STUDIES ON BACTERIA ISOLATES FROM URINE OF HEALTHY UNDERGRADUATE STUDENTS IN UNIBEN, NIGERIA

BY

CYNTHIA CHINWEDU ONWUEMENE

LSC2104003

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF LIFE SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY, EDO STATE,
NIGERIA.

NOVEMBER, 2025.

STUDIES ON BACTERIA ISOLATES FROM URINE OF HEALTHY UNDERGRADUATE STUDENTS IN UNIBEN, NIGERIA

BY

CYNTHIA CHINWEDU ONWUEMENE

LSC2104003

A 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.) DEGREE. IN UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by Cynthia Chinwedu Onwuemene in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

_____ _____

Prof. (Mrs.) O.I Enabulele **Date**
(Supervisor)

APPROVAL

I certify that this work has been accepted in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. (Mr.) E. O. Igbinosa **Date**
(Head of Department)

DEDICATION

This project is dedicated to Almighty God and my supervisor Prof. (Mrs.) O. I. Enabulele, my parents and siblings for their support and encouragement during my journey,

ACKNOWLEGDEMENTS

I would like to thank God for his continued grace and guidance, for how good he has been to me throughout my schooling and for his protection throughout the research project. I would also like to express my sincere thanks to my supervisor Prof. (Mrs.) O. I Enabulele and my sincere thanks to Dr. A. S. Aziegbemhin for his continuous guidance, support and knowledge impacted, it was really great working under you sir. I would like to thank my fellow students, friends and colleagues for the time we spent together and all the memories it brought. Special thanks to the head of department Prof. (Mr.) E. O. Igbinosa and other staff of the Department of Microbiology for their support. I extend my heartfelt gratitude to my parents, Sir and Lady C. C. Onwuemene, My uncle and aunt Mr. and Mrs. Ofili Okocha for their unwavering support, love and encouragement throughout my academic journey.

Finally, I acknowledge all authors and researchers whose works were referenced in this project, your contributions to the field of microbiology have been invaluable in shaping my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Antibiotic resistance has emerged as a major global health concern, particularly in bacterial pathogens associated with urine from asymptomatic students. This study investigated bacteria isolates from urine samples of healthy students, focusing on their prevalence, virulence characteristics and antibiotic resistance. Thirty-five (35) isolates were obtained and identified using selective media and biochemical tests, while virulence factors including haemolysis, capsule formation, serum resistance and gelatinase production were assessed by standard laboratory methods. Antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method. Results showed that *Escherichia coli* as the most prevalent bacteria 6 (26 %), followed by *Staphylococcus aureus* 3 (17 %), *Streptococcus* sp. 4 (13 %), *Salmonella* sp.3 (12 %) while *Micrococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* 2 (8 %) respectively. All isolates exhibited haemolytic activity, predominantly β-haemolysin, except *Klebsiella pneumoniae* and *Enterococcus faecalis*. All isolates except *Micrococcus* sp., *Pseudomonas aeruginosa* and *Enterococcus faecalis* produced capsule. Serum resistance assays revealed that *E. coli*, *Streptococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Micrococcus* sp. were resistant, while *S. aureus*, *Enterococcus faecalis* and *Salmonella* sp. were sensitive. Gelatinase activity was confirmed in *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Salmonella* sp., but absent in *Streptococcus* sp. *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Micrococcus* sp. Antibiotic testing showed the bacteria isolates were not resistant to levofloxacin and gentamycin, while resistance was observed against other antibiotics tested. The pathogenic potential of bacterial isolates recovered from healthy individuals emphasized the need for continuous monitoring of antimicrobial resistance to prevent dissemination.

CHAPTER ONE

INRODUCTION

1.1 Background of Study

Antibiotic resistance is becoming a major global health crisis, making it harder to treat infections with the drugs we rely on. Even in healthy people, like university students, these bacteria can quietly carry resistance genes, acting like secret hideouts that fuel the spread of antibiotic resistance in the community (Kebira *et al*., 2009). This hidden problem can make it tougher to keep infections under control down the line.

University students often have their health needs overlooked. Although they are usually healthy, certain factors increase their risk of spreading antibiotic-resistant bacteria. These include poor hygiene, crowded dorm living, stress that weakens immunity and taking antibiotics without medical advice. Studies show that many young adults use antibiotics without prescriptions, often because the drugs are easy to get, shared among friends, or because they are unaware of the dangers (Badiger *et al*., 2012). This kind of misuse puts pressure on bacteria, helping resistant strains grow and stick around, even in people who feel perfectly fine. It's worrying but not well-studied that healthy people can carry antibiotic-resistant bacteria in their urine without any symptoms. These silent carriers can unknowingly pass these tough bacteria to others and they might face bigger problems if they get an infection later on. Unfortunately, there is paucity research on the resistance patterns of bacteria in the urine of healthy individual, especially university students in low- and middle-income countries, even though this issue matters a lot. Understanding the resistance patterns of bacterial isolates from urine samples of apparently healthy individuals is essential for several reasons. First, it provides insight into the silent spread of resistance outside of healthcare settings. Additionally, it helps to inform public health policies, including antimicrobial stewardship programs and awareness campaigns., furthermore, it lays the groundwork for future research into resistance gene transmission, reservoirs and risk factors associated with carriage in healthy populations. This study focused on the types of bacteria found in the urine of healthy university students, their resistance to antibiotics and their virulence factor.

1.2 Aim and objectives

The aim of this work is to determine the antibiotic resistance patterns of bacterial isolates from urine samples of apparently healthy university students

The specific objectives of this study were to;

i. isolate and identify bacteria from urine samples of apparently healthy university students
ii. determined the susceptibility pattern of the bacterial to antibacterial agents
iii. determined the virulence factors (gelatinase production, haemolysis activity, serum resistance assay and capsule formation) of the bacteria isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Antibiotic Resistance

Antibiotics have saved millions of lives by treating infections that were once deadly. However, bacteria are increasingly developing ways to resist these drugs, a problem known as antibiotic resistance (AMR). This means infections that used to be easy to treat can become life-threatening again. Resistant bacteria spread quickly across borders through travel, trade, food, water and the environment, making AMR a global threat. The impact is already severe: AMR directly caused 1.27 million deaths in 2019 and contributed to nearly 5 million more worldwide (Murray *et al*., 2022). In Europe, it causes more than 35,000 deaths each year (European Centre for Disease Prevention and Control [ECDC], 2022), while in the United States, there are over 2.8 million resistant infections annually, leading to at least 35,000 deaths (Centers for Disease Control and Prevention [CDC], 2019). Common illnesses like urinary tract infections (UTIs), pneumonia and sepsis are becoming harder to treat. In some regions, over half of UTIs caused by *E. coli* no longer respond to first-line antibiotics (World Health Organization [WHO], 2021). Multidrug-resistant tuberculosis (MDR-TB) also remains a major problem, with nearly half a million cases reported worldwide each year (WHO, 2020).

Studying antibiotic resistance in apparently healthy individuals is essential because they may carry resistant bacteria without showing symptoms, serving as silent reservoirs that contribute to the spread of antimicrobial resistance (AMR). These carriers can unknowingly transmit resistant strains through direct contact, food, water, or the environment, making it difficult to control outbreaks (Holmes *et al*., 2016). While AMR is often studied in hospitals, research shows that healthy individuals in the community can also harbor resistant bacteria, highlighting that resistance is a wider public health issue (van Schaik, 2015). Surveillance in healthy populations can act as an early warning system, allowing the detection of emerging resistance before it causes large-scale infections (WHO, 2021). Moreover, if carriers later develop infections, these resistant bacteria may complicate treatment, increasing the risk of failure and poor outcomes (Prestinaci *et al*., 2015). Recent studies demonstrate that apparently healthy university students can carry antibiotic-resistant bacteria in their urinary tract, even without showing symptoms. For example, a 2023 study in Ethiopia found that 13.5 % of asymptomatic female students had

significant bacteriuria, with most bacterial isolates showing high resistance to commonly used antibiotics such as penicillin (97 %), amoxicillin-clavulanate (92 %) and trimethoprim-sulfamethoxazole (79 %). Overall, nearly 88% of isolates were multidrug resistant, with *Staphylococcus saprophyticus* and *Escherichia coli* being the most common pathogens (Reda *et al*., 2023). Similar findings have been observed among symptomatic university students. A study from northern Ethiopia reported that *E. coli* was the leading uropathogen, with 73 % of isolates displaying multidrug resistance and 26 % producing extended-spectrum beta-lactamases (ESBLs), complicating treatment options (Gebremariam *et al*., 2019). Comparable data from West Africa also highlight the issue: a study in Ghana found asymptomatic bacteriuria among about 10 % of students, with nitrofurantoin and gentamicin showing good activity against isolates, in contrast to poor performance by many beta-lactams (Boye *et al*., 2016). Taken together, these findings suggest that even apparently healthy students can act as reservoirs for resistant urinary pathogens, particularly *E. coli* and *S. saprophyticus*, contributing to the community-level spread of antibiotic resistance The implications for university health services are significant. International guidelines emphasize that screening or treating healthy, non-pregnant students for asymptomatic bacteriuria is unnecessary and potentially harmful, as it increases resistance without improving outcomes (Nicolle *et al*., 2019). Instead, campus health services should prioritize building a local antibiogram to guide empiric therapy, given the poor performance of older first-line agents like ampicillin, amoxicillin-clavulanate and trimethoprim-sulfamethoxazole. Safer empiric options, such as nitrofurantoin or fosfomycin (where available), should be considered based on local susceptibility data (Reda *et al*., 2023; Gebremariam *et al*., 2019).

