annually, with projections suggesting up to 10 million deaths by 2050 if current trends persist (World Health Organization, 2020; O'Neill, 2016). The global burden of AMR is driven by the increasing prevalence of multidrug-resistant (MDR) bacteria, which exhibit resistance to multiple classes of antibiotics, complicating treatment and increasing healthcare costs (Laxminarayan *et al*., 2013). Uropathogens, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are among the key contributors to AMR, particularly in community and hospital settings (Flores-Mireles *et al*., 2015).

Common antibiotics affected by resistance in uropathogens include β-lactams (e.g., ampicillin, amoxicillin), tetracyclines, sulfonamides (e.g., co-trimoxazole), fluoroquinolones (e.g., ciprofloxacin), and aminoglycosides (e.g., gentamicin). For instance, *E. coli*, the most prevalent uropathogen, shows resistance rates of 50–80% to ampicillin and 30–50% to co-trimoxazole in many regions, particularly in low- and middle-income countries (LMICs) (Tandogdu and Wagenlehner, 2016). *S. aureus*, including methicillin-resistant strains (MRSA), exhibits high resistance to penicillin and methicillin, with increasing resistance to non-β-lactam antibiotics like erythromycin and clindamycin (Turner *et al*., 2019).

Fluoroquinolone resistance is also rising, with global studies reporting 20–40% resistance rates among uropathogens, limiting their use as first-line treatments for UTIs (Gupta *et al*., 2011). The spread of extended-spectrum β-lactamase (ESBL)-producing bacteria, such as *Klebsiella* spp. and *E. coli*, further exacerbates the challenge, as these strains resist third-generation cephalosporins, necessitating the use of last-resort antibiotics like carbapenems (Pitout and Laupland, 2008). The global rise in AMR underscores the need for surveillance and antimicrobial stewardship to preserve the efficacy of existing antibiotics and guide treatment strategies.

2.13.2 AMR in Nigeria

In Nigeria, the prevalence of AMR among uropathogens is alarmingly high, driven by widespread antibiotic misuse, inadequate infection control, and limited diagnostic infrastructure. Studies across Nigeria report significant resistance to commonly used antibiotics among bacterial isolates from urine samples. For example, a study in a tertiary hospital in Lagos found that *S. aureus* isolates from UTI patients exhibited 80–100% resistance to ampicillin, 60–75% to tetracycline, and 50–70% to co-trimoxazole (Nwadike *et al*., 2014). Similarly, *E. coli* isolates from urine samples in Ile-Ife showed 85% resistance to ampicillin, 65% to co-trimoxazole, and 40% to ciprofloxacin, indicating a high burden of MDR uropathogens (Odetoyin *et al*., 2019). Fluoroquinolone resistance, particularly to ciprofloxacin, is increasingly reported, with resistance rates ranging from 30–50% among uropathogens in Nigerian hospitals (Iregbu and Nwajiobi-Princewill, 2013). These patterns limit treatment options, often requiring more expensive and less accessible antibiotics, such as carbapenems or vancomycin, which are not routinely available in community settings (Olalekan *et al*., 2020).

Several factors contribute to the high prevalence of AMR in Nigeria. Antibiotic misuse, including self-medication and over-the-counter availability, is a major driver. A study in Nigeria revealed that 70% of individuals purchase antibiotics without a prescription, often using them inappropriately for non-bacterial infections or incomplete courses (Olayinka *et al*., 2018). This practice promotes the selection of resistant strains, particularly in community settings like universities, where students may share antibiotics or use them without medical supervision (Ogbolu *et al*., 2018). Over-the-counter availability is facilitated by lax regulatory enforcement, allowing pharmacies and informal vendors to dispense antibiotics freely (O'Neill, 2016). Additionally, poor infection control measures, such as inadequate sanitation in healthcare facilities and community settings, contribute to the spread of resistant bacteria (Adeyemo *et al*., 2021). Limited access to diagnostic tools, such as culture and susceptibility testing, further exacerbates AMR, as empirical treatment often relies on broad-spectrum antibiotics, increasing selective pressure for resistance (Iregbu and Nwajiobi-Princewill, 2013). These factors highlight the urgent need for targeted interventions, including antimicrobial stewardship programs and improved surveillance, to address AMR in Nigeria, particularly in populations like university students who may serve as reservoirs for resistant bacteria

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

The study adopted a **cross-sectional descriptive research design** to investigate the prevalence of *Methicillin-resistant Staphylococcus aureus* (MRSA) and other bacterial isolates in urine samples collected from selected students at the University of Benin, Nigeria. This design was considered appropriate because it enables the assessment of microbial profiles and bacterial loads in urine samples at a single point in time without manipulation of variables. The cross-sectional approach allowed for the **identification of bacterial pathogens,** including MRSA, determination of their **antimicrobial susceptibility patterns,** and quantification of bacterial counts expressed as colony-forming units per milliliter (CFU/mL) among the study population. Data collection involved the administration of questionnaires, urine sample collection, and subsequent **microbiological, biochemical, and molecular analyses.** These procedures provided a comprehensive evaluation of the prevalence and resistance patterns of uropathogens in a community setting, thereby addressing the objectives of the study effectively.

