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THE PREVALENCE AND IDENTIFICATION OF ANTIBIOTICS RESISTANCE GENES IN COMMON UROPATHOGENS ATTENDING ATTAT HOSPITAL, ETHIOPIA

MSC THESIS

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The Prevalence and Identification of Antibiotics Resistance Genes in Common Uropathogens Attending Attat Hospital, Ethiopia

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DEDICATION

All of my brothers, sisters, and parents, who have helped and encouraged me throughout this stage of my study, are honored by this work. Mostly to God, the source of all life, who gave me the health, strength, and favor necessary to be able to see this through to completion.

DECLARATION

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ABBREVIATIONS AND ACRONOMY

UTI	Urinary Tract Infection
CFU	Colony-Forming Units
E. coli	Escherichia coli
NA	Nutrient Agar
IMViC	Indole, Methyl Red, Voges-Proskaur, and Citrate
NCCLS	National Committee for Clinical Laboratory Standards
UPE	Uropathogenic E. Coli
ARG	Antibiotics Resistance Genes
MD	Multi Drug Resistance
BA	Blood Agar
AMR	Antimicrobial Resistance
VRS	Vancomycin Resistance Staphlococcus Aeurus
tet(A)	tetracycline resistance gene
blaSHV	betalactam resistance gene (pencillin)
SHV	sulf-hydryl variable active site
MSA	Manitol Salt Agar

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ABSTRACT

Urinary tract infections (UTIs) are infections of the urinary framework (the kidneys, ureters, bladder, and urethra). UTI occurs when bacteria, primarily from the genital area or the stomach-related tract, stick to the entrance of the urethra and start to spread. In Ethiopia and the Gurage Zone specifically, there is a significant research deficit regarding the identification and prevalence of antibiotic resistance genes in common urinary tract bacterial infections. The aim of this study designed to estimate the prevalence of uropathogenic bacterial urinary tract infections and identify individuals with genes associated with antibiotic resistance at the Attat Hospital, Ethiopia. A cross-sectional study involved 384 across-section were involved in the current study patients from the Attat Hospital. Culture on Blood, Macconkey and Nutrient agar was carried out after a dipstick urine examination. Using the gram stain and biochemical testing, pathogenic bacteria were isolated and recognized. The Kirby Bauer disc diffusion method for determining antibiotic sensitivity and resistance employed erythromycin, cefepime, tetracycline, clarithromycin, vacomycin, pencilin, and ciprofloxacin. Genomic extraction and PCR amplification for detection of tetraA , vanA and blaSHV antibiotics resistance gene were done. According to the proportion of participants who experienced UTIs during the research period, the prevalence was 75.0%. Staphylococcus aureus *was the sole Gram-positive bacteria found; other Gram-negative bacteria included* Escherichia coli, Klebsiella pneumonia, Entrobactor aerogenes, Protus valigaris, Proteus mirabilis, *and* Pseudomonas aeruginosa. *The most often isolated bacterium was* Escherichia coli *(49.3%), whereas* P. aeuruginosa *was the least frequent (2.8%). The most effective antibiotic for all bacteria was ciprofloxacin, and the isolates tested negative for tetracycline. The molecular investigation identified three different genes with* E. coli, K. pnumoniae, *and* S. aeurus, *respectively: 53.8% tetA, 54.2% blaSHV, and 78.6% vanA. In order to prevent multidrug resistance, which would otherwise have an influence on the rising cost of care, the study advises regular surveillance and investigation of antibiotic use in the therapy of UTI. The need for future molecular analysis of antibiotic resistance genes cannot be overstated.*

Key words*: Antibiotic resistance pattern, Antibiotic Resistance gene, Prevalence, Urinary tract infection.*

1. INTRODUCTION

1.1. Background of the Study

A urinary tract disease could be a condition in which one or more sections of the urinary framework (the kidneys, ureters, bladder, and urethra) came to be contaminated (Saeed *et al*., 2020). Usually, urinary tract infections (UTIs) are classified as being either upper or lower, depending mostly on the anatomical area of the contamination (John *et al*., 2016). UTI occurs when bacteria primarily from the genital area or the stomach-related tract, stick to the entrance of the urethra and start to spread. UTIs are predicted annualy to impact 150–250 million individuals globaly (Zavala *et al*., 2020; Al-Khfaji *et al*., 2023; Manoharan, 2023) and result in more than $6 billion in yearly direct expenses for medical care (Zeng *et al*., 2022).