2.2 Antibiotic Resistance in Urinary Tract Infections

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting millions of people annually. They occur when harmful microorganisms, usually bacteria, invade parts of the urinary system such as the urethra, bladder, or kidneys, leading to inflammation and discomfort (Nicolle, 2023). In most cases, UTIs are caused by uropathogenic *Escherichia coli* (UPEC), which originates from the gut and spreads to the urinary tract. Other bacteria, including *Klebsiella* and *Proteus* species, may also be involved, especially in complicated or hospital-acquired infections (Gupta *et al*., 2022). Several risk factors contribute

to the development of UTIs. Women are disproportionately affected due to anatomical differences, particularly a shorter urethra and its proximity to the anus. Sexual activity, use of spermicides, menopause and hormonal changes further increase susceptibility (Mayo Clinic, 2023). Additional risks include urinary tract blockages such as kidney stones, prolonged catheter use and weakened immunity linked to chronic conditions like diabetes (Cleveland Clinic, 2024). Men are less commonly affected, but cases tend to increase with age, often associated with prostate enlargement or urinary retention (Nicolle, 2023). Urinary tract infections remain one of the most common bacterial infections across the lifespan and their global incidence is climbing. In 2019 there were an estimated 0.4 billion incident UTI cases, roughly a 60% rise since 1990, underscoring how frequently these infections occur in both community and healthcare settings (Murray *et al*., 2022). Critically, antimicrobial resistance (AMR) amplifies the toll of what would otherwise be readily treatable infections: 64,890 deaths were directly attributable to AMR in UTIs in 2019, with an additional ~260,000 deaths associated with resistant uropathogens meaning resistant bacteria were present and likely contributed to poor outcomes (Murray *et al*., 2022).

Antibiotic resistance in urinary tract infections, especially those caused by *Escherichia coli*, is a growing problem in healthcare. One major reason is the production of special enzymes called β-lactamases (including ESBLs and carbapenemases). These enzymes break down important antibiotics such as penicillins, cephalosporins and carbapenems, making them useless against the bacteria (Bush and Bradford, 2020). Resistance can also happen when bacteria change the structure of their target sites. Mutations in DNA gyrase and topoisomerase IV reduce the effectiveness of fluoroquinolones, leading to treatment failure (Gajdács, 2019).

Another way bacteria resist treatment is through efflux pumps, like AcrAB-TolC in *E. coli*, which act like tiny pumps to push antibiotics out of the cell before they can work (Delmar *et al*., 2021). Some bacteria also reduce drug entry by altering or losing porin channels in their outer membrane, which prevents antibiotics from getting inside (Dadgostar, 2019). In the case of aminoglycosides, resistance often develops because of enzymes that modify and inactivate the drug, stopping it from reaching its target (Gajdács, 2019).

Resistance genes can also spread quickly between bacteria through mobile genetic elements like plasmids, integrons and transposons. This means once one bacterium becomes resistant, it can share that ability with others, increasing the number of multidrug-resistant strains in hospitals

and communities (Beceiro *et al*., 2021). Finally, some bacteria form biofilms, which are protective layers that shield them from antibiotics and the immune system, helping them survive in the urinary tract (Flores-Mireles *et al*., 2022).

2.3 Bacteria Commonly Isolated from Urine

Urinary tract infections (UTIs) are caused by a variety of bacterial species, with *Escherichia coli* being the most predominant pathogen. Uropathogenic *E. coli* (UPEC) accounts for nearly 70–90 % of uncomplicated UTIs due to its ability to produce adhesins, hemolysins and biofilms that help it colonize the urinary tract. However, its increasing resistance to trimethoprim-sulfamethoxazole, fluoroquinolones and β-lactams, particularly through extended-spectrum β-lactamase (ESBL) production, has made treatment more challenging (Lee *et al*., 2021).

Klebsiella pneumoniae is another important uropathogen, especially in complicated or hospital-acquired UTIs. Its thick polysaccharide capsule enhances virulence and helps it evade immune defenses. Unfortunately, it frequently carries resistance genes, including those encoding ESBLs and carbapenemases (KPC), making it one of the most problematic multidrug-resistant organisms in urinary infections (Ranjan *et al*., 2021).

2.3.1 Proteus mirabilis

This is commonly linked to complicated and catheter-associated UTIs. It produces urease, which raises urine pH and promotes kidney stone formation, increasing the risk of recurrent infections. This organism is intrinsically resistant to nitrofurantoin and shows rising resistance to cephalosporins and fluoroquinolones, with ESBL strains also emerging (Wang *et al*., 2020).

2.3.2 Enterococcus faecalis

It is a significant cause of hospital-acquired UTIs, particularly in patients with catheters. Its resilience in harsh environments and persistence on medical surfaces make it a difficult pathogen to eradicate. Alarmingly, some strains exhibit resistance to β-lactams and aminoglycosides and vancomycin-resistant enterococci (VRE) are becoming a critical clinical concern (Flores-Mireles *et al*., 2022).

2.3.3 Staphylococcus saprophyticus

It plays a unique role as the second most common cause of community-acquired UTIs, especially in young, sexually active women. While it typically causes uncomplicated cystitis and remains generally susceptible to common antibiotics, resistance to trimethoprim-sulfamethoxazole and fluoroquinolones has been reported in some regions (Lee *et al*., 2021).

2.3.4 Pseudomonas aeruginosa

This is an opportunistic pathogen most often associated with hospital-acquired and catheter-related UTIs, particularly in immunocompromised patients. Its ability to form biofilms and its intrinsic resistance mechanisms, including efflux pumps and reduced membrane permeability, make it highly resistant to multiple antibiotic classes. Increasing resistance to carbapenems, aminoglycosides and fluoroquinolones further complicate treatment strategies (Ranjan *et al*., 2021).

2.4 Patterns of Antibiotic Resistance in Urinary Isolates

Escherichia coli remains the most common cause of community and healthcare-associated UTIs, but its resistance to many first-line antibiotics has steadily increased. Studies report high resistance rates to ampicillin and trimethoprim-sulfamethoxazole (TMP-SMX), with fluoroquinolone resistance also rising due to the spread of high-risk clones such as ST131 (Flores-Mireles *et al*., 2019; Dadgostar, 2019). By contrast, nitrofurantoin and fosfomycin generally remain effective for uncomplicated cystitis, though their use in pyelonephritis is not recommended because of poor tissue penetration (Gupta *et al*., 2021). *Klebsiella pneumoniae* shows even higher rates of extended-spectrum β-lactamase (ESBL) production and emerging carbapenem resistance in several regions (Tamma and Rodriguez-Baño, 2017). Other isolates, such as *Proteus mirabilis*, exhibit intrinsic nitrofurantoin resistance, while *Pseudomonas aeruginosa* often demonstrates multidrug resistance through efflux pumps and β-lactamases (Tacconelli *et al*., 2018). Enterococci, particularly *E. faecalis*, frequently retain susceptibility to ampicillin and nitrofurantoin in the urinary tract, though vancomycin-resistant enterococci (VRE) complicate treatment (O'Driscoll and Crank, 2015). *Staphylococcus saprophyticus*, usually susceptible to nitrofurantoin and TMP-SMX, remains a relevant pathogen in young women but has shown emerging biofilm-associated resistance (Lee *et al*., 2018).

Multiple surveillance reports confirm that *E. coli* resistance to TMP-SMX exceeds 20–30% in many countries, while fluoroquinolone resistance has sharply increased in both community and hospital isolates (Kahlmeter *et al*., 2021). ESBL-producing *E. coli* strains have risen globally, with prevalence climbing steadily over the past decade (Tamma and Rodriguez-Baño, 2017). Despite this, nitrofurantoin and fosfomycin remain effective options for lower UTIs, though inappropriate for pyelonephritis (Gupta *et al*., 2021). For complicated infections caused by ESBL-*E. coli*, carbapenems remain the most reliable option, but step-down to oral agents such as fluoroquinolones or TMP-SMX is recommended if susceptibility is confirmed (Rodríguez-Baño *et al*., 2018).

K. pneumoniae frequently carries ESBL and carbapenemase genes (e.g., KPC, NDM, OXA), making it one of the most challenging urinary pathogens to treat (Munoz-Price *et al*., 2019). *Proteus mirabilis*, a known stone-forming pathogen, is intrinsically resistant to nitrofurantoin and should be treated with other oral or intravenous agents (O'Hara *et al*., 2000). *Pseudomonas aeruginosa* often shows resistance to multiple drug classes, requiring susceptibility-guided therapy with antipseudomonal β-lactams (Tacconelli *et al*., 2018). Enterococcal UTIs, mostly caused by *E. faecalis*, are often susceptible to amoxicillin and nitrofurantoin, though *E. faecium* isolates show higher rates of vancomycin resistance (O'Driscoll and Crank, 2015). *S. saprophyticus* generally remains susceptible to nitrofurantoin, though resistant strains are being increasingly reported (Lee *et al*., 2018).