3.2 Study Area

The research was conducted at the University of Benin, located in Benin City, Edo State, Nigeria, within the Ovia North East Local Government Area (LGA). Ovia North East LGA is a prominent administrative division in Benin City, characterized by a mix of residential, educational, and commercial developments. The University of Benin, a federal tertiary institution, is a major educational hub in the region, hosting a diverse student population living in on-campus hostels and off-campus accommodations. The study area was selected due to its large student population, dense living conditions in hostels, and the potential for

environmental factors, such as shared sanitary facilities, to influence the prevalence of bacterial pathogens in urine samples.

The study specifically targeted students residing in selected hostels on the University of Benin's main campus (Ugbowo Campus). Five hostels were chosen based on their accessibility, cooperation of hostel management, population size, and willingness to participate in the study. These hostels included Hall 1, Hall 2, Hall 3, Hall 4, and NDDC Hostel, which collectively house a significant proportion of the university's student population. These hostels reflect typical living conditions, including shared bathrooms and variable hygiene practices, which are relevant to the study's focus on UTI-associated pathogens and antimicrobial resistance.

3.3 Study Population

The target population consisted of undergraduate students aged 16–30 years residing in the selected hostels at the University of Benin. A total of sixty (60) students were randomly selected from the five hostels to ensure equal representation and minimize selection bias. The sample included both male and female students to capture a broad spectrum of bacterial carriage patterns in urine samples. Participation was voluntary and subject to obtaining informed consent from the students or, where applicable, their guardians (for students under 18 years). Ethical approval was obtained from the University of Benin Ethics Committee, and permission was granted by the hostel management and university authorities prior to sample collection.

3.4 Sample Collection Procedure

Urine samples were collected from the selected students under aseptic conditions to prevent contamination. Each participant was provided with a sterile, wide-mouthed, screw-capped urine container and instructed to collect a midstream clean-catch urine sample. Approximately 10–20 mL of midstream urine was collected in the sterile container, which was labeled with a unique identification code corresponding to the participant and hostel. The samples were and transported immediately to the Microbiology Laboratory at the University of Benin for analysis.

3.5.Sterilization of Materials

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160^{o}C for 1 hr. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.6.Preparation and Sterilization of media

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, stirring glass rod and measuring cylinder. All glassware which include MacCartney bottles, Petri dishes, test tubes, conical flasks, measuring cylinders and pipettes, were sterilized at 160 ^{o}C for 1 hr in a hot-air-oven before use. The media used in this study were sterilized at 121 ^{o}C for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 ^{o}C. Aseptic conditions were ensured during inoculation and subculturing

3.7.Preparation of Culture Media

The media used were prepared according to the manufacturer's instructions. Media used were Nutrient Agar, Manittol Salt agar and Oxacillin Resistance Screening Agar Base

3.7.1. Preparation of Nutrient agar

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium will be the placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask will be allowed to cool.

3.7.2.Preparation of Manittol Salt agar

111 grams of agar was suspended in 1000 ml distilled water and heated to boiling, to dissolve the medium completely. It was then mixed properly and distributed in conical flasks. The medium was sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 mins and then left to cool before dispension on sterile petri dishes.

3.7.3. Preparation of Oxacillin Resistance Screening Agar Base (ORSAB)

51.75 grams of the dehydrated ORSAB powder was accurately weighed and suspended in 500 ml of purified distilled water. The mixture was heated to boiling with continuous stirring to ensure complete dissolution of the medium. Following dissolution, the medium was sterilized by autoclaving at a pressure of 15 pounds per square inch (equivalent to 121°C) for 15 minutes. After autoclaving, the medium was allowed to cool to a temperature range of 45–50°C. At this point, the rehydrated contents of one vial of XA Selective Supplement (FD191) were aseptically added to the cooled medium. The mixture was gently swirled to ensure thorough and even distribution of the selective supplement. The supplemented medium was then poured into sterile Petri dishes (approximately 15–20 ml per plate) under aseptic

conditions and allowed to solidify at room temperature. Once solidified, the plates were stored at 4°C in sealed containers until ready for use.

3.8 Isolation and Enumeration of Bacterial Isolates

Upon arrival at the Microbiology Laboratory, urine samples were processed for microbiological analysis to determine the total heterotrophic bacterial load, isolate potential uropathogens, and identify methicillin-resistant *Staphylococcus aureus* (MRSA) and other bacterial contaminants. Each urine sample was thoroughly mixed by gentle inversion to ensure homogeneity. A 1 mL aliquot of each urine sample was aseptically transferred into a test tube containing 9 mL of sterile normal saline to create a 10^{-1} dilution. Serial tenfold dilutions were prepared up to 10^{-4}, as needed, to achieve countable colony numbers for accurate enumeration (Cheesbrough, 2006).

For bacterial enumeration, the pour plate technique was employed. From each dilution, 1 mL was aseptically dispensed into sterile Petri dishes labeled appropriately. Approximately 15–20 mL of molten Nutrient Agar (for total bacterial counts) and Mannitol Salt Agar (for *S. aureus* isolation), cooled to approximately 45°C, was poured into each plate. The contents were gently swirled to ensure even distribution of the inoculum within the agar medium and allowed to solidify. The plates were incubated in an inverted position at 37°C for 24–48 hours (Cheesbrough, 2006).

