ASSESSMENT OF VANCOMYCIN RESISTANT *Staphylococcus aureus* FROM URINE SAMPLES OF SELECTED STUDENTS IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

BY

Efose Gertrude AISAGBONHI (Miss)

LSC2103905

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

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 REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was successfully carried out by **EFOSE GERTRUDE AISAGBONHI (MISS)** with matriculation number **LSC2103905,** of the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria, under my supervision.

_____ _____

DR. (MRS) J.Z SAIDU **DATE**

(Project Supervisor)

_____ _____

Prof. E.O IGBINOSA **DATE**

(Head of Department)

DEDICATION

This report is dedicated to Almighty God for his Strength, directions and guidance all through the course of my study and this work.

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ABSTRACT

Vancomycin-resistant *staphylococcus aureus* (VRSA) is a strain of *S. aureus* that has developed overtime due to the overuse of vancomycin as an antibiotic for the treatment against *S.aureus x*infections which have reduced the available options of managing the pathogen that causes a lot of infections including UTIs. The aim of this study is to determine the prevalence of Vancomycin-Resistant *Staphylococcus aureus* (VRSA) from urine samples of selected students in the University of Benin, Edo State. A cross-sectional descriptive design was used, and sixty urine samples were collected and analyzed using standard microbiological and biochemical methods to isolate and identify vancomycin-resistant *S.aureus*(VRSA) and assess their antibiotic susceptibility patterns. The results revealed that 23.3% of samples showed leukocytes, while *Staphylococcus aureus* (33%) was the most predominant isolate, followed by *Escherichia coli* (21%) and *Pseudomonas* spp. (11%). The overall VRSA prevalence was 6.7%, with a higher rate among females (5.0%) than males (1.7%). The isolates exhibited high resistance to β-lactam antibiotics, while most remained susceptible to ofloxacin and ceftriaxone. The findings emphasize a significant occurrence of antimicrobial resistance particularly vancomycin-resistant *S. aureus*, among university students. It concludes that improved hygiene practices, rational antimicrobial use, and continuous antimicrobial surveillance are vital to controlling the spread of resistant strains in the university communities.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Staphylococcus aureus (*S.aureus*) is a Gram-positive, spherical bacterium that can cause numerous human infections, including skin and nasal infections, food poisoning, bacteremia, and urinary tract infections (UTIs) (Vasudevan and Ranganathan, 2015). UTIs are common in both males and females and can affect either the upper or lower urinary tract. However, they occur more frequently in females because their shorter urethra provides bacteria from the perianal region with a quicker route to the bladder, increasing the likelihood of infection (Al-Mathkhury and Abdul-Ghaffar,2011).

In recent years, highly virulent strains of S. aureus have emerged with resistance to antibiotics such as methicillin, resulting in the rise of methicillin-resistant Staphylococcus aureus (MRSA). This has increased reliance on vancomycin as a final treatment option for infections caused by these resistant strains (Alghamdi et al., 2023). Vancomycin is a glycopeptide antibiotic effective against staphylococci, streptococci, and other Gram-positive bacteria. It is the primary therapy for MRSA infections because it is bactericidal against most staphylococcal and nonenterococcal streptococcal strains. Its antimicrobial action mainly involves blocking the production of peptidoglycan, a key component of the bacterial cell wall. Vancomycin binds strongly to the terminal D-alanyl-D-alanine groups of cell wall precursors, preventing their incorporation into the growing peptidoglycan chain. This interruption stops proper peptide cross-linking and ultimately inhibits cell wall formation (Gardete *et al.,* 2014).

Vancomycin-resistant *Staphylococcus aureus* (VRSA) is a form of *S. aureus* that has emerged largely because of the prolonged and excessive use of vancomycin to treat *S. aureus* infections, which has limited the available treatment options for managing this pathogen, including cases of

urinary tract infection (Li et al., 2022). Resistance in *S. aureus* develops through two main pathways: Vancomycin-Intermediate *S. aureus* (VISA) and Vancomycin-Resistant *S. aureus* (VRSA). VISA strains, first identified in 1997, show decreased susceptibility by thickening their cell walls, providing additional binding sites that capture vancomycin before it reaches its target. In contrast, true VRSA acquires the vanA gene, usually from Enterococci, which changes the cell wall target from D-Ala-D-Ala to D-Ala-D-Lac. This modification greatly reduces vancomycin's ability to bind effectively, making the drug clinically ineffective. Because this genetic trait can spread between bacteria, it presents a significant public health concern (Ghahremani *et al*., 2015).

VRSA is usually very rare and easily goes undetected but despite its rarity, it is of grave concern because it renders one of the last-line treatment options against multidrug-resistant *S. aureus,* particularly Methicillin-Resistant *S. aureus* ineffective (Ahmed *et al.,* 2024).Studying VRSA prevalence causing UTIs among students addresses the knowledge gap regarding VRSA prevalence among students who reside at the university hostel and are at high risk contracting of UTIs. It contributes to antimicrobial resistance surveillance among student populations. It may help guide empirical therapy for UTIs and inform infection prevention strategies and early detection of VRSA in community settings aid to control its spread before it becomes widespread.

1.2 AIMS AND OBJECTIVES

The aim of this study was to determine the prevalence of Vancomycin-Resistant Staphylococcus aureus (VRSA) in urine samples collected from selected students at the University of Benin, Edo State.

The specific objectives were to:

1. Isolate and identify Staphylococcus aureus from urine samples of selected students
2. Assess the antibiotic susceptibility patterns of the S. aureus isolates
3. Evaluate vancomycin resistance among the isolates using standard laboratory procedures.

4. Detect the prevalence of VRSA in relation to age groups among the selected students

CHAPTER TWO

LITRERATURE REVIEW

This chapter reviews relevant literature on *Staphylococcus aureus,* mechanisms of vancomycin resistance, global and Nigerian prevalence of VRSA, and its significance in urinary tract infections.

2.1. Staphylococcus aureus

Staphylococcus aureus, often called "golden staph," is a gram-positive, spherical bacterium within the family Staphylococcaceae and the genus Staphylococcus. It is non-motile, does not form spores, and is a facultative anaerobe that tests positive for both catalase and coagulase (Boucher et al., 2017). Under the microscope, it typically appears in grape-like clusters. Staphylococcal species are widely distributed across the world, and S. aureus commonly colonizes the upper respiratory tract, gastrointestinal tract, and urogenital tract of around 20 to 30 percent of people who serve as long-term carriers (Gulzar and Zehra, 2018).

S. aureus is a significant pathogen responsible for many diseases in humans and animals. It can inhabit the skin, the mucosal surfaces of the anterior nostrils, the gastrointestinal tract, the perineal area, the urogenital tract, the pharynx, and other body sites (Choo and Chambers, 2016). The organism is also present in the environment and in various foods derived from animals. Because it tolerates drying and harsh conditions, S. aureus can survive in stressful environments. In humans, infections caused by this bacterium range from mild skin conditions to severe and potentially fatal illnesses such as endocarditis, meningitis, pneumonia, osteomyelitis, gastroenteritis, septicemia, and toxic shock syndrome.