2.5 Mechanisms of Antibiotic Resistance in Urinary Isolates

Antibiotics are often called "miracle drugs" because they changed the course of medicine forever. Infections that once claimed millions of lives like pneumonia, tuberculosis, or sepsis became treatable within days. But today, that miracle is slipping away. Around the world, bacteria are becoming resistant to antibiotics, making common infections harder and sometimes impossible, to cure. This problem, known as antibiotic resistance, is quietly growing into one of the biggest health threats of our time. The numbers are sobering. In 2019 alone, antibiotic-resistant infections were directly responsible for an estimated 1.27 million deaths and contributed to nearly 5 million deaths worldwide (Murray *et al*., 2022). Everyday illnesses like urinary tract infections, which affect around 400 million people globally each year, are becoming more complicated because many bacteria no longer respond to standard antibiotics. Even simple

medical procedures like a C-section or hip replacement rely on antibiotics to prevent infection. Without them, these routine treatments could once again become life-threatening. What makes antibiotic resistance especially alarming is how easily it spreads.

Resistant bacteria travel through people, animals, food and the environment, meaning that what starts as a local problem can quickly turn global. For patients, this often means longer hospital stays, stronger and more toxic drugs, higher medical costs and sometimes no cure at all. That's why antibiotic resistance is often called a "silent pandemic." It doesn't make headlines like COVID-19, but its impact is enormous and growing. If we don't act, the world risks entering a post-antibiotic era, where minor injuries or routine infections could once again kill. Protecting antibiotics requires all of us using them only when needed, supporting infection prevention and investing in new treatments to make sure these life-saving medicines remain effective for future generations.

2.5.1 Fluoroquinolones

Fluoroquinolones, such as ciprofloxacin and levofloxacin, are commonly prescribed for urinary tract infections because of their broad activity and high urinary concentrations. They act by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication. Resistance, however, has become widespread and is primarily caused by mutations in the quinolone resistance-determining regions (QRDRs) of the gyrA and parC genes, which reduce drug affinity for their targets (Rezaei *et al*., 2024). In addition, plasmid-mediated quinolone resistance (PMQR) genes, including qnr, aac(6′)-Ib-cr and efflux pump genes such as oqxAB and qepA, contribute to low-level resistance and promote the selection of chromosomal mutations (Machuca *et al*., 2016; Gomi *et al*., 2025). Efflux pump overexpression and porin loss further strengthen resistance, making clinical infections harder to treat. Recent surveillance shows fluoroquinolone resistance in *E. coli* isolates from complicated UTIs can reach almost 50%, with high prevalence in global clones ST131 and ST1193 (García-Meniño *et al*., 2024).

2.5.2 Aminoglycosides

Aminoglycosides, including gentamicin, amikacin and tobramycin, bind the 30S ribosomal subunit, causing misreading of mRNA and inhibiting protein synthesis. Resistance to this class is mediated mainly by aminoglycoside-modifying enzymes (AMEs), which include

acetyltransferases (AAC), nucleotidyltransferases (ANT) and phosphotransferases (APH) that chemically modify the drug and block ribosomal binding (Foudraine *et al*., 2021). A more concerning mechanism is the emergence of 16S rRNA methyltransferases such as ArmA and RmtB, which methylate the aminoglycoside binding site and confer very high-level resistance to nearly all clinically important aminoglycosides (Wachino *et al*., 2020). These resistance genes are increasingly found in Enterobacterales isolates co-carrying carbapenemases, severely limiting therapeutic options (Li *et al*., 2024).

2.5.3 β-Lactams and Cephalosporins

β-lactams, including penicillins, cephalosporins and carbapenems, are widely used in UTI management and act by binding penicillin-binding proteins (PBPs) to inhibit cell wall synthesis. Resistance is most commonly mediated by β-lactamases. Extended-spectrum β-lactamases (ESBLs) such as CTX-M, TEM and SHV hydrolyze third-generation cephalosporins like cefotaxime and ceftazidime and are now highly prevalent among urinary *E. coli* isolates (Azzam *et al*., 2024). AmpC β-lactamases, either chromosomal or plasmid-mediated, provide additional resistance and are not effectively inhibited by clavulanate (IDSA, 2024). Carbapenemases such as KPC, NDM and OXA-48-like hydrolyze carbapenems and are frequently carried on mobile elements with other resistance genes, accelerating dissemination (Onyeji *et al*., 2024; Carroll *et al*., 2024). Resistance is further enhanced by porin loss, which reduces drug entry and synergizes with β-lactamase activity (Bologna *et al*., 2024).

2.5.4 Sulfonamides

Sulfonamides, particularly when combined with trimethoprim (TMP-SMX), are used in UTI therapy by targeting bacterial folate synthesis pathways. Resistance to this class is primarily due to the acquisition of sul genes (sul1, sul2, sul3 and sul4), which encode drug-resistant dihydropteroate synthases and dfr genes, which produce trimethoprim-resistant dihydrofolate reductases (Poey *et al*., 2024; de los Santos *et al*., 2021). These genes are frequently carried on integrons and transposons, which facilitate their rapid dissemination among Enterobacterales. Class 1 integrons, in particular, are strongly associated with TMP-SMX resistance in *E. coli* urinary isolates (Hemati *et al*., 2024).

2.5.5 Macrolides

Although macrolides are not commonly used for Gram-negative UTIs due to poor activity, they remain relevant for Gram-positive pathogens such as *Enterococcus faecalis* or *Staphylococcus saprophyticus*. Resistance mechanisms include ribosomal target modification by methyltransferases encoded by erm genes (ermA, ermB and ermC), which methylate the 23S rRNA and prevent antibiotic binding (Chen *et al*., 2023). Efflux pumps encoded by mef(A/E) and msr(D) also contribute by actively exporting macrolides, while mph genes encode macrolide-phosphotransferases that enzymatically inactivate the drug (Abdel-Karim *et al*., 2025; Ma *et al*., 2024). These resistance determinants are often detected in urinary isolates of *Enterococcus*, frequently alongside aminoglycoside resistance genes, which complicates therapy (Guan *et al*., 2024).

Table 1: Major mechanisms of antibiotic resistance in urinary pathogens

Antibiotic Class	Target / Mode of Action	Resistance Mechanisms
Fluoroquinolones	Inhibit DNA gyrase and topoisomerase IV	Target site mutations; plasmid-mediated resistance; efflux pump overexpression
Aminoglycosides	Bind 30S ribosomal subunit to inhibit protein synthesis	Aminoglycoside-modifying enzymes (AMEs); 16S rRNA methylation; efflux
β-lactams (Penicillins and Carbapenems)	Inhibit cell wall synthesis by binding PBPs	β-lactamase production (ESBLs, carbapenemases); porin mutations; efflux pumps
Cephalosporins	Similar to other β-lactams (PBP inhibition)	Extended-spectrum β-lactamases (ESBLs); AmpC β-lactamase
Sulfonamides and Trimethoprim (TMP-SMX)	Inhibit folate synthesis (*folP*, *folA*)	Target gene mutations; plasmid-borne resistant enzymes; integron-mediated resistance
Macrolides	Bind 50S ribosomal subunit, blocking elongation	Methylation of 23S rRNA; active efflux; intrinsic resistance

Source: Garcia-Menino (*et al*., 2024)

2.6 Contributing Factors to Antibiotic Resistance

2.6.1 Overuse and Misuse of Antibiotics in Human Medicine

The inappropriate use of antibiotics in healthcare is one of the strongest drivers of antimicrobial resistance (AMR). Studies indicate that nearly one-third of global antibiotic prescriptions are unnecessary, often given for viral infections such as influenza and the common cold (Khalil *et al*., 2025). In many low- and middle-income countries (LMICs), antibiotics are available without prescription, leading to self-medication and incomplete courses that encourage the survival of resistant strains (Ayukekbong *et al*., 2017; Cars *et al*., 2021).

2.6.2 Extensive Use of Antibiotics in Agriculture and Animal Husbandry

The agricultural sector is a major consumer of antibiotics worldwide, particularly in livestock and poultry production. Antibiotics are often administered not only for treatment but also for growth promotion and disease prevention under intensive farming conditions (Ma *et al*., 2021). These practices contribute to the emergence of resistant bacteria that can be transmitted to humans via direct contact, contaminated food, or the environment (Ardakani *et al*., 2023)

2.6.3 Lack of New Antibiotic Development

The discovery and development of novel antibiotics have slowed significantly over the past decades. Most new approvals are derivatives of existing classes rather than entirely new compounds, leaving few therapeutic options for multidrug-resistant infections (Ventola, 2015; Klein *et al*., 2024). High research costs and low economic returns further discourage pharmaceutical investment, creating an “innovation gap” that exacerbates the crisis (Aslam *et al*., 2018)

2.6.4 Global Travel and Trade

Resistant bacteria do not remain confined to one region. Modern air travel and international trade facilitate the rapid spread of resistant strains across borders. Surveillance studies demonstrate that antimicrobial-resistant pathogens are frequently detected in aircraft wastewater, highlighting the global transmission of resistance (Hendriksen *et al*., 2019). This globalization of AMR poses serious challenges to containment, especially in countries with weak surveillance (Munk *et al*., 2018).