The prevalence of UTIs varies greatly throughout Africa. Inadequate laboratory diagnoses are a problem in many parts of sub-Saharan Africa, impeding the efficient treatment of infectious diseases(Schmider *et al*., 2022). The average prevalence of UTIs throughout the nine sub-Saharan African nations was 32.12%, with South Africa ranking highest at 67.6%. Nigeria, Zambia, were next, with 43.65%, 38.25%, Ethiopia (37.47%), and Uganda (35.66%), Tanzania at 23.7%, Ghana at 19.2%, Kenya (18.53%), and Senegal at 5.1% (Mwang'onde and Mchami, 2022). According to Agaba *et al*. (2017), UTIs are the most prevalent hospital-acquired infection, accounting for up to 35-40%. Due to the important effort associated with UTI, enteric bacteria, in particular *Escherichia coli*, continue to be a significant concern in clinical microbiology laboratories. Sex, age, illness, being hospitalized, and obstruction are just a few of the irregularities and circumstances that affect the body's natural ability to fight off UTI infections (Hassali *et al*., 2018).

Women tend to have UTIs more frequently than men because germs can enter the bladder more easily in women than in men. Because of the female urethra's shorter and wider size and close proximity to the anus, bacteria can enter the bladder more easily in women (Abdulkadi and Aisha, 2018). Sexual activity and pregnancy further increase the risk of UTIs in women. Additionally, the use of diaphragms and the presence of indwelling catheters also contribute to the development of UTIs (Lee *et a*l., 2020).

UTIs are usually brought on by bacteria, which can also exist in the vagina, the digestive tract, or the area around the urethra, which is the opening to the urinary tract. These microorganisms typically enter the urethra before moving on to the bladder and kidneys. Usually, the body gets rid of the germs and doesn't exhibit any symptoms. Common signs of a UTI include burning sensation while urinating, frequent or intense urges to urinate even when only passing a small amount of urine, lower back pain or discomfort, murky dark, strange-smelling urine, and fever or chills (Okafor *et al.*, 2021).

There are numerous distinct UTI diagnostic criteria that vary depending on the patient and circumstance. There are numerous methods for performing urinalysis cultures. The use of sheep blood and MacConkey agar selective medium for routine urine culture is recommended for the diagnosis of UTI. The Clinical and Laboratory Standards Institute's approved standard is used to determine the drug resistance profile (Magiorakos *et al*., 2012).

According to Lima *et al*. (2020), resistant bacteria frequently operate as reservoirs for the various antimicrobial resistance genes that are present on mobile genetic components and can be transmitted to other clinically important bacteria via horizontal gene transfer mechanisms. Tetracycline resistance (tet (A), tet (B), tet (C), and tet (H)) and betalactam resistance are just a few examples of the several drug-resistance phenotypes and genotypes that *E. coli* ARGs are known to contain (Mohammadi *et al*., 2018). With a history spanning nearly 70 years, *Staphylococca*l resistance to antimicrobials, particularly vancomycin, is another global problem. Van genes, such as vanA, vanB, and vanD, have been identified and are linked to high levels of vancomycin resistance (Maharjan *et al*., 2021). Members of the Enterobacteriaceae family, including *Klebsiella pnumoniae*, frequently manufacture extended-spectrum beta-lactamases (ESBLs) that are encoded by genes including SHV, TEM, and CTX-M (Pishtiwan *et al*., 2019). In this study, the etiological agents, antibiotic resistance patterns, and molecular detection of antibiotic resistance genes in uropathogenic bacteria were the main research focuses. Understanding the prevalence and resistance profiles of UTIs in this scenario can help us develop better management and treatment plans for UTI patients.