Following incubation, colonies on the agar plates were counted using a digital colony counter. Plates with 30–300 colonies were selected for enumeration to ensure statistical reliability, as counts outside this range may be less accurate due to overcrowding or insufficient colonies (Gui *et al*., 2014). The total viable bacterial count was calculated using the formula:

$$Cfu/ml = \frac{number\ of\ colonies}{volume\ of\ plated\ (ml) x\ dilution\ factor}$$

(Gui *et al.,* 2014).

The bacterial load was expressed as colony-forming units per milliliter (CFU/mL) of urine. Distinct colonies were subcultured for further identification using standard microbiological techniques, including Gram staining, catalase, coagulase, and other biochemical tests to identify *S. aureus* and other uropathogens. Suspected *S. aureus* isolates were tested for methicillin resistance by plating inOxacillin Resistance Screening Agar Base (ORSAB).

3.9. Bacterial Identification

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation on nutrient agar and MacConkey agar. Biochemical tests were also carried out to further identify the bacterial isolates.

3.9.1 Gram staining

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective (Cheesbrough, 2010).

3.9.2. BIOCHEMICAL TEST

3.9.2.1. 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 (Fawole and Oso, 2004).

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

3.9.2.2. 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 (Fawole and Oso, 2004).

3.9.2.3. 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 (Cheesbrough, 2010).

3.9.2.4. Potassium Hydroxide (KOH) test

Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium. No reaction (absence of stringing) indicates a Gram-positive bacterium (Fawole and Oso, 2004).

3.9.2.6. Citrate utilization test

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate members of Enterobacteriaceae capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green (Cheesbrough, 2010).

3.9.2.7. Triple sugar iron (TSI) agar test

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H_2S) or gas (O_2), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through

the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H_2S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2) (Fawole and Oso, 2007).

3.10 Antibiotic Susceptibility Test

The identified colonies of bacteria were subjected to antibacterial susceptibility testing (AST) to determine their resistance or susceptibility profiles against commonly used antibiotics within the locality. The test was carried out using the standard disc diffusion method (Kirby–Bauer technique) on Mueller Hinton Agar (MHA), with antibiotic discs obtained from Oxoid, UK. This method was adopted in line with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

For the assay, bacterial cultures aged between 18 and 24 hours were prepared and standardized to the 0.5 McFarland turbidity standard, equivalent to approximately 1.5×10^8 CFU/mL. The standardized inoculum was streaked uniformly across the surface of sterile MHA plates using a sterile loop. Antibiotic discs were then carefully placed on the inoculated plates with sterile forceps, ensuring proper spacing to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 24 hours, after which the diameters of the zones of

inhibition around each disc were measured in millimeters using a meter rule. The antibiotics employed in this study, along with their disc concentrations, were as follows: Cefuroxime (30 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Cloxacillin (5 µg), Ofloxacin (5 µg), Ceftazidime (30 µg), Augmentin (30 µg; Amoxicillin–Clavulanic acid), and Oxacillin (1 µg). These antibiotics were selected to represent a wide spectrum of drug classes, including β-lactams, aminoglycosides, fluoroquinolones, and macrolides, thereby allowing for a comprehensive assessment of resistance patterns among the isolates. Interpretation of the susceptibility results followed the Clinical and Laboratory Standards Institute (CLSI) guidelines, with isolates categorized as Resistant (R), Intermediate (I) and Susceptible (S) when zones measured ≥ 17 mm (Bauer et al., 1966; Cheesbrough, 2000; CLSI, 2012).

3.10.1 Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Pure *S. aureus* colonies were streaked onto the surface of ORSAB plates and incubated aerobically at 35–37°C for 24 to 48 hours. Following incubation, plates were examined for the presence of blue-colored colonies, which are characteristic of MRSA due to their ability to hydrolyze the chromogenic substrate in the presence of oxacillin (Becker *et al*., 2016). Colonies exhibiting this distinct blue pigmentation were recorded as presumptive MRSA isolates. To confirm methicillin resistance, presumptive MRSA isolates were subjected to the cefoxitin disc diffusion test, as cefoxitin is a reliable surrogate for detecting *mecA*-mediated resistance. A 30 µg cefoxitin disc was placed on Mueller-Hinton Agar plates inoculated with a standardized suspension of each isolate (0.5 McFarland standard), and zones of inhibition were measured after incubation at 35°C for 24 hours. Isolates with a zone diameter of ≤21 mm were classified as MRSA, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018).

3.10.2. Multiple Antibiotic Resistance (MAR) Index

According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source. In this study the MAR index was determined by employing the methods delineated by Chitanand *et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates.

$$MAR\ index = \frac{y}{nx}$$

$$where\ y = number\ of\ resistance\ scored,$$

$$n = number\ of\ isolates\ and$$

$$x = total\ number\ of\ antibioitics$$

It is a general established rule that MAR index greater than 0.2 is indicative of the fact that the bacterium originates from areas where antibiotics have been abused (or regularly used) or worse still from areas of high-risk source of contamination.