2.6.5 Poor Infection Prevention and Control Practices

Weak infection prevention and control (IPC) in healthcare settings accelerates the spread of resistant organisms. Overcrowding, inadequate sanitation, limited hygiene practices and insufficient protective equipment contribute to hospital-acquired infections caused by pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Acinetobacter baumannii* (Ho *et al*., 2024). Strengthening IPC and water, sanitation and hygiene (WASH) infrastructure is crucial to reducing infection rates and limiting antibiotic use (World Health Organization [WHO], 2023).

2.6.6 Environmental Contamination

Wastewater and effluents from hospitals, pharmaceutical industries and agricultural facilities often contain antibiotics and resistant bacteria. These contaminants enter rivers, lakes and soil ecosystems, creating selective pressure that fosters resistance in environmental microbial communities (Larsson and Flach, 2022). Environmental reservoirs serve as gene pools, enabling resistance genes to transfer to clinically relevant pathogens via horizontal gene transfer (La Rosa *et al*., 2025).

2.6.7 Weak Regulatory and Stewardship Systems

In countries with limited enforcement of prescription regulations, antibiotics can be easily purchased without medical oversight. This unregulated access promotes self-medication and irrational prescribing (O'Neill, 2016; Booton *et al*., 2021). Even in regulated settings, inadequate antibiotic stewardship programs within healthcare facilities contribute to inappropriate prescribing practices (Abdul-Aziz *et al*., 2020)

2.6.8 Socio-Economic and Behavioral Factors

Poverty, low health literacy and poor access to healthcare play a critical role in resistance dynamics. Patients in LMICs often rely on informal markets, share medications, or interrupt treatments due to financial constraints (Gebretekle *et al*., 2021). Misinformation and cultural practices also drive irrational antibiotic use, particularly in rural communities (Auta *et al*., 2019)

2.6.9 Horizontal Gene Transfer Among Bacteria

Beyond human practices, bacterial genetics strongly influence resistance dynamics. Resistance genes are often located on mobile genetic elements such as plasmids, transposons and bacteriophages, facilitating rapid horizontal transfer between bacterial species (Partridge *et al*., 2018). This mechanism explains the emergence of multidrug-resistant organisms such as extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (Mathers *et al*., 2015)

2.6.10 Climate Change and Environmental Shifts

Emerging evidence suggests that rising global temperatures and ecological changes influence the spread of resistant pathogens. Climate change can expand the geographic range of bacteria and accelerate their replication rates, indirectly promoting resistance selection (Wuijts *et al*., 2017). Moreover, climate-driven flooding and droughts may exacerbate antibiotic contamination in water systems (Kaba *et al*., 2020)

2.7 Public Health Implications

One of the most concerning aspects of antibiotic resistance is the silent spread of resistant bacteria in communities. Many individuals, including healthy carriers, may harbor resistant strains without showing clinical symptoms, thereby serving as reservoirs for transmission. This asymptomatic carriage poses a hidden risk because resistant organisms can be transferred through direct contact, contaminated food, or environmental exposure, leading to community-wide dissemination before outbreaks are detected (Holmes *et al*., 2016; Murray *et al*., 2022).

The potential impact on future treatment options is equally significant. As more bacterial strains develop multidrug resistance, conventional antibiotics lose their efficacy, narrowing therapeutic choices and forcing reliance on last-line or more toxic alternatives. This trend increases the likelihood of treatment failures, prolonged illness, higher healthcare costs and mortality, especially in vulnerable populations such as children, the elderly and immunocompromised individuals (Laxminarayan *et al*., 2020; Cassini *et al*., 2019). If resistant bacteria continue to spread unchecked, minor infections that were once easily treatable may become life-threatening, undermining decades of medical progress.

To mitigate these risks, the importance of surveillance and monitoring in healthy populations cannot be overstated. While most resistance research focuses on clinical cases, evidence shows

that resistant bacteria are also present among healthy carriers, including university students and community dwellers (Tacconelli *et al*., 2018). Routine surveillance of such groups enables early detection of emerging resistance patterns, informs antibiotic stewardship strategies and guides targeted interventions to prevent widespread outbreaks. Effective monitoring at both local and global levels also strengthens preparedness against future public health emergencies, ensuring that resistance trends are recognized and addressed proactively rather than reactively (World Health Organization [WHO], 2021).

2.8 Strategies for the Control and Prevention of Antibiotic Resistance

2.8.1 Responsible Use of Antibiotics (Antibiotic Stewardship)

The responsible use of antibiotics, also known as antimicrobial stewardship, is critical to preserving the effectiveness of existing drugs. Stewardship involves prescribing antibiotics only when necessary, using the right drug, at the correct dose and for the appropriate duration (Dyar *et al*., 2017). In hospitals, antimicrobial stewardship programs (ASPs) reduce inappropriate prescriptions, limit the spread of resistant bacteria and improve patient outcomes. For example, structured stewardship interventions have been shown to reduce broad-spectrum antibiotic use and decrease the prevalence of multidrug-resistant organisms (Pulcini *et al*., 2019). In community settings, discouraging self-medication and over-the-counter antibiotic use is equally important to ensure that treatment decisions are guided by healthcare professionals.

2.8.2 Health Education and Awareness Programs

Education plays a central role in shaping long-term behaviors related to antibiotic use. Students, particularly in universities and colleges, are an important target group because they represent a population that often engages in self-medication and may have misconceptions about antibiotics (Huttner *et al*., 2010). Health education campaigns in schools and universities can raise awareness about the dangers of misuse, the importance of completing prescribed courses and the role of hygiene in preventing infections. Introducing modules on antimicrobial resistance into school curricula ensures that young people develop a deeper understanding of responsible antibiotic use (Dyar *et al*., 2017). Moreover, peer-led campaigns and social media–based interventions have been shown to be effective in reaching younger populations and fostering behavior change (Kakkar *et al*., 2018).

2.8.3 Strengthening Policies to Regulate Antibiotic Sales

Weak regulatory systems contribute significantly to antibiotic misuse, particularly in low- and middle-income countries where over-the-counter sales remain common (Laxminarayan *et al*., 2016). Strengthening policies to regulate antibiotic distribution ensures that these drugs are only dispensed with a valid prescription, reducing the likelihood of inappropriate use. Enforcement of prescription-only policies, coupled with stricter monitoring of pharmacies, is essential to curb misuse (WHO, 2021). At the same time, policies should ensure equitable access to antibiotics for patients who genuinely need them, particularly in rural or underserved areas. International experiences, such as restrictions implemented in parts of Europe, demonstrate that strong regulation can effectively reduce community-level antibiotic consumption and resistance rates (Willyard, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Location

This study was carried out at the Laboratory Department of Microbiology, Faculty of Life Science, University of Benin.

3.2 Collection of bacterial isolates from clinical sources

Thirty-five (35) bacteria isolates from urine were collected with a sterile test tubes containing a prepared nutrient broth from my supervisor as a kind gift gesture.

3.3 Inoculation and incubation

Thirty-five sterile test tubes were labeled and with the aid of a sterile inoculating loop, the bacteria isolate was inoculated into a prepared nutrient broth medium in a sterile test tube. It was then incubated at 37 ^{o}C for 18 - 24 hours

3.4 Identification of the bacteria isolates

Enrichment of the bacteria was done by inoculated into a prepared nutrient broth medium in a sterile test tube. It was then incubated at 37 ^{o}C for 18 - 24 hours. The pre-enriched culture was then streaked on the surfaces of selective media (Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar (MSA) agar and incubated for 24hrs at 37 ^{o}C (Elanthamil *et al*., 2018).

3.5 Standardization of the Isolates

MacFarland standard (0.5) was prepared by mixing 0.05ml of 1% barium chloride ($BaCl_2$) with 9.95ml of 1% Sulfuric acid(H_2SO_2) to form barium sulphate suspension. The turbid solution (Mc Farland standard) formed was transferred into a test tube for comparison with different bacterial inoculums suspension (Cheesbrough, 2004)

3.6 Antibiotics susceptibility test

Kirby-bauer disc diffusion technique was used as to determine the antibacterial activity of isolated bacteria. 20ml Mueller Hinton agar plates were prepared following the manufacturer's instructions. 1ml aliquot of each test organism suspension (standardized) was transferred onto the well-dried Mueller Hinton agar plates and was spread evenly following slow rotation of the

plates and excess was decanted. The plates were allowed to dry, with the aid of sterile forceps, antibiotic disc was impregnated in the well-dried Mueller Hinton agar plates. The antibiotics disk contains Levofloxacin (20mg), cefuroxime (30mg), Gentamycin (10g), Rifampicin(20mg), Streptomycin (30mg), Azithromycin (10mg) Ciprofloxacin (10mg) Erythromycin (30mg) The plates were incubated for 24h. At 37 °C. The resultant visible zones of inhibition were measured in millimetres (mm). Zones were interpreted using the NCCL. (Cheesbrough, 2002).