(Pollitt *et al*., 2018).

Staphylococcus aureus is a highly adaptable bacterium that has progressively developed resistance to many commonly used antibiotics. Over recent decades, resistance has increased as

the organism has evolved and as antibiotics have often been misused (Cui et al., 2017). The bacterium acquires resistance genes through horizontal transfer of mobile genetic elements, enabling it to withstand different classes of drugs. Resistance may also arise from changes in drug-binding sites on target molecules or from enhanced activity of endogenous efflux pumps (Pal et al., 2021).

Urinary tract infections (UTIs) are widespread microbial infections that affect structures within the urinary system, including the kidneys, bladder, urethra and prostate. These infections have significant direct and indirect effects on individuals and represent a global health concern, increasingly contributing to morbidity. Most UTIs—about 90 percent—are caused by Gram-negative bacteria, with *Escherichia coli* responsible for 65 to 90 percent of cases. Other common uropathogens include *Enterococcus* species, *Klebsiella pneumoniae, Citrobacter* species, *Pseudomonas aeruginosa* and coagulase-negative *staphylococci* (McLellan et al., 2016).

Although UTIs caused by S. aureus are relatively uncommon, the bacterium can still ascend the urinary tract and cause infection in certain people. The success of S. aureus as a human pathogen is largely linked to its remarkable ability to adapt and acquire resistance to antibiotics. More than half of S. aureus infections today are attributed to methicillin-resistant S. aureus (MRSA). The organism's resistance mechanisms are associated with plasmid-mediated production of β-lactamases and other related processes (*Gnanamani et al.,* 2017)

Until the 1970s, vancomycin was the main antibiotic used to treat MRSA infections. However, its increased use eventually contributed to the emergence of two types of glycopeptide-resistant *S. aureus*. The first type, vancomycin-intermediate *S. aureus* (VISA), develops a thicker and less cross-linked cell wall, causing glycopeptides to become trapped at the cell surface. The second type, vancomycin-resistant *S. aureus* (VRSA), shows a much higher level of resistance (Wilhelm, 1991). Although these strains remain relatively uncommon, their detection in multiple countries highlights their global significance. Growing evidence suggests that their occurrence may rise, as reports of vancomycin-resistant infections continue to increase each year. Instances of *S. aureus* showing heteroresistance to vancomycin have also been documented, which may facilitate the development of stronger resistance over time. Research findings indicate that VRSA prevalence varies widely, ranging from about 1.3 percent to up to 20 percent (Selim *et al.* 2022).

2.2. Vancomycin as a last-resort antibiotic

Vancomycin is one of the earliest antibiotics used in medicine and has been applied clinically for almost six decades. It was first isolated in 1957 by Dr. Kornield, an organic chemist at Eli Lilly, from *Streptomyces orientalis* found in the forests of Borneo (Holmes et al., 2015). Vancomycin is a glycopeptide antibiotic that is effective against Gram-positive organisms including *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Pneumococcus*, *Listeria*, *Corynebacterium*, and *Clostridium* species. Today, it is primarily prescribed for infections caused by MRSA and for patients who cannot tolerate semisynthetic penicillins or cephalosporins (Sharma and Hammerschlag, 2019).

Vancomycin kills susceptible bacteria by disrupting normal cell wall formation. Bacterial cells rely on their cell wall to protect them from bursting due to internal osmotic pressure. During cell growth, the peptidoglycan layer must expand, which occurs through the addition of the precursor lipid II and the actions of penicillin-binding proteins (PBPs) that carry out transglycosylation and

transpeptidation (Alvarez et al., 2019). Vancomycin, being hydrophilic, binds to the D-alanyl-D-alanine (D-Ala–D-Ala) termini of lipid II via hydrogen bonding. This attachment alters the structure of the precursor, preventing its addition to the developing peptidoglycan chain and blocking the subsequent cross-linking step. The result is breakdown of the cell wall and bacterial cell death (Cong et al., 2020). However, vancomycin's large molecular structure prevents it from crossing the outer membrane of Gram-negative bacteria, giving it little activity against these organisms (Yan et al., 2019).

Extensive use of vancomycin as a first-line treatment for MRSA has contributed to the emergence of resistant strains, including vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA). The first VISA strain, with a minimum inhibitory concentration (MIC) of 8 μg/mL, was documented in Japan in 1997, and the first VRSA case was identified in a diabetic patient in the United States in 2002. Earlier laboratory studies proposed several resistance mechanisms in MRSA, with the most notable being reduced cell wall permeability and increased cell wall thickness, which together limit access of vancomycin to its targets (Batabyal et al., 2012). Another resistance pathway involves plasmid-borne genes (vanA, vanB, vanD, vanE, vanF, and vanG) thought to originate from enterococci. Recent findings also show that VISA strains grow more slowly and possess a significantly thicker cell wall compared with fully susceptible strains. Heterogeneous VISA (hVISA) display MIC values within the susceptible range (≤ 2 μg/mL) but contain subpopulations with resistant characteristics. Infections caused by VISA and hVISA are linked to higher rates of treatment failure with vancomycin, longer hospital stays, increased risk of persistent infection, and greater healthcare costs (Shariati et al., 2020).

2.3 Emergence and Mechanism of Vancomycin Resistance

The clinical identification of reduced vancomycin susceptibility in Staphylococcus aureus emerged in the 1990s, following reports of strains with intermediate resistance. The first widely recognized vancomycin-intermediate S. aureus (VISA) cases were documented in Japan and the United States during the late 1990s (Dayan et al., 2016). In 2002, the United States recorded the first well-confirmed clinical infection caused by a vancomycin-resistant S. aureus (VRSA) strain, showing high-level resistance associated with the vanA genotype. This case involved a patient with extensive healthcare exposure and a simultaneous infection with vancomycin-resistant enterococci (VRE). These initial findings demonstrated that S. aureus can develop strong vancomycin resistance and highlighted the need for closer monitoring (Chang et al., 2003).

High-level vancomycin resistance in S. aureus, characteristic of the classical VRSA phenotype, typically develops through the horizontal transfer of the vanA operon—often located on Tn1546-like genetic elements—from VRE. The vanA operon modifies the bacterial cell-wall target from D-Ala-D-Ala to D-Ala-D-Lac, significantly decreasing vancomycin's ability to bind effectively (Ghalehnoo, 2019).