CHAPTER FOUR

RESULTS

The results of the urinalysis conducted on urine samples collected from 60 selected students are presented in Table 4.1. Of the samples, 24% tested positive for leukocytes, suggesting possible urinary tract infections. Nitrites were detected in 17% of samples, supporting this finding. Protein was present in 15% of samples, and blood (hematuria) appeared in 10%, indicating potential urinary tract irritation or damage. Elevated urobilinogen was found in 6.7% of samples. The urine pH ranged from 5.0 to 8.0, with an average of 6.2, and specific gravity varied between 1.005 and 1.030, with a mean of 1.018, reflecting varied hydration levels. Ascorbic acid was elevated in 6.7% of samples, which may affect test accuracy. Ketones were detected in 3.3% of samples, possibly due to fasting or diet. Bilirubin and glucose were present in 1.7% and 3.3% of samples, respectively, suggesting potential liver issues or undiagnosed diabetes. Most samples (71.7%) were pale yellow, 20% amber, and 8.3% cloudy. These findings indicate a significant portion of students may have urinary abnormalities requiring further investigation.

The result of the mean total viable count (TVC) of urine samples according to age group and sex is presented in **Table 4.2**. Female samples generally recorded higher TVC values than male samples across all age ranges. The highest mean TVC for females ($5.2 \pm 0.4 \times 10^5$ CFU/mL) was recorded in the 21–25 age group, while the lowest ($4.0 \pm 0.1 \times 10^5$ CFU/mL) was observed in the 31–35 age group. Among males, the mean TVC ranged from $3.5 \pm 0.2 \times 10^5$ CFU/mL to $4.1 \pm 0.3 \times 10^5$ CFU/mL, also peaking in the 26–30 age group.

The result of the mean total ***Staphylococcus*** count on Mannitol Salt Agar (MSA) is presented in **Table 4.3.** Across all age groups, female samples showed higher Staphylococcus counts than male samples. The 21–25 age group had the highest mean count for females ($3.8 \pm 0.3 \times 10^5$ CFU/mL), while the lowest for males was observed in the 31–35 age group ($2.8 \pm 0.1 \times 10^5$ CFU/mL).

Table 4.4 outlines the cultural, morphological, and biochemical characteristics of the isolated bacteria. A total of six distinct bacteria were identified from the urine samples: *Staphylococcus aureus*, *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Pseudomonas spp.*, and *Enterococcus faecalis*.

The result showing the prevalence of bacterial isolates in all urine samples (n = 60) is presented in **figure 4.1.** *Staphylococcus aureus* was the most prevalent isolate (33%), followed by *Escherichia coli* (21%), and *Pseudomonas sp.* (11%). Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 16.7%, while *Klebsiella sp.*, *Proteus sp.*, and *Enterococcus sp.* were less prevalent.

The distribution of bacterial isolates by sex is shown in Table 4.5. Female participants exhibited a higher prevalence of all bacterial isolates. For instance, *Staphylococcus aureus* was found in 36.1% of female samples compared to 29.2% in male samples. Similar trends were observed for *E. coli*, MRSA, and *Pseudomonas spp*.

The result indicating the percentage prevalence of MRSA in urine samples based on age and sex is presented in **Table 4.6**. The overall prevalence of MRSA was 16.7%, with the highest occurrence in the 21–25 age group. Female samples showed a higher MRSA detection rate (11.7%) compared to male samples (5.0%).

The result of the antibiotic susceptibility pattern of the bacterial isolates is presented in **Table 4.7.** The isolates exhibited varying degrees of resistance and susceptibility to the tested antibiotics. Notably, *Staphylococcus aureus* showed strong resistance to Cloxacillin and Oxacillin, while *Proteus sp.* and *Klebsiella sp.* were generally susceptible to Ofloxacin and Ceftriaxone. The susceptibility profiles were interpreted using standard zones of inhibition: Susceptible (≥ 17 mm), Intermediate (11–16 mm), and Resistant (≤ 10 mm).

The result of the MAR index of bacterial isolates is presented in **Table 4.7.** The highest MAR index (0.44) was observed in *Staphylococcus aureus*, indicating significant multidrug resistance. *Escherichia coli*, *Pseudomonas sp.*, and *Enterococcus sp.* each had a MAR index of 0.22, while *Klebsiella sp.* and *Proteus sp.* showed the lowest MAR index of 0.11.

Table 4.1: Urinalysis Test Results for Urine Samples from Selected Students (n = 60).

Parameter	No. Positive (n)	Percentage (%)	Permissible Limit / Normal Range
Leukocytes (LEU)	14	23.3	0 – 10 WBC/µL (Negative)
Nitrites (NIT)	10	16.7	Negative
Urobilinogen (URO)	4	6.7	0.2 – 1.0 mg/dL
Protein (PRO)	9	15.0	<150 mg/day (≤10 mg/dL in spot urine)
pH	–	–	4.6 – 8.0
Blood (BLO)	6	10.0	0 – 5 RBC/µL (Negative)
Specific Gravity (SG)	–	–	1.005 – 1.030
Ascorbic Acid (ASCOR)	4	6.7	<40 mg/dL
Ketones (KET)	2	3.3	Negative (<0.6 mmol/L)
Bilirubin (BILI)	1	1.7	Negative (<0.02 mg/dL)
Glucose (GLU)	2	3.3	Negative (<15 mg/dL)