3.7 Gelatinase production test

The gelatinase production test was carried out by inoculating pure bacterial isolates onto nutrient agar supplemented with 1% gelatin and incubating the plates at 37 °C for 72 hours. Following incubation, the presence of a clear zone around the inoculation spot indicated the enzymatic hydrolysis of gelatin, confirming gelatinase activity (Ristow and Welch, 2016).

3.8 Hemolysis

Pure cultures of bacterial isolates were grown on the surface of 5% defibrinated sheep blood agar that is made with nutrient agar and incubated at 37 ^{o}C for 72hrs. Lysing of the red blood cells is indicated by a clear halo around the inoculum spot is indicative of haemolysin production (Ristow and Welch, 2016).

3.9 Serum Resistance Assay

The serum resistance assay was carried out by incubating bacterial isolates in pooled normal human serum (NHS), which served as a source of complement. Test organisms were grown from single colonies in Mueller–Hinton broth. The bacteria were incubated with serum within an interval of 30, 60 and 120 minutes and plated on Mueller–Hinton agar to determine cfu counts. (Necchi *et al*. (2017).

3.10 Capsule Formation test

The capsule staining procedure was carried out using Congo Red as the negative stain. A small drop of Congo Red was placed on a clean glass slide and a loopful of bacterial culture was added aseptically and gently mixed with the stain. The mixture was then spread into a thin smear by dragging another clean slide across the surface at an angle and was allowed to stand for 5–7 minutes. The smear was left to air dry completely without heat fixation to avoid shrinking or destroying the capsules. After drying, the smear was flooded with crystal violet stain for about 1

minute to stain the bacterial cells while leaving the capsules unstained. The excess stain was drained off by tilting the slide at a 45 °C angle and counter stained with 20 % copper sulphate solution and the slide was allowed to air dry. Finally, the preparation was examined microscopically under the oil immersion objective (100×), where encapsulated cells appeared as dark-stained bacterial cells surrounded by clear halos representing the capsules against a darker background (Sofos, 2009).

CHAPTER FOUR

RESULTS

Figure 4.1 shows the distribution of bacterial isolates from the urine samples of apparently healthy students. *Escherichia coli* as the most prevalent bacteria 6 (26%), followed by *Staphylococcus aureus* 3 (17%), *Streptococcus* sp. 4 (13%), *Salmonella* sp. 3 (12%) while *Micrococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* 2 (8%) respectively.

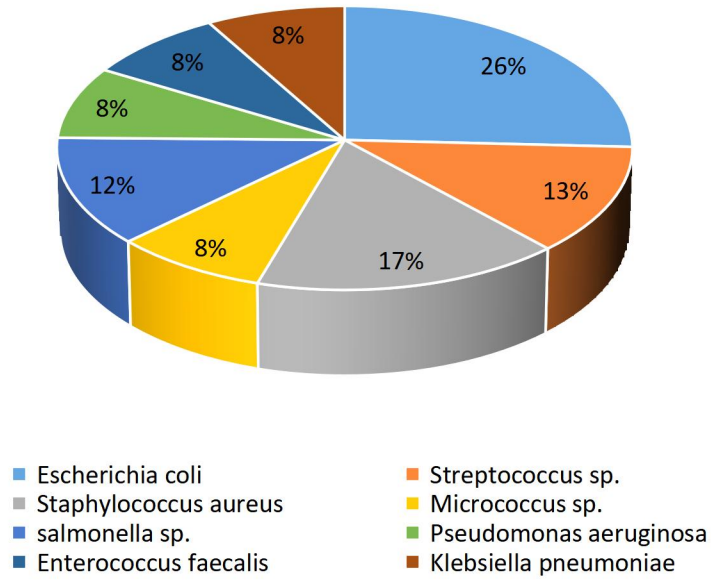

Figure 1: Percentage frequency of isolates from urine

Table 4.1 shows the Haemolysis activity of the bacteria isolates from urine sample. The result revealed that all the bacterial isolates from urine samples exhibited haemolytic activity, though with variations in the type of haemolysin produced. The predominant haemolysin type observed was β-haemolysin, produced by *E. coli, Streptococcus* sp., *S. aureus* and *Salmonella* sp., while *Micrococcus* sp. produced α-haemolysin and no haemolysis in *Klebsiella pneumoniae* and *Enterococcus faecalis*

Table 4.1: Haemolysis activity of the isolates from urine sample

Isolates	n (%)	Type of haemolysin n (α), n (ß), n(γ)	Percentage (%)
Escherichia coli	6 (25)	0(0), 6(ß), 0(0)	(100)
Streptococcus sp.	3 (12)	0(0), 3(ß), 0(0)	(100)
Staphylococcus aureus	4 (16)	0(0), 4(ß), 0(0)	(100)
Micrococcus sp.	2 (8)	2(α), 0(0), 0(0)	(100)
salmonella sp.	3 (12)	0(0), 3(ß), 0(0)	(100)
P. aeruginosa	2(8)	0(0), 2(ß), 0(0)	2(100)
Enterococcus faecalis	2(8)	0(0), 0(0), 0(0)	0(0)
Klebsiella pneumoniae	2(8)	0(0), 0(0), 0(0)	0(0)

Table 4.2 show the outcome of the capsule formation test for the bacterial isolates obtained from urine samples. Microscopic examination revealed that *Escherichia coli*, *Streptococcus* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella* sp. demonstrated dark-stained cells surrounded by distinct clear halos, which is indicative of capsule production. In contrast, *Micrococcus* sp., *Pseudomonas aeruginosa* and *Enterococcus faecalis* showed only dark-stained cells without visible halos, indicating the absence of capsule formation.

Table 4.2: Capsule formation of the isolates from urine sample

Isolates	**n(%)**
Escherichia coli	6(100)
Streptococcus sp.	3(100)
Staphylococcus aureus	4(100)
Micrococcus sp.	0(0)
salmonella sp.	3(100)
P. aeruginosa	0(0)
Enterococcus faecalis	0(0)
Klebsiella pneumoniae	2(100)

Table 4.3 revealed variable survival patterns among the bacterial isolates following exposure to normal human serum. *Escherichia coli*, *Streptococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Micrococcus* sp. maintained relatively high viable counts after 120 minutes of incubation, showing only minor reductions from their baseline cfu counts. This indicates resistance to complement-mediated killing and suggests the presence of surface structures such as capsules, outer membrane proteins, or other serum resistance factors that protect against complement attack. In contrast, *Staphylococcus aureus*, *Enterococcus faecalis* and *Salmonella* sp. exhibited marked reductions in cfu counts after 120 minutes, reflecting sensitivity to complement-mediated lysis. The serum-sensitive nature of these isolates may be linked to the absence or reduced expression of protective surface components.

Table 4.3: Serum resistance assay of isolates from urine sample

Isolates	**Time (M)**		
	(0 cfu/ml)	**(120) cfu/ml**	
Escherichia coli	1.2×10^6	9.0×10^5	Resistant
Streptococcus sp.	1.3×10^6	8.8×10^5	Resistant
Staphylococcus aureus	1.1×10^6	1.5×10^5	Sensitive
Micrococcus sp.	1.5×10^6	9.5×10^5	Resistant
salmonella sp.	1.0×10^6	5.0×10^5	Sensitive
P. aeruginosa	1.2×10^6	1.2×10^5	Resistant
E. faecalis	1.1×10^6	1.1×10^4	Sensitive
K. pneumoniae	1.2×10^6	1.2×10^6	Resistant

The gelatinase production test revealed that *Escherichia coli*, *Staphylococcus aureus*, *P. aeruginosa* and *Salmonella* sp. exhibited clear zones around the colonies, confirming gelatin hydrolysis and indicating gelatinase activity. In contrast, *Streptococcus* sp. *Enterococcus faecalis*, *K. pneumoniae* and *Micrococcus* sp. showed no visible zones of clearance, suggesting the absence of gelatinase production. Gelatinase is an extracellular metalloproteinase that degrades gelatin, collagen and other host proteins, facilitating bacterial invasion and tissue dissemination

Table 4.4: Gelatinase production test of isolates from urine sample

Isolates	n(%)	Gelatinase production	
		n(%) +	n(%) -
Escherichia coli	6 (25)	6(100)	3(100)
Streptococcus sp.	3 (12)	0(0)	3(100)
Staphylococcus aureus	4 (16)	4(100)	0(0)
Micrococcus sp.	2 (8)	0(0)	2(100)
salmonella sp.	3 (12)	3(100)	0(0)
P. aeruginosa	2(8)	2(100)	0(0)
E. faecalis	2(8)	0(0)	2(100)
K. pneumoniae	2(8)	0(0)	2(100)

4.6 Antibiotic resistance of the bacteria isolates from urine

Table 4.5: Showing Antibiotic resistance of the bacteria isolates from urine

Isolates	n (%)	LEV	CN	CEF	RD	CTZ	S	AZM	AMX	CPX	E
Escherichia coli	6(100)	6(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	6(100)	0(0.0)
Streptococcus sp.	3(100)	3(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100)	0(0.0)
S. aureus	4(100)	4(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(100)	0(0.0)
Micrococcus sp.	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)
Salmonella sp.	3(100)	3(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100)	0(0.0)
P. aeruginosa	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)
E. faecalis	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)
K. pneumoniae	2(100)	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	2(100)	0(0.0)

KEY: LEV-Levofloxacin, CN-Gentamycin, CEF-Cefuroxime, RD-Rifampicin, CTZ-Ceftazidime, S-Streptomycin, AZM-Azithromycin, AMX-Amoxil, CPX-Ciprofloxacin, E-Erythromycin

0(0.0) – Resistance, (100) – Susceptible

CHAPTER FIVE

DISCUSSION

This study focused on antibiotic resistance pattern and virulence factors of bacteria isolates from urine of apparently healthy university students. conventional microbiological techniques were used to identify and characterize the bacteria isolates from urine gifted as kind gesture from my supervisor. Nutrient broth was used for enrichment and incubated at 37 °C for 18–24 hours was effective in supporting the growth of the isolates which is in consonance consistent with the findings of Cheesbrough (2004, 2006), who highlighted the importance of preliminary enrichment in clinical bacteriology for maximizing recovery rates of viable organisms. Similar approached have been reported in studies focusing on urinary tract pathogens, where enrichment prior to selective plating improved detection rates of *Escherichia coli* and *Klebsiella* sp. (Okonko *et al*., 2009; Oluremi *et al*., 2011).