In contrast, the VISA phenotype typically arises by stepwise chromosomal mutations that produce a thicker, poorly cross-linked cell wall that sequesters vancomycin molecules and reduces their effective activity (a non-van gene mechanism). Molecular and genomic analyses of clinical VRSA isolates have repeatedly shown vanA or vanA-like elements integrated into staphylococcal plasmids or transposons, confirming enterococcal origin in many case. (Zhu *et al*., 2008)

Laboratory definitions and confirmation steps reflect these biological differences. Reference-method broth microdilution (and confirmatory testing at reference labs) are used to classify

isolates: vancomycin-susceptible *S. aureus* (VSSA) typically as MIC ≤2 μg/mL, VISA shows MICs of 4–8 μg/mL, and VRSA is reported at MICs ≥16 μg/mL. Because mixed cultures (e.g., co-isolated MRSA + VRE) can produce misleading results, public-health guidance emphasizes confirming any elevated vancomycin MIC in *S. aureus* by repeat MIC testing, purity checks, and, where indicated, PCR for van genes (Swenson *et al*., 2009). Although VRSA remains uncommon relative to MRSA, recent systematic reviews and meta-analyses show substantial heterogeneity between regions and study methods, and several analyses report non-negligible pooled prevalences in some settings (David and Daum, 2017). This heterogeneity reflects differences in sample sources, laboratory methods, and true geographic variation; nevertheless, the confirmed ability of *S. aureus* to acquire vanA and the clinical reports of treatment failures make VRSA a pathogen of high clinical concern and justify continued surveillance and stewardship efforts worldwide (Wu *et al*., 2021).

2.4 Global and Regional Prevalence of VRSA

Globally, VRSA/VISA/hVISA remain uncommon compared with MRSA, but systematic analyses show the prevalence has increased in some regions over time and that there is substantial regional heterogeneity. A global analysis and systematic reviews report low single-digit pooled prevalences overall but a measurable rise in recent decades, underscoring the need for continued surveillance (Aref *et al.,* 2020).

In Africa and Nigeria the picture is mixed but concerning. A recent Nigeria meta-analysis (98 studies, pooled data) reported that vancomycin resistance among *S. aureus* isolates was pooled at ~13% (wide heterogeneity across studies and sample types), and the authors note that a few high-prevalence studies strongly influenced the pooled estimate — removal of outliers reduced the

pooled estimate substantially. This means vancomycin resistance has been documented repeatedly in Nigeria but varies by location and study design. (Ezeh *et al.,* 2023)

Recent hospital/region studies illustrate that local rates can be substantial in some settings: for example, an antibiotic-resistance study from a tertiary facility in Calabar (2023/2024 report) examined MRSA isolates and reported elevated vancomycin resistance among clinical isolates, and an Enugu study reported 9.1% VRSA and 24.2% VISA among MRSA isolates tested in that series. These are hospital-based figures (not population surveys) and therefore reflect clinical contexts where antibiotic exposure is higher; they demonstrate why local surveillance (including community populations such as students) is important. (Umoh *et al.,* 2024)

2.5 Urinary Tract Infections and Female Susceptibility

Urinary tract infections are among the most widespread bacterial infections globally, affecting individuals of all ages and sexes. They occur when the urethra, bladder, ureters, or kidneys become infected, and they lead to millions of healthcare consultations each year. Approximately 150 million people worldwide are estimated to experience a UTI annually (Chan et al., 2021). These infections place a significant burden on healthcare systems and contribute heavily to antibiotic use. While Gram-negative bacteria, especially *Escherichia coli*, cause the majority of UTIs, Gram-positive organisms such as *Enterococcus* species and *Staphylococcus aureus* are increasingly recognized as important pathogens in both community-acquired and hospital-associated infections (Flores-Mireles et al., 2015).

Females experience UTIs far more frequently than males owing to a combination of anatomical, physiological, and behavioral factors. The female urethra is shorter (about 4 cm) and positioned closer to the anus, facilitating the ascension of uropathogens into the bladder. Additional factors such as sexual activity, use of diaphragms and spermicides, pregnancy, hormonal changes, and

poor personal hygiene further increase the risk (Bent *et al*., 2002). Consequently, young and sexually active women, such as female university students represent one of the most vulnerable groups for recurrent or uncomplicated cystitis.

While *E. coli* remains the dominant uropathogen, *Staphylococcus aureus* has been increasingly identified as a causative agent of both community-acquired and hospital-associated UTIs. *S. aureus* bacteriuria may arise through ascending infection, especially following instrumentation or catheterization, or through hematogenous spread from another infection site . Though less frequent than enteric bacteria, *S. aureus* is clinically significant because many isolates exhibit multidrug resistance, including methicillin and, in some reports, reduced susceptibility or resistance to vancomycin (Mancuso *et al*., 2023). The occurrence of such resistant strains in urinary isolates complicates empirical therapy and may lead to treatment failure if not accurately detected.

Given the biological susceptibility of females and the growing global concern over antimicrobial resistance, evaluating vancomycin-resistant *S. aureus* in female urine samples is vital.
Understanding its prevalence among female university students provides insight into community-level colonization or infection and contributes to improved surveillance and antibiotic stewardship in Nigeria.

2.6 Public Health Significance of Vancomycin-Resistant *Staphylococcus aureus* (VRSA)

Vancomycin-resistant *Staphylococcus aureus* (VRSA) represents one of the gravest threats to infection control and antimicrobial therapy worldwide. Because vancomycin has long been considered the ―last-line‖ agent for treating methicillin-resistant *S. aureus* (MRSA) infections, the emergence of VRSA severely limits treatment options and increases morbidity and mortality

Infections caused by VRSA are associated with prolonged hospital stays, increased healthcare costs, and a higher risk of adverse outcomes compared with infections caused by vancomycin-susceptible strains (Monaco *et al*., 2016).

The public health concern arises not only from individual treatment failure but also from the potential for horizontal gene transfer and wider dissemination. The *vanA* operon responsible for high-level resistance can be transferred from enterococci to *S. aureus* through plasmid exchange, illustrating that resistance genes can spread between species under selective antibiotic pressure (Weinstein and Fridkin, 2001). Such genetic exchange may occur in healthcare environments where patients receive multiple antibiotics or harbor mixed infections, enabling *S. aureus* to acquire additional resistance traits.

Epidemiological surveillance studies emphasize that VRSA isolates are usually multidrug resistant, displaying concurrent resistance to macrolides, fluoroquinolones, and aminoglycosides (Yousefi*et al*., 2017). This complicates empirical therapy, narrows the range of effective agents, and underscores the necessity for antimicrobial-stewardship programs to limit unnecessary glycopeptide use. Infection-prevention measures such as screening, isolation of colonized patients, and strict hand hygiene remain critical to reduce nosocomial transmission.

Globally, the detection of VRSA—even in low numbers—signals a potential erosion of the effectiveness of glycopeptides. Continuous laboratory surveillance, accurate detection of VISA/VRSA, and molecular characterization of resistance mechanisms are essential to guide clinical management and to inform antibiotic-policy frameworks (Blechman *et al.,* 2024). For Nigeria and other low- and middle-income countries, strengthening diagnostic capacity and antimicrobial stewardship is especially urgent, as over-the-counter antibiotic use and limited infection-control resources heighten the risk of resistant-strain dissemination.