Table 4.2: Mean Total Viable Count (TVC) (× 10^5CFU/mL) of Urine Samples Based on Age and Sex

Age Range (Years)	No. of Samples	Female	Male
16–20	18	4.5 ± 0.3	3.8 ± 0.2
21–25	24	5.2 ± 0.4	4.0 ± 0.3
26–30	12	4.8 ± 0.2	4.1 ± 0.3
31–35	6	4.0 ± 0.1	3.5 ± 0.2

Values are represented as mean ± standard deviation

Table 4.3: Mean Total *Staphylococcus* Count on Mannitol Salt Agar (MSA)

Age Range (Years)	No. of Samples	Female	Male
16–20	18	3.5 ± 0.2	2.9 ± 0.3
21–25	24	3.8 ± 0.3	3.0 ± 0.2
26–30	12	3.6 ± 0.2	3.1 ± 0.1
31–35	6	3.2 ± 0.2	2.8 ± 0.1

Values are represented as mean ± standard deviation

Table 4.4: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolate

Characteristic	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Elevation	Raised	Flat	Raised	Raised	Raised	Raised
Margin	Entire	Undulate	Undulate	Wavy	Entire	Entire
Color	Cream	Cream	Green	Pale Yellow	Cream	Mucoid/Cream
Shape	Circular	Irregular	Irregular	Circular	Circular	Circular
Size	Medium	Large	Medium	Medium	Small	Large
Gram Stain	+	-	-	-	+	-
Cell Type	Cocci	Rod	Rod	Rod	Cocci	Rod
Arrangement	Clusters	Disperse	Disperse	Clusters	Chains	Capsules
Color (Gram Reaction)	Purple	Pink	Pink	Pink	Purple	Pink
KOH String Test	-	+	-	+	-	+
Catalase	+	+	+	+	-	+
Indole	-	+	-	+	-	-
Citrate	-	-	+	+	-	+
Oxidase	-	-	+	-	-	-
Glucose	+	+	+	+	+	+
Sucrose	+	-	-	+	+	+
Lactose	+	+	-	-	+	+
Gas Formation	-	+	-	+	-	+
H_2S Formation	-	-	-	+	-	-
TSI (Slant/Butt)	K/A	A/AG	K/AG	K/AH_2S	A/A	A/AG
Identity	*Staphylococcus aureus*	*E. coli*	*Pseudomonas* sp.	*Proteus* sp.	*Enterococcus* sp.	*Klebsiella* sp.

Key: (-) negative test; (+) positive test; (A) Acid; (K) Alkaline; (G) Gas production (bubbles); (H_2S) Hydrogen sulphide (black precipitate); (KOH) Potassium hydroxide test; (TSI) Triple sugar iron test