The identification of *Escherichia coli* on Eosin Methylene Blue agar (EMB) based on its characteristic green metallic sheen remains a well-established approach and our findings corroborate the diagnostic utility of EMB reported by Aibinu *et al*. (2004) and Orhue *et al*. (2015) also observed the distinctive sheen in clinical isolates, confirming EMB as a reliable medium for presumptive *E. coli* identification. However, studies employing chromogenic media (e.g., CHROMagar) have suggested higher specificity and faster turnaround times compared to EMB (Manaia *et al*., 2018), indicating that while classical EMB remains effective, more recent innovations may offer diagnostic advantages in modern laboratories.

Similarly, the isolation of *Salmonella* sp. on Salmonella-Shigella agar which yielded colorless colonies with black centers is in agreement with earlier reports where H_2S production served as a critical diagnostic feature (Singh *et al*., 2014). Nonetheless, other researchers have argued that SS agar has lower sensitivity compared to more selective media such as Xylose Lysine Deoxycholate (XLD) agar (Andrews-Polymenis *et al*., 2010), suggesting that while our findings are valid, alternative media could enhance recovery of *Salmonella* in mixed cultures.

The identification of *Micrococcus luteus* through colony pigmentation, Gram staining and biochemical reactions (catalase- and oxidase-positive, non-motile) aligns with classical taxonomic descriptions by Kloos *et al*. (1974). More recent studies (Anyanwu *et al*., 2017;

Vancanneyt *et al*., 2004) confirm that pigmentation and Gram reaction remain valid markers, though molecular approaches such as 16S rRNA sequencing now provide more accurate differentiation from closely related species. Thus, while this finding agrees with earlier phenotypic studies, they highlighted the limitations of morphology-based identification when compared with contemporary molecular diagnostics.

Staphylococcus aureus yielded golden-yellow colonies on Mannitol Salt Agar and align with the findings by Shittu and Lin (2006) and Udo *et al*. (2013), who validated MSA as a selective and differential medium. However, as noted by Kateete *et al*. (2010), false positives may arise from other coagulase-positive staphylococci, indicating that confirmatory biochemical or molecular tests are necessary for absolute accuracy.

REFERENCES

Abdul-Aziz, M. H., *et al*. (2020). Antimicrobial stewardship in hospitals: Challenges and future directions. *Clinical Infectious Diseases, 71*(9), 2671–2676

Abdulkareem, S. A., Khalil, H. S., Abdulkadir, M. and Mohammed, S. (2023). Prevalence of ciprofloxacin-resistant *Escherichia coli* and associated resistance genes in urinary tract infections. *Journal of Global Antimicrobial Resistance, 33,* 28–34. https://doi.org/10.1016/j.jgar.2022.12.012

Akinyemi, K. O., Alabi, S. A., Taiwo, M. A. and Oyefolu, A. O. B. (2010). Antimicrobial resistance profile and virulence factors of *Salmonella enterica* serovars isolated from food handlers and food. *Journal of Infection in Developing Countries, 4*(9), 546–554.

Alemayehu, A., Teklemariam, Z. and Beyene, G. (2023). Prevalence, bacterial isolates and antimicrobial susceptibility patterns of urinary tract infections among symptomatic university students at Haramaya University, Eastern Ethiopia: A cross-sectional study. *Frontiers in Cellular and Infection Microbiology,* 13*,* 1269214

Al-Groom, R., El-Hossary, E. M. and Hassan, H. (2025). Extended-spectrum β-lactamase-producing Enterobacterales in urinary tract infections: Trends in prevalence and antimicrobial resistance. *Frontiers in Cellular and Infection Microbiology, 15,* 1498321. https://doi.org/10.3389/fcimb.2025.1498321

Ali, I., Rafaque, Z., Ahmed, S., Malik, S., Dasti, J. I. and Zafar, A. (2019). Virulence determinants and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) strains isolated from urinary tract infections in Pakistan. *Frontiers in Microbiology, 10,* 1508.

Ardakani, Z., *et al*. (2023). Evaluating the contribution of antimicrobial use in farmed animals to AMR in humans. *One Health, 16,* 100580

Ayukekbong, J. A., Ntemgwa, M. and Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries. *Journal of Clinical Medicine, 6*(2), 19

Badiger, S., Kundapur, R., Jain, A., Kumar, A., Pattanshetty, S., Thakolkaran, N., … Ullal, N. (2012). Self-medication patterns among medical students in South India. *Australasian Medical Journal, 5*(4), 217–220.

Beceiro, A., Tomás, M. and Bou, G. (2021). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clinical Microbiology Reviews, 34*(4), e0011420

Bobba, R., Mehta, A. and Sundaram, S. (2023). Genetic basis of rifampicin resistance among Gram-negative uropathogens. *Infection, Genetics and Evolution, 112,* 105414. https://doi.org/10.1016/j.meegid.2023.105414

Boye, A., *et al*. (2016). *Prevalence of asymptomatic bacteriuria in a sub-population of a university in Ghana*. University of Cape Coast Institutional Repository.

Cassini, A., Högberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S., ... and Suetens, C. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet Infectious Diseases, 19*(1), 56–66

Centers for Disease Control and Prevention (CDC). (2019). *Antibiotic Resistance Threats in the United States, 2019*. U.S. Department of Health and Human Services.

Chowdhury, A., Sarker, M. M. R. and Rahman, M. (2024). Spread of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales: Implications for urinary tract infections. *Journal of Infection and Public Health, 17(2),* 207–215. https://doi.org/10.1016/j.jiph.2023.10.004

Cleveland Clinic. (2024). Urinary tract infections (UTIs). Retrieved from https://my.clevelandclinic.org/health/diseases/9135-urinary-tract-infections

Colgan, R. and Williams, M. (2011). Diagnosis and treatment of acute pyelonephritis in women. *American Family Physician, 84*(5), 519-526

Dadgostar, P. (2019). Antimicrobial resistance: Implications and costs. *Infection and Drug Resistance, 12*, 3903–3910

Dela Cruz, T. E. and Torres, J. M. O. (2016). *Gelatin hydrolysis test protocol*. American Society for Microbiology. https://asm.org/asm/media/protocol-images/gelatin-hydrolysis-test-protocol.pdf

Delmar, J. A., Su, C. C. and Yu, E. W. (2021). Structural mechanisms of heavy-metal extrusion by the Cus efflux system of *Escherichia coli*. *Nature Reviews Microbiology, 19*(1), 31–45. https://doi.org/10.1038/s41579-020-0404-8

Dyar, O. J., Huttner, B., Schouten, J. and Pulcini, C. (2017). What is antimicrobial stewardship? *Clinical Microbiology and Infection, 23*(11), 793–798

El Baz, R., Abouelkhair, M. A. and Abdelhady, H. (2025). Mechanisms of aminoglycoside resistance among multidrug-resistant Enterobacterales. *Pathogens, 14(2),* 145. https://doi.org/10.3390/pathogens14020145

Elsayed, S. M., Khalifa, E. and Yousef, R. (2024). Global trends in fluoroquinolone resistance among uropathogenic *Escherichia coli*: A systematic review and meta-analysis. *Antibiotics, 13(1),* 57. https://doi.org/10.3390/antibiotics13010057

European Centre for Disease Prevention and Control (ECDC). (2022). *Antimicrobial resistance surveillance in Europe 2022 – 2020 data*

Farahi, N., Rezaei, A. and Shahini, S. (2018). Fluoroquinolone resistance in *Escherichia coli*: Mechanisms and clinical impact. *Infection and Drug Resistance, 11,* 209–218. https://doi.org/10.2147/IDR.S150282

Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269–284.

Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. (2022). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 20*(4), 221–238

Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269-284

Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269–284.

Foster, T. J. (2019). The remarkable ability of *Staphylococcus aureus* to evade and overcome the host immune system. *International Journal of Medical Microbiology, 309*(6), 402–409.