This review has examined existing studies related to *Staphylococcus aureus* and its growing resistance to vancomycin. *S. aureus* is a versatile pathogen capable of causing a wide range of infections, including skin infections, pneumonia, septicemia, and urinary tract infections. Its ability to develop resistance mechanisms, particularly to β-lactam and glycopeptide antibiotics, has complicated clinical management and contributed to its global significance as a public-health threat.

Vancomycin has long served as the last-resort antibiotic for treating infections caused by methicillin-resistant *S. aureus* (MRSA). However, the emergence of vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA and VRSA) has reduced the reliability of this drug, as confirmed by laboratory and clinical reports since the late 1990s. (Beckeley *et al.,* 2021). The mechanisms of resistance—either through thickened cell walls (VISA) or acquisition of the *vanA* operon from enterococci (VRSA)—highlight the organism's remarkable adaptability. (Howden *et al.,* 2014)

Although VRSA remains relatively rare compared with MRSA, evidence from global and regional studies indicates a slow but steady increase in detection rates, especially in Africa and parts of Asia. In Nigeria, several reports and meta-analyses have documented vancomycin resistance among clinical *S. aureus* isolates, including those from urine samples. These findings emphasize the need for continuous surveillance and antimicrobial-stewardship efforts.

Females are disproportionately affected by urinary tract infections due to anatomical and behavioral factors, and *S. aureus*—though less common than *E. coli*—remains an important opportunistic uropathogen. The occurrence of multidrug-resistant or vancomycin-resistant *S.*

aureus in urine presents a potential risk for treatment failure and community spread.

In summary, the reviewed literature underscores the clinical and epidemiological importance of VRSA, particularly in female populations vulnerable to urinary tract infections. It also establishes a clear rationale for the present study—to determine the prevalence of methicillin- and vancomycin-resistant *Staphylococcus aureus* from urine samples among selected students at the University of Benin, Edo State, Nigeria, thereby contributing to local data on antimicrobial resistance and informing infection-control strategies.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was done at the University of Benin, Benin City, Edo State, Nigeria. The University of Benin being a federal tertiary institution holds a large population of students living in on-campus hostels and off-campus accommodations. The study area was chosen because of its large student population, crowded living conditions in hostels, and the likeliness for environmental factors, such as shared sanitary facilities, to lead to the high rate of bacterial pathogens in urine samples.

The study was focused on students living in the various hostels on the University's main campus (Ugbowo Campus). Four hostels were chosen due to their accessibility, agreement of hostel management, population size, and amount students willing to participate in the study. These hostels included Hall 1, Hall 2, Hall 3 and NDDC Hostel, which hold a significant fraction of the university's student population. The hostels provide necessary living conditions such as shared bathrooms and toilet facilities, which are required because the study's concentration on UTIs and antimicrobial resistance.

3.2 Research Design

A cross-sectional descriptive research design was usedto investigate the prevalence of *Vancomycin-resistant Staphylococcus aureus* (VRSA) and other bacterial isolates in urine samples collected from chosen students at the University of Benin, Nigeria. This design was

considered appropriate because it provides the test of microbial profiles and bacterial loads in urine samples without manipulation of variables. The research design allowed for the identification of bacterial pathogens**,** including VRSA, determination of their antimicrobial susceptibility patterns**,** and quantification of bacterial counts expressed as colony-forming units per milliliter (CFU/mL) among the study population. Distribution of questionnaires, urine sample collection, and microbiological, biochemical, and molecular analyses were used as data source**.** These steps provided a comprehensive understanding of the high existence and resistance mechanism of uropathogens in a community setting, hence executing the purpose of the study accurately.

3.3 Study Population

The study population included undergraduate students of the age group 16–30 years living in the hostels at the University of Benin. In total sixty students were randomly selected from the five hostels to make sure there was equal representation and distribution without any bias. The sample included both male and female students to show a broad spectrum of bacterial carriage patterns in urine samples. All participation was done voluntarily and informed consent was given by the students or, in some cases, their guardians for students under 18 years (minors). Ethical approval was obtained from the University of Benin Ethics Committee, and permission was granted by the hostel management and university authorities before the samples were collected.

3.4 Sample Collection Procedure

Urine samples were gotten from the selected students under aseptic conditions to prevent contamination. All students that participated were 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 there transported immediately to the Microbiology Laboratory at the University of Benin for analysis.

3.5. Sterilization of Materials

All the glasswares and instruments used such as Petri-dishes, conical flask, round bottom flask and bottles were washed, rinsed and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160oC for 1 hr. They were allowed to cool after sterilization before usage. An aseptic working environment was maintained throughout with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.6. Preparation and Sterilization of media

The media used in this study were sterilized at 121 oC for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 oC. Aseptic conditions were ensured during inoculation and subculturing

3.7. Preparation of Culture Media

Media used in the study, including Brain Heart Infusion Agar (BHI), nutrient agar, and mannitol salt agar, were prepared according to the manufacturer's instructions.

3.7.1. Preparation of Nutrient agar

28 g of nutrient agar was put into 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve. The medium was then kept in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was left to cool down.

3.7.2. Preparation of Manittol Salt agar

111 grams of agar was dispensed in 1000 ml distilled water and heated to boiling, to fully dissolve the medium. It was then distributed in conical flasks. The medium was sterilized by autoclaving at 121 °C for 15 mins and then left to cool down before pouring into sterile petri dishes.

3.7.3. Preparation of Brain Heart Infusion Agar

52 grams of brain heart infusion agar powder was put in 1l of distilled water. The mixture was heated to boiling with continuous stirring to completely dissolve the medium. the medium was afterwards sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to a temperature range of 45–50°C and was not allowed to solidify after the autoclaving process. After cooling down, 6ml of 1mg\ml of vancomycin stock was aseptically added to the medium. The mixture was carefully swirled to allow for even distribution of the antibiotic. The medium with antibiotic was then dispensed into sterile Petri dishes under aseptic conditions and allowed to solidify at room temperature. After which, the plates were stored in sealed containers at 4°C till need 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 vancomycin-resistant *Staphylococcus aureus* (VRSA) 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, 2005).

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, 2005).

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 = number of colonies/volume of plated (ml) x dilution factor (Equation 1)

(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 vancomycin resistance by plating in Brain Heart Infusion Agar (BHI) fixed with vancomycin.

3.9. Bacterial Identification

The bacterial isolates were categorized based on their colonial morphological characteristics such as shape, size, elevation, opacity, margin and color on the agar plates.

Biochemical tests were also carried out to further identify the bacterial isolates and they include catalase test, citrate utilization test, oxidase test, indole test, KOH test and Triple sugar iron fermentation.

3.9.1 Gram staining

Thin smears of the bacterial isolates were made from the pure culture on clean, grease free slide. The smears were air dried and then heat fixed by passing the slide over flame. The smears were flooded with crystal violet for 60 seconds, drained and flooded with gram's iodine for another 60 seconds then rinsed with distilled water. The smears are then decolorized using 95% alcohol for 30 seconds and quickly rinsed with distilled water. The smears were then counter stained with safranin for 60 seconds, rinsed with distilled water and allow to air dried. The slides were examined under the oil immersion(X100) objective. The gram positive cells appear blue while the gram negative cells were indicated with a pink coloration.