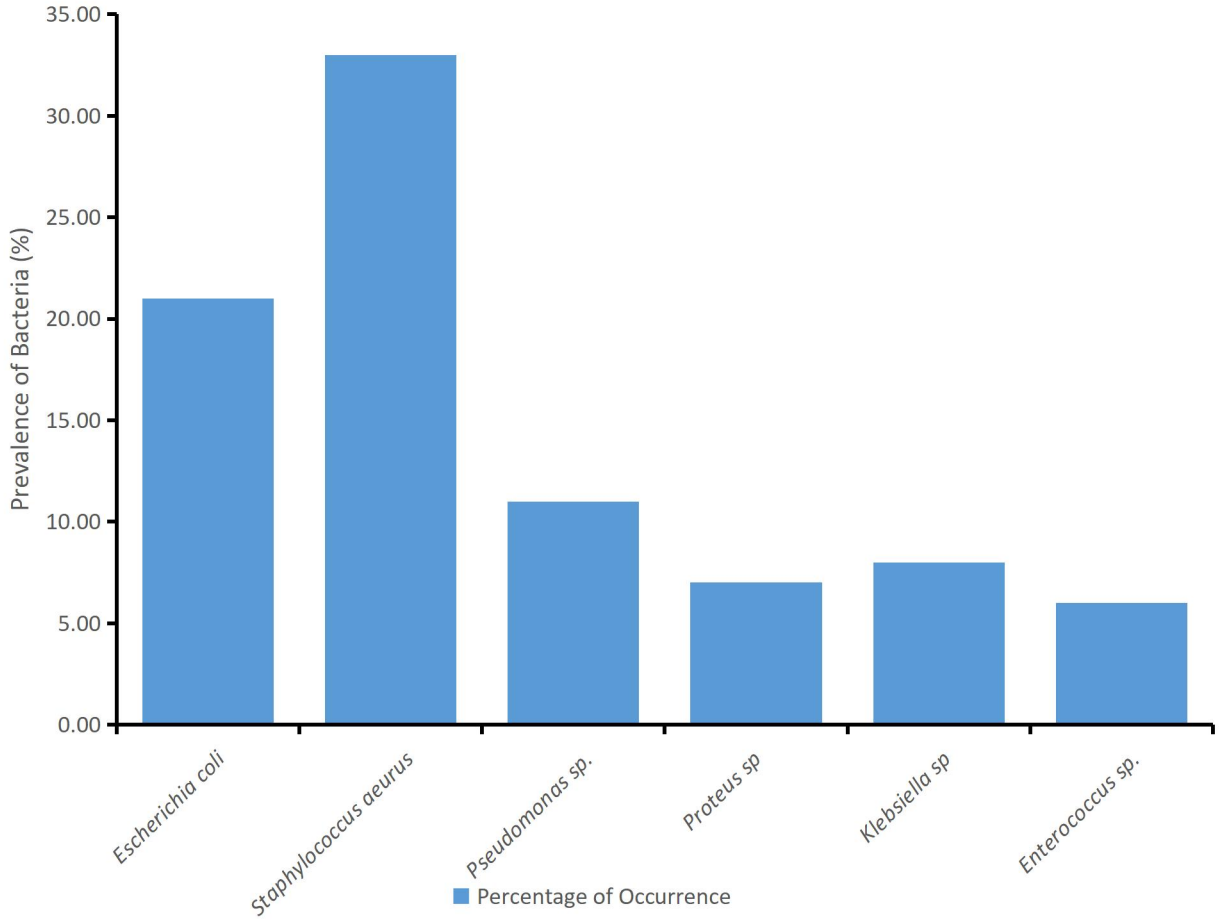

Figure 4.1: Prevalence of Bacterial Isolates in Urine Samples (n = 60)

Table 4.5: Distribution of Bacterial Isolates Based on Sex

Bacterial Isolate	Female (n = 36)	Prevalence (%)	Male (n = 24)	Prevalence (%)
Staphylococcus aureus	13	36.1	7	29.2
Escherichia coli	8	22.2	5	20.8
MRSA	7	19.4	3	12.5
Pseudomonas spp.	4	11.1	3	12.5
Klebsiella spp.	3	8.3	2	8.3
Enterococcus faecalis	2	5.6	1	4.2
Proteus spp.	2	5.6	2	8.3

Table 4.6: Percentage Prevalence of MRSA in Urine Samples by Age and Sex

Age Range (Years)	No. of Samples	Female	Male	Total
16–20	18	2 (11.1%)	1 (5.6%)	3 (16.7%)
21–25	24	3 (12.5%)	1 (4.2%)	4 (16.7%)
26–30	12	1 (8.3%)	1 (8.3%)	2 (16.7%)
31–35	6	1 (16.7%)	0 (0.0%)	1 (16.7%)
Total	60	7 (11.7%)	3 (5.0%)	10 (16.7%)

Table 4.8. Antibiotic Susceptibility Pattern of the Bacterial Isolates (Zone of Inhibition in mm)

Isolate	Cefuroxime (CRX)	Gentamycin (CN)	Ceftriaxone (CTR)	Erythromycin (E)	Cloxacillin (CXC)	Ofloxacin (OFX)	Ceftazidime (CAZ)	Augmentin (AU)	Oxacillin (OX)	MAR Index
Escherichia coli	12 (I)	14 (I)	20 (S)	15 (I)	10 (R)	16 (I)	18 (S)	10 (R)	20(S)	0.22
Staphylococcus aureus	10 (R)	18 (S)	12 (I)	14 (I)	0 (R)	22 (S)	9 (R)	16 (I)	6 (R)	0.44
***Proteus* sp.**	14 (I)	18 (S)	21 (S)	13 (I)	10 (R)	18 (S)	19 (S)	12 (I)	16 (I)	0.11
***Pseudomonas* sp.**	10 (R)	20 (S)	18 (S)	10 (R)	12 (I)	19 (S)	17 (S)	14 (I)	15 (I)	0.22
***Klebsiella* sp.**	12 (I)	16 (I)	20 (S)	14 (I)	10 (R)	20 (S)	18 (S)	12 (I)	16 (I)	0.11
***Enterococcus* sp.**	11 (I)	14 (I)	17 (S)	13 (I)	9 (R)	17 (S)	16 (I)	10 (R)	14 (I)	0.22

Key:

- **S**: Susceptible (≥ 17 mm)
- **I**: Intermediate (11–16 mm)
- **R**: Resistant (≤ 10 mm)
- Cefuroxime (CRX) – 30 µg
- Gentamicin (CN) – 10 µg
- Ceftriaxone (CTR) – 30 µg
- Erythromycin (E) – 15 µg
- Cloxacillin (CXC) – 5 µg
- Ofloxacin (OFX) – 5 µg
- Ceftazidime (CAZ) – 30 µg
- Augmentin (AU) – 30 µg (Amoxicillin–Clavulanic acid)
- Oxacillin (OX) – 1 µg

CHAPTER FIVE

DISCUSSION

Urinary tract infections (UTIs) remain one of the most common bacterial infections affecting individuals of all age groups, particularly females (Czajkowski *et al*., 2021). In recent years, the emergence of antibiotic-resistant organisms such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) has added complexity to the treatment and control of UTIs. Methicillin-Resistant *Staphylococcus aureus* (MRSA), once confined largely to hospitals, is now increasingly being identified in community-acquired infections, including among healthy individuals. This study seeks to investigate the prevalence of MRSA in urine samples collected from selected students in University of Benin Edo State, Nigeria.