Foxman, B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors and disease burden. *Infectious Disease Clinics of North America, 28*(1), 1-13

Gajdács, M. (2019). The continuing threat of methicillin-resistant *Staphylococcus aureus* (MRSA). *Antibiotics, 8*(2), 52.

Gebremariam, T. T., *et al*. (2019). Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia. *BMC Infectious Diseases, 19*(1), 659. https://doi.org/10.1186/s12879-019-4272-y

Getanda, P., Kamau, S. and Mureithi, D. (2024). The role of antimicrobial stewardship programs in controlling multidrug-resistant urinary pathogens. *BMC Infectious Diseases, 24,* 782. https://doi.org/10.1186/s12879-024-09212-7

Gilmore, M. S., Clewell, D. B., Ike, Y. and Shankar, N. (2017). *Enterococci: From commensals to leading causes of drug resistant infection.* Massachusetts Eye and Ear Infirmary.

González, M., Torres, P. and Alarcón, C. (2025). Molecular epidemiology of rifampicin resistance in Enterobacterales. *Antimicrobial Agents and Chemotherapy, 69(3),* e01933-24. https://doi.org/10.1128/aac.01933-24

Gupta, K., Bhadelia, N. and Soper, D. E. (2017). Urinary tract infections in women: Diagnostic and management strategies. *New England Journal of Medicine, 376*(6), 562–574.

Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., Moran, G. J., Nicolle, L. E., Raz, R., Schaeffer, A. J. and Soper, D. E. (2022). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2022 update by the Infectious Diseases Society of America and the European

Society for Microbiology and Infectious Diseases. *Clinical Infectious Diseases, 74*(5), 897–907

Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., ... and Soper, D. E. (2021). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women. *Clinical Infectious Diseases, 72*(6), e349–e398

Halldórsdóttir, H., Magnusdottir, H. and Bjarnadottir, A. (2024). Epidemiology of ESBL-producing Enterobacterales in urinary tract infections: A global review. *Clinical Microbiology Reviews, 37(1),* e00245-23. https://doi.org/10.1128/cmr.00245-23

He, Y., Wang, H. and Xu, X. (2024). Aminoglycoside resistance in uropathogens: Current status and emerging challenges. *Frontiers in Microbiology, 15,* 1385219. https://doi.org/10.3389/fmicb.2024.1385219

Holmes, A. H., Moore, L. S. P., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J. and Piddock, L. J. V. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176–187

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., ... and Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet, 387*(10014), 176–187

Huttner, B., Goossens, H., Verheij, T. and Harbarth, S. (2010). Characteristics and outcomes of public campaigns aimed at improving the use of antibiotics in outpatients in high-income countries. *The Lancet Infectious Diseases, 10*(1), 17–31

Irfan, M., Idrees, F. and Aslam, M. (2022). Antibiotic misuse and resistance development: A review of stewardship practices. *Infection and Drug Resistance, 15,* 1579–1591. https://doi.org/10.2147/IDR.S354302

Ivanova, A., Petrova, M. and Dimitrov, T. (2024). Rising macrolide resistance among Enterobacterales: Clinical implications for azithromycin use. *Journal of Global Antimicrobial Resistance, 36,* 87–95. https://doi.org/10.1016/j.jgar.2024.03.010

Kahlmeter, G., Giske, C. G., Kirn, T. J. and Turnidge, J. D. (2021). Antimicrobial susceptibility testing in Europe and the United States: An historical review. *Clinical Microbiology Reviews, 34*(2), e00233-20

Kakkar, M., Walia, K., Vong, S., Chatterjee, P. and Sharma, A. (2018). Antibiotic resistance and its containment in India. *BMJ, 362*,

Kebira, A. N., Ochola, P. and Khamadi, S. A. (2009). Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections. *Journal of Applied Biosciences, 22*(2), 1320–1325.

Kleinschmidt, S., Munderloh, U. G. and Dobrindt, U. (2021). Serum resistance as a pathogenicity factor of uropathogenic *Escherichia coli*. *Virulence, 12*(1), 1599–1610.

Kline, K. A. and Lewis, A. L. (2016). Gram-positive uropathogens, polymicrobial urinary tract infection and the emerging microbiota of the urinary tract. *Microbiology Spectrum, 4*(2).

Kloos, W. E. and Musselwhite, M. S. (2016). The genus *Micrococcus*. In M. Dworkin *et al*. (Eds.), *The Prokaryotes* (pp. 961–971). Springer.

Lamichhane, B., Shrestha, R. and Adhikari, P. (2024). Genetic determinants of macrolide resistance in Enterobacteriaceae from urinary tract infections. *Antibiotics, 13(4),* 632. https://doi.org/10.3390/antibiotics13040632

Larsson, D. G. J. and Flach, C.-F. (2022). Antibiotic resistance in the environment. *Nature Reviews Microbiology, 20,* 257–269

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., … Cars, O. (2013). Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases*, *13*(12), 1057–1098

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Rottingen, J. A., Klugman, K. and Davies, S. (2020). Access to effective antimicrobials: a worldwide challenge. *The Lancet, 387*(10014), 168–175

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Rottingen, J. A., Klugman, K. and Davies, S. (2016). Access to effective antimicrobials: A worldwide challenge. *The Lancet, 387*(10014), 168–175

Lee, D. S., Lee, S. J. and Choe, H. S. (2021). Community-acquired urinary tract infection by *Escherichia coli* in the era of antibiotic resistance. *BioMed Research International, 2021*, 1–10

Linhares, I., Raposo, T., Rodrigues, A. and Almeida, A. (2013). Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: A ten-year surveillance study (2000–2009). *BMC Infectious Diseases, 13,* 19.

Magistro, G. and Stief, C. G. (2019). The role of the bacterial capsule in urinary tract infection. *International Journal of Urology, 26*(6), 532–539.

Magliano, E., Grazioli, V., Deflorio, L., Leuci, A. I., Mattina, R., Romano, P. and Cocuzza, C. E. (2012). Gender and age-dependent etiology of community-acquired urinary tract infections. *The Scientific World Journal, 2012,* 349597.

Mareș, M. (2024). Antimicrobial resistance patterns of uropathogenic *Escherichia coli* in Europe: 2010–2023. *European Journal of Clinical Microbiology and Infectious Diseases, 43(5),* 881–890. https://doi.org/10.1007/s10096-023-04659-9

Mathers, A. J., Peirano, G. and Pitout, J. D. D. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clinical Microbiology Reviews, 28*(3), 565–591

Munoz-Price, L. S., Poirel, L., Bonomo, R. A., Schwaber, M. J., Daikos, G. L., Cormican, M., ... and Paterson, D. L. (2019). Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *The Lancet Infectious Diseases, 19*(6), e18–e27

Murray, C. J. L., *et al*. (2022). *Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis.* The Lancet, 399(10325), 629–655

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... and Global Burden of Disease Antimicrobial Resistance Collaborators. (2022). Global burden

of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... and Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet, 399*(10325), 629–655

Necchi, F., Saul, A. and Rondini, S. (2017). Development of a serological assay to detect serum bactericidal activity against *Escherichia coli*. *Clinical and Vaccine Immunology, 24*(5), e00031-17.

Necchi, F., Saul, A. and Rondini, S. (2017). Development of a serum bactericidal assay to evaluate antibody responses against *Salmonella Paratyphi A*. *PLoS ONE, 12*(2), e0172163.

Nicolle, L. E. (2023). Urinary tract infections in adults: Epidemiology and treatment. *The New England Journal of Medicine, 389*(3), 243–254

Nicolle, L. E., Gupta, K., Bradley, S. F., Colgan, R., DeMuri, G. P., Drekonja, D., Eckert, L. O., Geerlings, S. E., Köves, B., Hooton, T. M., Juthani-Mehta, M., Knight, S. L., Saint, S., Schaeffer, A. J., Soper, D. E. and Tenke, P. (2019). Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. *Clinical Infectious Diseases, 68*(10), e83–e110. https://doi.org/10.1093/cid/ciy1121

Park, J., Lee, H. and Kim, S. (2025). Antibiotic resistance trends in urinary tract pathogens: A 10-year surveillance in South Korea. *Journal of Antimicrobial Chemotherapy, 80(2),* 298–307. https://doi.org/10.1093/jac/dkae003

Partridge, S. R., Kwong, S. M., Firth, N. and Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews, 31*(4), e00088-17

Prestinaci, F., Pezzotti, P. and Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, *109*(7), 309–318

Pulcini, C., Binda, F., Lamkang, A. S., Trett, A., Charani, E., Goff, D. A., ... and Mendelson, M. (2019). Developing core elements and checklist items for global hospital antimicrobial stewardship programmes: A consensus approach. *Clinical Microbiology and Infection, 25*(1), 20–25

Ranjan, K. P., Ranjan, N. and Chakraborty, A. (2021). Emerging multidrug-resistant uropathogens: A growing threat to public health. *Infection and Drug Resistance, 14*, 4215–4227

Reda, B. K., *et al*. (2023). Antibiogram of uropathogens and associated risk factors among asymptomatic female college students in Dessie town, Northeast Ethiopia. *PLOS ONE, 18*(7), e0287260. https://doi.org/10.1371/journal.pone.0287260

Rezaei, R., Ahmadi, M. and Hosseini, N. (2024). Co-selection of fluoroquinolone and β-lactam resistance in uropathogenic Enterobacterales. *Microbial Drug Resistance, 30(1),* 55–65. https://doi.org/10.1089/mdr.2023.0123

Ristow, L. C., Welch, R. A. and Hultgren, S. J. (2016). Hemolysin of uropathogenic *Escherichia coli*: A pore-forming toxin with dual roles in pathogenesis. *International Journal of Medical Microbiology, 306*(8), 472–479.