3.9.2. BIOCHEMICAL TEST

3.9.2.1. Catalase Test

Catalase test was carried to identify the absence or presence of the enzyme catalase. It is done by making a suspension of fresh culture of the test organisms using sterile distilled water on a clean glass microscope slide and few drops of 3% hydrogen peroxide (H_2O_2) were added on the smeared slide. Formation of bubbles indicates positive result. Lack of bubbles indicates negative result.

3.9.2.2. Oxidase Test

The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electrons between electron donors in the bacteria and a redox dye known as tetramethyl-*p*-phenylene-diamine. The dye would be reduced to deep purple colour if yielded to positive reactions.

Several reagents can be used for this study but Kovacs oxidase reagent: 1% tetra-methyl-p-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. It was allowed and observed for colour development within 30 seconds. The appearance of a deep purple-blue/blue colour indicated oxidase production and the negative result was when no colour changed (Fawole and Oso, 2007)..

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, 2005).

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 (Roberts and Sandle, 2008).

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, 2005).

3.9.2.7. Triple sugar iron (TSI) agar test

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 H2S, 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 H2S production.Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO2 and H2). The use of this test is to find out an organism's ability to ferment sugars and to produce hydrogen sulphide (H2S) or gas (O2), or both.

3.10 Detection of Vancomycin-Resistant *Staphylococcus aureus* (VRSA)

Pure *S. aureus* colonies were streaked onto the surface of brain heart infusion agar plates containing vancomycin and incubated aerobically at 35–37°C for 24 to 48 hours. Following incubation, plates were examined for the presence or absence of visible colonies, which is used as an indicator of vancomycin resistance. Plates that showed distinct, creamy or golden, opaque colonies of *Staphylococcus aureus* were considered positive for growth, indicating possible vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates. While plates showed no visible growth after incubation were recorded as negative, indicating the absence of VRSA and suggesting that the isolates were susceptible to vancomycin.

3.10.1 Antibiotic Susceptibility Test

The bacterial colonies identified were utilized to assess the susceptibility and resistance of the isolates through standard Antibacterial Susceptibility Testing (AST). This analysis determined their response to commonly used antibiotics in the study area. The antibiotics tested included Cefuroxime (30 µg), Cloxacillin (5 µg), Gentamicin (10 µg), Ofloxacin (5 µg), Oxacillin (1 µg), Ceftazidime (30 µg), Erythromycin (15 µg), Ceftriaxone (30 µg), and Augmentin (30 µg). The antibiotic discs, manufactured by Oxoid, UK, were used for the disc diffusion method applied in this study. For the AST procedure, bacterial cultures grown for 18–24 hours were streaked onto Mueller-Hinton Agar (MHA) plates. The inoculum was adjusted to match a 1.5 x 10^8 CFU/mL McFarland standard, ensuring consistency in bacterial density.

Using sterile forceps, antibiotic discs were carefully placed on the inoculated MHA plates. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones around each disc was measured in millimeters using a ruler. The results were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The 2020 CLSI standards classified the bacterial isolates' responses to antibiotics as Resistant (R), Intermediate (I), or Sensitive (S), providing insight into their susceptibility patterns.

3.10.2. Multiple Antibiotic Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) Index is used to assess the antibiotic resistance profile of bacterial isolates. It represents the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics tested. The MAR index helps to determine whether an isolate originated from a high-risk source of contamination where antibiotics are frequently used. A MAR index value greater than 0.2 indicates that the bacterial isolate may have originated from an environment where antibiotics are often misused or heavily applied, such as hospitals or farms, while a value less than or equal to 0.2 suggests that the isolate likely came from a source with limited or infrequent antibiotic exposure.

The formula below was used to decipher MAR index of bacterial isolates.

MAR index=y/nx

Where y = number of resistance scored

n = number of isolates and

x = total number of antibiotics

CHAPTER FOUR

RESULT

4.0 RESULTS

The results of the urinalysis conducted on urine samples collected from 60 selected students are presented in Table 4.1. The results revealed that 23.3% of the samples tested positive for leukocytes, indicating possible urinary tract infection. Nitrites were detected in 16.7% of samples, further suggesting bacterial activity. Protein was found in 15% of the samples, while blood (hematuria) was present in 10%, signifying potential irritation or inflammation of the urinary tract. Urobilinogen was elevated in 6.7% of the samples, while 6.7% showed the presence of ascorbic acid, which could interfere with reagent strip readings. Ketones and glucose were detected in 3.3% of the samples, respectively, while bilirubin was observed in 1.7%, indicating possible hepatic involvement. The urine pH values ranged from 5.0 to 8.0, which falls within the normal physiological range of 4.6 to 8.0. The specific gravity ranged from 1.005 to 1.030, reflecting varied hydration levels among participants.

Table 4.2 shows the mean total viable count (TVC) of urine samples based on age and sex. The results indicated that female samples consistently recorded higher bacterial loads than male samples across all age groups. The highest mean count for females ($5.2 \pm 0.4 \times 10^5$ CFU/mL) was recorded among participants aged 21–25 years, 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 range.

The mean total *Staphylococcus* count on Mannitol Salt Agar (MSA) for the urine samples according to age and sex is presented in Table 4.3. Across all age categories, female samples

exhibited higher *Staphylococcus* counts compared to male samples. The highest mean count for females ($3.8 \pm 0.3 \times 10^5$ CFU/mL) occurred in the 21–25 age group, whereas the lowest for males ($2.8 \pm 0.1 \times 10^5$ CFU/mL) was recorded in the 31–35 age group.

Table 4.4 outlines the cultural, morphological, and biochemical characteristics of the bacterial isolates obtained from urine samples. Six distinct bacterial species were identified based on their cultural, morphological and biochemical reactions: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* sp., *Proteus* sp., *Enterococcus* sp. and *Klebsiella* sp.

The prevalence of bacterial isolates recovered from urine samples is presented in Figure 4.1. The results show that *Staphylococcus aureus* was the most prevalent isolate (33%), followed by *Escherichia coli* (21%) and *Pseudomonas* sp. (11%). Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 10% of isolates, while *Klebsiella* sp., *Proteus* sp., and *Enterococcus* sp. were less frequent.

Table 4.5 presents the distribution of bacterial isolates from urine samples based on sex. Female participants exhibited higher prevalence rates for all bacterial isolates compared to males. Specifically, *Staphylococcus aureus* was identified in 36.1% of female samples and 29.2% of male samples. *E. coli* was present in 22.2% of female samples and 20.8% of male samples. *Pseudomonas* spp., *Klebsiella* spp., and *Proteus* spp. were detected in both sexes at lower but comparable frequencies

Table 4.6 shows the percentage prevalence of Vancomycin-Resistant *Staphylococcus aureus* (VRSA) in urine samples based on age and sex. The overall prevalence of VRSA was 6.7%, with the highest detection rate observed in the 21–25 age group. Female samples exhibited a higher percentage of VRSA (5.0%) compared to males (1.7%).