The urinalysis results revealed that 14 (23.3%) of urine samples were positive for leukocytes, and 10 (16.7%) were positive for nitrites, suggesting a significant proportion of students may have urinary tract infections (UTIs). These findings align with studies indicating that leukocytes and nitrites are reliable indicators of bacterial infection in the urinary tract (Flores-Mireles *et al*., 2015; Masajtis-Zagajewska and Nowicki, 2017). The presence of protein (15%) and blood (10%) in samples further supports the possibility of urinary tract irritation or damage, consistent with UTI pathology or other renal issues (Foxman, 2014). The detection of elevated urobilinogen (6%), bilirubin (1.7%), and glucose (3.3%) suggests potential liver dysfunction or undiagnosed diabetes in a small subset of the population, warranting further clinical evaluation (Abebayehu*et al*., 2023). The variation in urine pH (5.0–8.0, mean 6.2) and specific gravity (1.005–1.030, mean 1.018) reflects differences in hydration status, which may influence bacterial growth and UTI risk. The high ascorbic acid levels in

6.7% of samples could interfere with test accuracy, as noted in previous studies (Flores-Mireles *et al*., 2015). The predominance of pale yellow urine (72%) is typical of healthy hydration, while cloudy urine (8%) may indicate bacterial contamination or other abnormalities (Iregbu and Nwajiobi-Princewill, 2013). These results suggest that a notable proportion of students may have undiagnosed urinary abnormalities, highlighting the need for routine health screenings in university settings.

The mean total viable count (TVC) of urine samples showed higher bacterial loads in female students compared to males across all age groups, with the highest mean TVC for females (5.2×10^5 CFU/mL) in the 21–25 age group. This finding is consistent with studies reporting higher UTI prevalence in females due to anatomical factors, such as a shorter urethra, which facilitates bacterial ascension (Foxman, 2014). The higher TVC in the 21–25 age group may reflect increased risk factors, such as sexual activity or poor hygiene practices, common among university students in this age range (Elorfaly*et al*., 2024). Male samples showed lower TVC values ($3.5–4.1 \times 10^5$ CFU/mL), with a peak in the 26–30 age group, possibly due to differences in exposure to risk factors or hygiene practices (Odetoyin *et al*., 2019).

The mean *Staphylococcus* count on Mannitol Salt Agar also indicated higher counts in females (e.g., 3.8×10^5 CFU/mL in the 21–25 age group) compared to males (2.8×10^5 CFU/mL in the 31–35 age group). This aligns with the higher prevalence of *S. aureus* in female urine samples and may be attributed to perineal colonization, which is more common in females (Tong *et al*., 2015). These bacterial counts, exceeding the threshold for significant bacteriuria ($\geq 10^5$ CFU/mL), suggest active infection or colonization in a substantial portion of the study population, necessitating further investigation into risk factors and clinical implications (Flores-Mireles *et al*., 2015).

The prevalence of bacterial isolates showed *Staphylococcus aureus* as the most common uropathogen (33%), followed by *Escherichia coli* (21%) and *Pseudomonas* spp. (11%). This distribution is notable, as *E. coli* is typically the predominant uropathogen globally (70–90% of community-acquired UTIs), while *S. aureus* is less common (5–10%) (Flores-Mireles *et al*., 2015). The prevalence of *E. coli* (21%), though lower than expected, aligns with Nigerian studies reporting a shift toward gram-positive uropathogens in community settings (Ekwealor *et al*., 2016) and correlates with findings by Poonam and Ulka (2013), as well as other researchers (Nadia *et al*., 2004; Bloomberg *et al*., 2005; Ojo and Anibijuwon, 2010; Boekitwetan *et al*., 2012; Geofrey *et al*., 2013). Other isolates, including *Pseudomonas* spp. (11%), *Klebsiella* spp. (8%), *Proteus* spp. (7%), and *Enterococcus faecalis* (6%), further highlight the diversity of uropathogens, consistent with regional studies (Iregbu and Nwajiobi-Princewill, 2013). The higher prevalence of all isolates in females (e.g., *S. aureus*: 36.1% vs. 29.2%; *E. coli*: 22.2% vs. 20.8%) is attributed to anatomical and behavioral factors (Foxman, 2014).

The overall prevalence of MRSA in urine samples was 16.7%, with a higher detection rate in females (11.7%) compared to males (5.0%). The highest MRSA prevalence was observed in the 21–25 age group, which corresponds to the peak TVC and *Staphylococcus* counts, suggesting a correlation between bacterial load and resistance in this demographic (Olalekan *et al*., 2020). This prevalence is within the range reported for community-acquired MRSA in Nigeria (10–20%) but lower than hospital-based studies (25–40%) (Olalekan *et al*., 2020; Nwadike *et al*., 2014). The presence of MRSA in a healthy student population is concerning, as it indicates a community reservoir for resistant strains, potentially driven by antibiotic misuse or poor hygiene practices in hostels (David and Daum, 2010). The higher MRSA prevalence in females

may be linked to increased perineal colonization or behavioral factors, such as frequent antibiotic use for UTIs (Adeyemo *et al*., 2021). These findings underscore the need for targeted surveillance and infection control measures in university settings to curb the spread of MRSA.