Rodríguez-Martínez, J. M., Cano, M. E., Velasco, C., Martínez-Martínez, L. and Pascual, A. (2016). Plasmid-mediated quinolone resistance: An update. *Journal of Infection and Chemotherapy, 22(3),* 159–167. https://doi.org/10.1016/j.jiac.2015.12.007

Rosini, R., Margarit, I. and Rinaudo, C. D. (2019). The protective role of capsular polysaccharides in Gram-positive bacterial infections. *Frontiers in Immunology, 10,* 2753.

Rupprecht, T. A., Pfister, H. W. and Angele, B. (2017). Pathogenesis of CNS infections caused by commensals: The example of *Micrococcus luteus*. *Frontiers in Neurology, 8,* 484.

Salah, F., Ahmed, R. and Younis, M. (2019). Emergence of ciprofloxacin and cephalosporin co-resistance in urinary isolates of *Escherichia coli*. *Infection and Drug Resistance, 12,* 2801–2811. https://doi.org/10.2147/IDR.S220577

Salam, M. A., Rahman, M. S. and Haque, R. (2023). Antimicrobial resistance in urinary pathogens: Implications for treatment guidelines. *Frontiers in Public Health, 11,* 1165742. https://doi.org/10.3389/fpubh.2023.1165742

Sanchez, G. V., *et al*. (2022). Antimicrobial stewardship in college and university health settings. *Current Treatment Options in Infectious Diseases, 14*(3), 226–237. https://doi.org/10.1007/s40506-022-00260-1

Shittu, A. O. and Lin, J. (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BMC Infectious Diseases, 6*(125), 1–9.

Smeltzer, M. S. (2016). Staphylococcal virulence factors and their role in disease. *Microbiology Spectrum, 4*(2).

Sofos, J. N. (2009). Staining techniques in microbiology: Principles and applications. *Journal of Microbiological Methods, 78*(2), 93–103

Subashchandrabose, S. and Mobley, H. L. T. (2015). Virulence and fitness determinants of uropathogenic *Escherichia coli*. *Microbiology Spectrum, 3*(4).

Suwandi, A., Fink, K. and McSorley, S. J. (2019). *Salmonella's* strategies for overcoming host innate and adaptive immune responses. *Pathogens, 8*(5), 251.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., ... and Magrini, N. (2018). Discovery, research and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases, 18*(3), 318–327

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., ... and Magrini, N. (2018). Discovery, research and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases, 18*(3), 318–327

Talan, D. A. and Stamm, W. E. (1999). Urinary tract infection in adults. *The New England Journal of Medicine, 340*(6), 438-450

Tong, S. Y. C., Davis, J. S., Eichenberger, E., Holland, T. L. and Fowler, V. G. (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations and management. *Clinical Microbiology Reviews, 28*(3), 603–661.

Toyting, D., Okeke, I. and Bello, A. (2024). Community antibiotic misuse and its role in rising resistance in urinary tract infections. *Tropical Medicine and Infectious Disease, 9(6),* 312. https://doi.org/10.3390/tropicalmed9060312

van Schaik, W. (2015). The human gut resistome. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670), 20140087

Ventola, C. L. (2015). The antibiotic resistance crisis: Causes and threats. *PandT, 40*(4), 277–283

Wang, H., Zhong, Z., Luo, Y. and Cox, E. (2020). *Proteus mirabilis* infections and their resistance mechanisms. *Frontiers in Microbiology, 11*, 593923

Weidenmaier, C. and Lee, J. C. (2017). Structure and function of surface polysaccharides of *Staphylococcus aureus*. *Current Topics in Microbiology and Immunology, 409,* 57–93.

Willyard, C. (2017). The drug-resistant bacteria that pose the greatest health threats. *Nature, 543*(7643), 15

Wilson, B. R. and Ho, M. (2019). Capsule-mediated immune evasion in bacterial infections. *Trends in Microbiology, 27*(6), 436–446.

World Health Organization (WHO). (2021). *Global antimicrobial resistance and use surveillance system (GLASS) report 2021*

World Health Organization. (2020). *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

World Health Organization. (2021). *Global antimicrobial resistance and use surveillance system (GLASS) report 2021*. World Health Organization

APPENDIX I

Table 7: Cultural characteristics, Morphological characteristics and Biochemical characteristics Of bacterial isolates from urine of male undergraduate

Cultural characteristics				
Colour	cream	Cream	Cream	Cream
Shape	Irregular	Round	Circular	Circular
Elevation	Flat	Raise	Raise	Raise
Margin	Undulate	Smooth	Entire	Entire
Size	Large	medium	medium	Large
Morphological characteristics				
KOH	+ve	-ve	-ve	-ve
Gram stain	-ve	+ve	+ve	+ve
Cell mophology	Rod	Cocci	Cocci	Rod
Cellarrangement	Singly	Cluster	Cluster	Singly
Biochemical characteristics				
Catalase	+	+	+	+
Indole	+	+	+	-
Oxidase	-	-	-	-
Voges-proskauer	Variable	-	-	-
Spore forming		-	-	-
Glucose	+	+	+	+
Lactose	+	+	+	-
Sucrose	+	+	+	-
Mannitol	+	+	+	-
H2S production	-	-	+	+
Identity	*E.coli*	*S. aureus*	*Streptococcus*	*Salmonella*

	sp.	sp.

APPENDIX II

PREPARATION OF MEDIA

Nutrient Agar28g of powdered nutrient agar were dissolved in 1000ml of deionized water allowed to soak for 10minutes and then sterilized with an antoclaving for 15minutes at 121^0C allowed to cool and pour into petri dishes.

Eosin Methylene Blue

37.5g of powdered EMB agar was dissolved in 1000ml of deionized water. Allow to soak for 10minute, swirl to mix and sterilize by autoclaving at 121^0C for 15minutes. Allow to cool at 47^0C and pour into petri dishes.

Peptone water / Broth

3.8g of powdered peptone water was dissolved in 280ml of distilled water allow to soak for 10minutes and 5ml was dispensed into 5 labelled sterile test-tubes then sterilized by autoclaving for 15minutes at 121^0C

Thio sulphate citrate Bile Salt(TCBS)

88g of powdered TCBS cholera medium agar was dissolved in 1000ml of deionized water, allow to soak for 10minutes, swirl to mix the bring to the boil and cool to 45^0C and pour into petri dishes

Sammon citrate Agar

24g of powdered SCA was dissolved in 1000ml of deionized water, soak for 10minutes and swirl to mix. Dispense into test tubes by adding 5ml and sterilized by autoclaving at 121^0C for 15minutes. The medium is set as a slope ensuring that the slant is over a butt about 3cm deep.

Procedure for sub culturing

Pure isolates were obtained by selecting discrete colonies and having them subcultured onto petri dishes containing freshly prepared NA media. The bacteria isolates were also transferred by streak method onto free plates respectively.

MORPHOLOGICAL IDENTIFICATION

Gram Staining

1. A thin smear was prepared on clean glass allowed to air dried and then flame it.
2. The smear was stained with crystal violet for 60 seconds.
3. Rapidly wash off the stain with clean water for 5 seconds.

4. Tip off all the water and cover the smear with lugol's iodine for 60 seconds and washed off under slowly running tap.
5. Decolourized using 90% ehanol and washed immediately with clean water.
6. The smear was covered with safranine reagent for 30 seconds then washed off the stain slowly under running tap.
7. The slide was blot dry using paper towel.
8. The strained cell were examined microscopically with oil immersion using only 100 objective lens.
9. Gram positive cells stain purple while gram negative cells stain pink or red.

BIOCHEMICAL TEST

Sugar Fermentation (Glucose)

The smear solution were 1% of glucose. The sugar glucose was prepared and sterilized with nutrient agar at 121^0C for 15minutes. Phenol red was used as indicator for acid production. The colony was inoculated on the nutrient agar containing the glucose. The presence of dark color shows the organism can ferment glucose (Cheesbrough, 2004).

Oxidase test

Procedure: place a piece of filter paper in a clean petri dish and add 2 0r 3 drops of freshly prepared oxidase reagent. Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper. Positive colonies turn bluish – purple (Cheesbrough, 2004).

Catalase test

Procedure: 1ml hydrogen peroxide solution was poured in a test tube. A sterile glass rod was used to collect or remove several colonies of the test organism and immersed. In the hydrogen peroxide solution, the test tubes was observed for immediate bubbling of gases which indicate a positive reaction (Cheesbrough, 2004).

Citrate utilization

Procedure: prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8^0C). Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.Incubate at 35^0C for 48hours, look for a bright blue color in the medium (Cheesbrough, 2004).