Figure 4.2 illustrates the percentage prevalence of VRSA among male and female participants. The results show that VRSA occurrence was higher in females (8.3%) than in males (4.2%), suggesting a gender-related disparity in resistance pattern distribution.

The antibiotic susceptibility pattern of bacterial isolates, expressed as the zones of inhibition (in millimeters), along with their Multiple Antibiotic Resistance (MAR) indices is presented in table Table 4.7. The isolates displayed varied resistance patterns. *Staphylococcus aureus* exhibited the highest MAR index (0.44), showing marked resistance to Cloxacillin, Oxacillin, and Ceftazidime. *E. coli*, *Pseudomonas* sp., and *Enterococcus* sp. each had moderate MAR indices of 0.22, indicating resistance to multiple antibiotic classes. *Proteus* sp. and *Klebsiella* sp. showed the lowest MAR indices (0.11), suggesting limited resistance. Notably, most isolates were susceptible to Ofloxacin and Ceftriaxone, which recorded larger inhibition zones.

Table 4.1: Urinalysis of 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) of Urine Samples Based on Age and Sex

Age Range (Years)	No. of Samples	Female	Male
16–20	15	4.5 ± 0.3	3.8 ± 0.2
21–25	20	5.2 ± 0.4	4.0 ± 0.3
26–30	15	4.8 ± 0.2	4.1 ± 0.3
31–35	10	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)

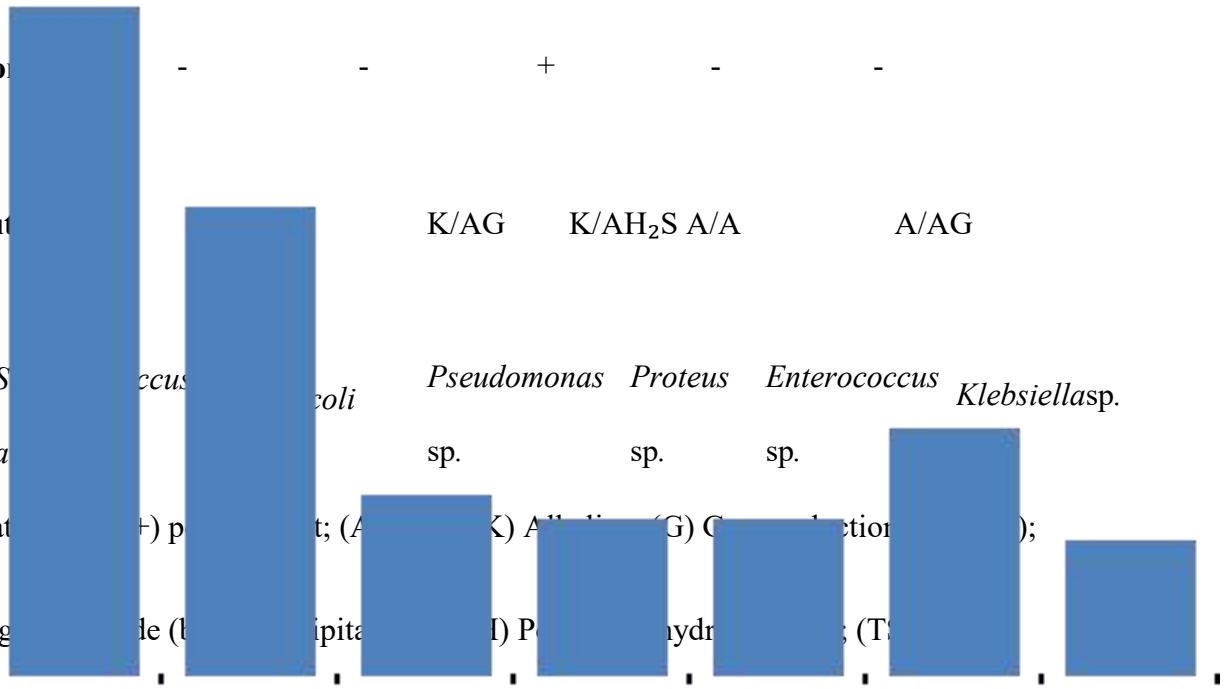
Age Range (Years)	No. of Samples	Female	Male
16–20	15	3.5 ± 0.2	2.9 ± 0.3
21–25	20	3.8 ± 0.3	3.0 ± 0.2
26–30	15	3.6 ± 0.2	3.1 ± 0.1
31–35	10	3.2 ± 0.2	2.8 ± 0.1

Values are represented as mean ± standard deviation

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

Characteristic	Isolate 1	Isolate 2	Isolate 4	Isolate 5	Isolate 6	Isolate 7
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
Colour (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/But			K/AG	K/AH_2S A/A		A/AG
Identity	*S ccus*		*coli*	*Pseudomonas* sp.	*Proteus* sp.	*Enterococcus* sp.	*Klebsiella*sp.