The antibiotic susceptibility patterns revealed significant resistance among bacterial isolates, particularly *S. aureus*, which showed strong resistance to cloxacillin (0 mm) and oxacillin (6 mm), consistent with the 16.7% MRSA prevalence. This resistance is mediated by the *mecA* gene, which encodes penicillin-binding protein 2a (PBP2a), rendering β-lactam antibiotics ineffective (Peacock and Paterson, 2015). *S. aureus* also exhibited intermediate susceptibility to cefuroxime (10 mm) and erythromycin (14 mm), indicating limited treatment options (Turner *et al*., 2019). In contrast, *Proteus* spp. and *Klebsiella* spp. showed susceptibility to ofloxacin and ceftriaxone, aligning with studies suggesting these antibiotics remain effective against gram-negative uropathogens in Nigeria (Odetoyin *et al*., 2019). *Pseudomonas* spp. displayed resistance to cefuroxime and erythromycin, reflecting its intrinsic resistance mechanisms, such as efflux pumps (Lister *et al*., 2009). *Enterococcus faecalis* showed variable susceptibility, with resistance to cloxacillin and augmentin, consistent with its known resistance profile (Arias and Murray, 2012). These findings highlight the challenge of treating UTIs in this population due to widespread resistance, emphasizing the need for susceptibility-guided therapy.

The MAR index indicated significant multidrug resistance, with *S. aureus* exhibiting the highest MAR index (0.44), followed by *E. coli*, *Pseudomonas* spp., and *Enterococcus faecalis* (0.22 each), and *Klebsiella* spp. and *Proteus* spp. (0.11 each). An MAR index above 0.2 suggests exposure to multiple antibiotics, likely due to

misuse or overuse, which is prevalent in Nigeria (O'Neill, 2016). The high MAR index for *S. aureus* corroborates its MRSA prevalence and resistance to β-lactam antibiotics, posing a significant public health threat (Olalekan *et al*., 2020). The lower MAR indices for *Klebsiella* and *Proteus* spp. suggest they may be less exposed to selective pressure in this population, possibly due to targeted use of effective antibiotics like ofloxacin (Odetoyin *et al*., 2019). These results indicate a high burden of multidrug-resistant uropathogens among university students, necessitating antimicrobial stewardship programs to promote rational antibiotic use.

The findings have several implications for public health and clinical practice. The high prevalence of leukocytes, nitrites, and bacterial isolates, particularly *S. aureus* and MRSA, suggests a significant burden of UTIs and asymptomatic bacteriuria among University of Benin students. The higher bacterial loads and MRSA prevalence in females highlight the need for gender-specific interventions, such as hygiene education and routine UTI screening (Foxman, 2014). The widespread antibiotic resistance, particularly among *S. aureus*, underscores the urgency of implementing antimicrobial stewardship programs to curb misuse and preserve antibiotic efficacy (O'Neill, 2016). The presence of MRSA in a community setting like a university indicates a potential reservoir for transmission, necessitating improved sanitation in hostels and public health campaigns to raise awareness about antibiotic resistance (David and Daum, 2010). The diverse microbial profile and high MAR indices further emphasize the need for susceptibility testing to guide treatment, especially in resource-limited settings where empirical therapy is common (Iregbu and Nwajiobi-Princewill, 2013).

5.1. CONCLUSION

This study provides critical insights into the microbial composition, bacterial load, and antibiotic resistance patterns of uropathogens in urine samples from selected students at the University of Benin, Nigeria. The high prevalence of *Staphylococcus aureus*, *Escherichia coli*, and methicillin-resistant *Staphylococcus aureus* (MRSA), coupled with significant bacterial counts (up to 5.2×10^5 CFU/mL in females) and a high MAR index, underscores the substantial burden of urinary tract infections (UTIs) and antimicrobial resistance (AMR) in this community setting. The elevated resistance to β-lactam antibiotics, particularly in MRSA, highlights the urgent need for enhanced antimicrobial surveillance and prudent antibiotic use in university populations. Further research should focus on molecular characterization of resistance genes, such as *mecA*, and explore alternative strategies, such as targeted antimicrobial therapies and hygiene interventions, to address the growing challenge of antibiotic resistance in UTI management among students.

5.2. Recommendations

Based on the study's findings, the following recommendations are proposed to address the prevalence of bacterial uropathogens, MRSA, and antimicrobial resistance among University of Benin students:

1. Implement mandatory urinalysis and microbiological screening for students, particularly females aged 21–25, to detect asymptomatic bacteriuria and UTIs early, preventing complications and transmission of resistant strains .

2. Develop university-based programs to educate students on rational antibiotic use, restrict over-the-counter antibiotic access, and promote prescription-only dispensing to reduce selective pressure for resistance.
3. Upgrade shared bathroom facilities in university hostels and provide hygiene education to reduce bacterial transmission, addressing environmental risk factors identified in the study.
4. Conduct longitudinal studies with molecular techniques (e.g., PCR for *mecA* and *mecC* genes) to monitor AMR trends in community settings, informing national surveillance efforts .
5. Investigate behavioral and environmental factors, such as antibiotic use history and hygiene practices, to identify drivers of bacterial carriage and resistance, enabling targeted interventions.
6. Equip university health centers with microbiological facilities for culture and susceptibility testing to guide UTI treatment, minimizing reliance on empirical therapy.
7. Launch campaigns to raise awareness about AMR among students and local communities, emphasizing responsible antibiotic use and infection prevention strategies.

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