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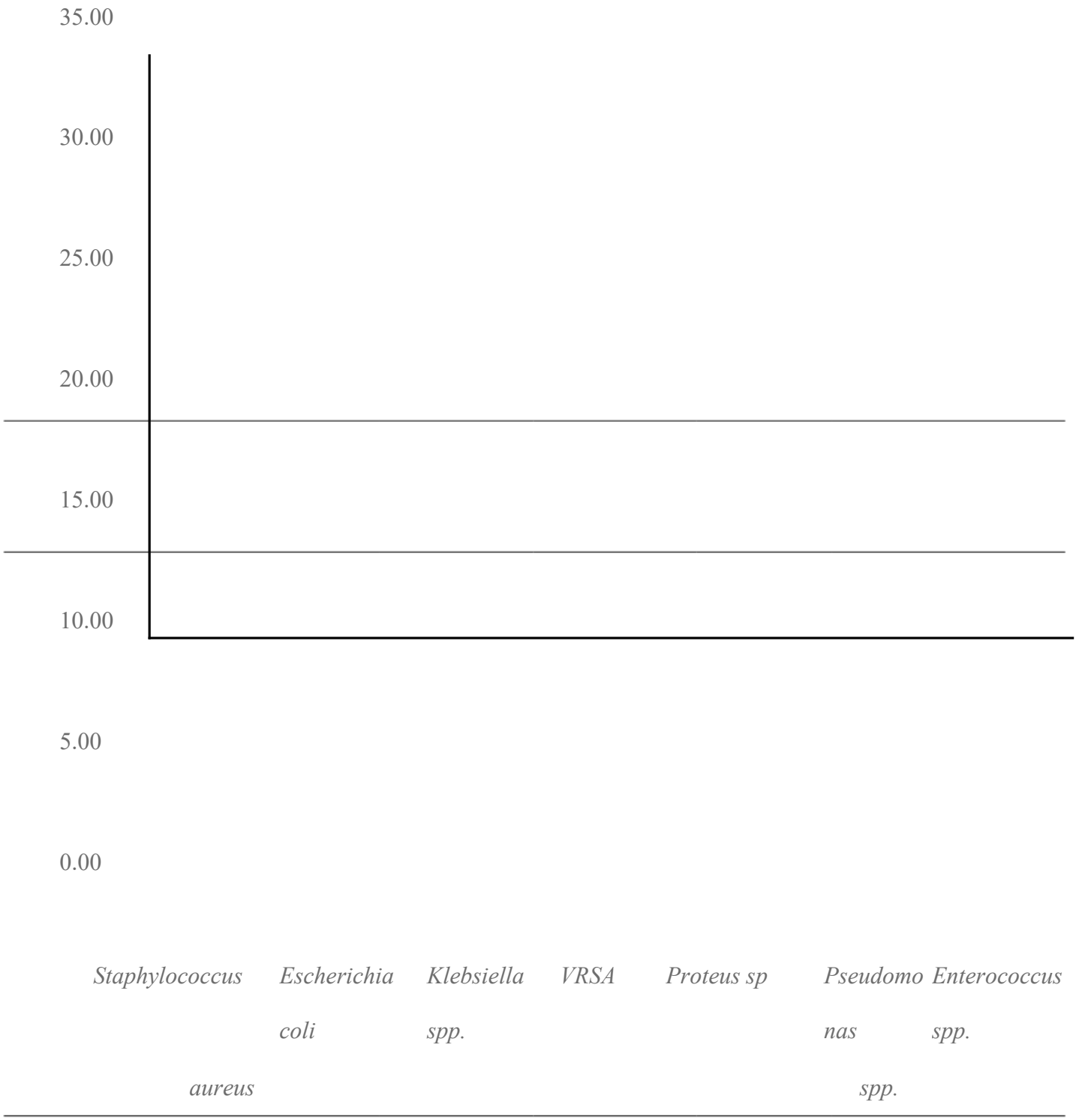

Figure 4.1: Prevalence of Bacterial Isolates in Urine Samples (n = 60)

Table 4.5: Distribution of Bacterial Isolates from Urine Samples 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
Vancomycin-Resistant *S. aureus* (VRSA)	3	8.3	1	4.2
Pseudomonas spp.	4	11.1	3	12.5
Klebsiella spp.	3	8.3	2	8.3
Enterococcus spp.	2	5.6	1	4.2
Proteus spp.	3	8.3	2	8.3

Table 4.6: Percentage Prevalence of Vancomycin-Resistant *Staphylococcus*

aureus **(VRSA) in Urine Samples by Age and Sex**

Age Range (Years)	No. of Samples	Female Positive for VRSA (%)	Male Positive for VRSA (%)	Total VRSA Positive (%)
16–20	15	1 (6.7%)	0 (0.0%)	1 (6.7%)
21–25	20	1 (5.0%)	1 (5.0%)	2 (10.0%)
26–30	15	1 (6.7%)	0 (0.0%)	1 (6.7%)
31–35	10	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	60	3 (5.0%)	1 (1.7%)	4 (6.7%)

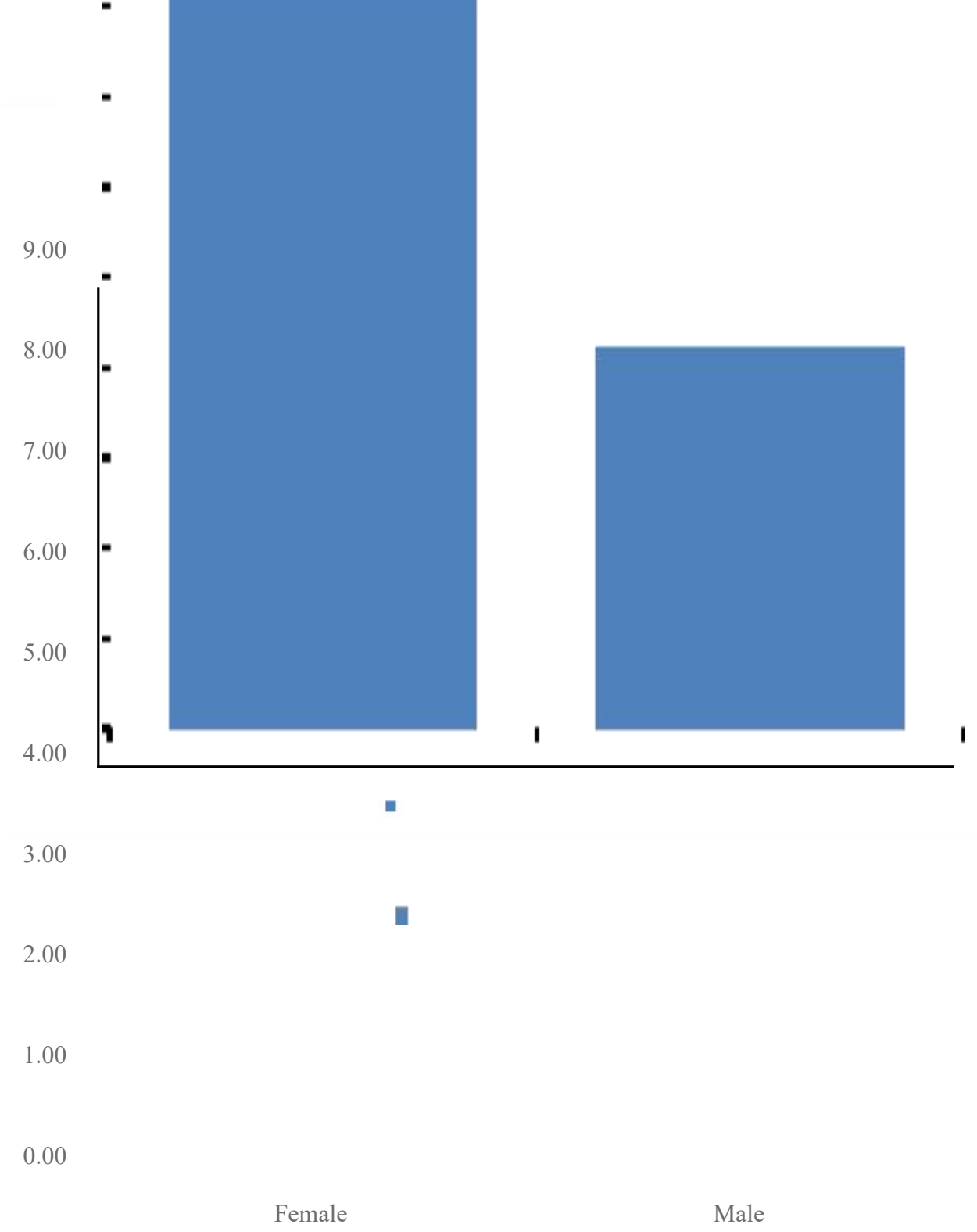

Figure 4.2.Percentage Prevalence of VRSA in Urine Sample

Table 4.7: Antibiotic Susceptibility Pattern of the Bacterial Isolates (Zone of Inhibition in mm) and MAR Index

Isolate	CRX	CN	CTR	E	CXC	OFX	CAZ	A
Escherichia coli	12 (I)	14 (I)	20 (S)	15 (I)	10 (R)	16 (I)	18 (S)	10
Staphylococcus aureus	10 (R)	18 (S)	12 (I)	14 (I)	0 (R)	22 (S)	9 (R)	16
Proteus spp.	14 (I)	18 (S)	21 (S)	13 (I)	10 (R)	18 (S)	19 (S)	12
Pseudomonas spp.	10 (R)	20 (S)	18 (S)	10 (R)	12 (I)	19 (S)	17 (S)	14
Klebsiella spp.	12 (I)	16 (I)	20 (S)	14 (I)	10 (R)	20 (S)	18 (S)	12
Enterococcus spp.	11 (I)	14 (I)	17 (S)	13 (I)	9 (R)	17 (S)	16 (I)	10

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

5.1 DISCUSSION

Urinary tract infections (UTIs) remain among the most prevalent bacterial infections affecting individuals globally, with females being disproportionately impacted due to anatomical and behavioral factors (Czajkowski *et al*., 2021). In this study, urine samples collected from 60 students of the University of Benin were examined to determine the prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA) and other uropathogens, as well as their antimicrobial susceptibility profiles. The findings reveal notable bacterial presence, multidrug resistance patterns, and gender-associated differences, emphasizing the growing challenge of antimicrobial resistance in community settings.

The urinalysis findings indicated that 23.3% of samples were positive for leukocytes and 16.7% for nitrites, both of which are reliable indicators of bacterial infection in the urinary tract (Flores-Mireles *et al*., 2015). The detection of protein (15%) and blood (10%) further suggests inflammation or possible damage to urinary tract tissues (Foxman, 2014). These findings collectively suggest a considerable occurrence of subclinical or active UTIs among students, potentially linked to poor hygiene, dehydration, or asymptomatic bacteriuria—conditions that are common in communal living environments such as hostels. The recorded pH and specific gravity values fall within normal ranges, suggesting no metabolic derangements that could have influenced bacterial growth.

The mean total viable count (TVC) results revealed that females exhibited higher bacterial loads (up to 5.2×10^5 CFU/mL) compared to males ($3.5–4.1 \times 10^5$ CFU/mL). This supports previous findings that females are more prone to UTIs because of their shorter urethral length and proximity to the anus, which facilitates bacterial ascension (Foxman, 2014). The highest counts

were recorded in the 21–25 age group, which corresponds to the most sexually active age range among university students (Elorfaly, 2024). The mean Staphylococcus count on Mannitol Salt Agar (MSA) also followed a similar pattern, with females recording higher loads, further corroborating the observed gender difference in infection rates. The distribution of bacterial isolates revealed *Staphylococcus aureus* as the predominant uropathogen (33%), followed by *Escherichia coli* (21%) and *Pseudomonas spp*. (11%). This is notable because *E. coli* is typically the most common uropathogen globally, but a shift toward Gram-positive organisms such as *S. aureus* has been reported in several community studies across Nigeria (Ekwealor *et al*., 2016). The prominence of *S. aureus* in this study may indicate increased skin and perineal colonization or contamination through poor hygiene practices. The recovery of other organisms such as *Klebsiella*, *Proteus*, and *Enterococcus* species further demonstrates the polymicrobial nature of urinary infections in this population, consistent with reports (Iregbu and Nwajiobi-Princewill, 2013). The detection of VRSA in 6.7% of the population, with a higher prevalence among females (5.0%) than males (1.7%), underscores the growing presence of vancomycin-resistant strains within the community. The age group 21–25 years exhibited the highest VRSA occurrence, aligning with the demographic that also recorded the highest total bacterial counts. The higher female VRSA rate may be associated with greater antibiotic exposure, frequent self-medication, or anatomical susceptibility (Adeyemo *et al*., 2021). Although VRSA is still considered rare globally (Ahmed *et al*., 2024), the identification of resistant strains in this study population indicates that resistance determinants may be spreading beyond hospital settings into the community, particularly among university students. Antibiotic susceptibility testing revealed high resistance to β-lactam antibiotics, especially cloxacillin and oxacillin, confirming the presence of methicillin and vancomycin-resistant *S. aureus* strains. The high multiple antibiotic resistance (MAR) index recorded for *S. aureus* (0.44) suggests that isolates have been exposed to several antibiotics, likely as a result of

indiscriminate antibiotic use (O'Neill, 2016). In contrast, lower MAR values in *Proteus* and *Klebsiella* species (0.11) imply reduced antibiotic pressure. The moderate resistance levels observed in *E. coli*, *Pseudomonas*, and *Enterococcus* species (MAR = 0.22) further indicate the circulation of multidrug-resistant strains within the population. The continued susceptibility of most isolates to ofloxacin and ceftriaxone suggests that these antibiotics remain effective for empirical treatment in this setting, consistent with undergone studies. (Odetoyin *et al*. 2019).

The overall results point to significant antimicrobial resistance among uropathogens, particularly S. aureus, and confirm that female students represent a high-risk group for urinary tract infections and carriage of resistant bacteria. The high VRSA prevalence, though moderate compared with hospital-based reports, raises public health concerns because of its potential for spread in the university community. The data also highlight the importance of antimicrobial stewardship, routine screening, and improved sanitation practices to reduce bacterial transmission and prevent the emergence of more resistant strains.

5.2. CONCLUSION

This study provides substantial evidence for the antibiotic resistance activity of the uropathogen like staphylococcus aureus particularly vancomycin-resistant staphylococcus aureus found in the collected urine samples among the students residing in the campus of the University of Benin, Nigeria. It shows severe issue of urinary tract infections (UTIs) and antimicrobial resistance in the institutions community setting. The growing resistance to β-lactam antibiotics, particularly in VRSA, presents the current need for improved antimicrobial surveillance and proper use of antibiotics in university populations. Further research should focus on molecular characterization of resistance genes and explore alternative strategies, such as targeted antimicrobial therapies and hygiene interventions, to work on the growing challenge of antibiotic resistance in UTI management among university students.

5.3. Recommendations

Based on the results of this study, several recommendations can be made:

1. Improve the public bathroom facilities in university hostels and provide good hygiene education to reduce bacterial transmission causing UTIs.
2. Conduct programs to educate students on proper usage of antibiotics use in order to prevent microbial resistance to antibiotics.
3. Research on students antibiotic use history and hygiene practices, to identify the cause of bacterial carriage and resistance and source for appropriate solutions.
4. Ensure the university health centers are stocked with microbiological facilities for culturing and susceptibility testing to provide UTI treatment.

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