1.2. Statement of the Problem

The common bacterial infection disease known as a urinary tract infection (UTI) can affect both sexes and Age groups. Although both sexes can contract the infection, women are more susceptible because of their anatomy and reproductive physiology. Typically, bacterial invasion of the urinary tract, comprising the upper and lower urinary tracts, results in the infection. *Escherichia coli*, one of the bacterial species, is responsible for 60% to 90% of illnesses. Treatment failures are mostly brought on by the adaptability and gene-based antibiotics of resistant bacteria. The prolonged periods of infectivity caused by the inability to treat the infection increase the number of infected people moving through the community and increasing the likelihood that a resistant strain may spread to the rest of the population.

Numerous factors, such as inappropriate antibiotic use, the availability of fake medications on the market, clinician noncompliance with standard treatment guidelines, and a lack of laboratory resources for culture and sensitivity, have been linked to the increasing antibiotic resistance genes of bacterial pathogens isolated from patients with urinary tract infections. Due to the presence of pathogenic bacteria that are resistant to antibiotics, the scientific community is searching for novel classes of antibiotic agents (Bharadwaj *et al*., 2022). This is part of an endeavor to find prospective sources of uropathogenic bacteria for the production of unique medications to get around the issue of rising drug resistance genes against the chosen pathogens. Understanding the prevalence and resistance characteristics of UTIs in this environment will allow us to better manage and treat UTI patients, but there is a huge research gap in the prevalence and identification of antibiotic resistance genes in common urinary tract urophatogens in Ethiopia, particularly in Gurage Zone. Therefore, the goal of this study was an investigation of UTI infections in patients at Attat Hospital in Gurage, Ethiopia, with a main focus on determining the causative agents, patterns of drug sensitivity, and molecular identification of antibiotic resistance genes in uropathogenic bacteria.

1.3. Objectives of the Study

1.3.1. General objective

The general objectives of this study was to asses the prevalence of uropathogens and identification of antibiotics resistance genes in common uropathogens attending Attat Hospital, Ethiopia.

1.3.2. Specific objectives

- To investigate the prevalence of urinary tract bacterial pathogens among patients attending Attat Hospital,
- To isolate and identify uropathogenic bacteria isolates based on their phenotypic characteristics,
- To determine antibiotic susceptibility and resistance pattern of bacterial isolates using disk diffusion, and
- To identify antibiotic resistance genes in bacterial pathogens isolated from urinary tract patients.

1.4. Significance of the Study

To effectively manage infections, knowing the prevalence of UTIs among patients provides insight into the severity of the infection and the age-associated risk factor. The study findings on the drug sensitivity and resistance of enteric bacteria were useful in informing policymakers and helping improve the standard of UTI diagnostic accuracy by clinicians, hence allowing for appropriate antibiotic therapy. To that effect, the present study focused on investigating the frequency and trends of UTI cases in the Attat Hospital and options for effective preventative measures like urinate after sexual activity, wipe front to back, drink plenty of fluids, explore birth control options, consume cranberries,and take probiotics.

2. LITERATURE REVIEW

2.1. Urinary Tract Infection (UTIs)

Bacteriuria, or the growth of bacteria in urine inside the urinary tract, is regarded as a urinary tract infection. If there are more than 10^5 organisms per milliliter, there is considered to be significant bacteriuria. Urinary tract infections continue to be a major clinical concern more than 50 years after the invention of antibiotic therapy, and they frequently cause patients to visit their general practitioner. Infections can affect the bladder, kidney, ureters, pelvis, parenchyma, or urethra, among other parts of the urinary tract. UTIs are more common in women than in men, and they often affect urinary tracts that are physically healthy (Tan & Chlebicki, 2016).

2.2. Urinary Tract System

According to Hickling *et al*. (2017), urine is produced and stored in the urinary system until it is ejected. The urinary system is divided into two main sections: the upper, which houses the kidney, renal pelvis, and ureters, and the lower, which houses the urinary bladder and urethra. The fundamental function of the urinary system is to filter waste and extra fluid from the blood, and urine is a liquid waste product that is removed from the body by urination.

2.2.1. Kidney

The two kidneys, which eliminate the majority of metabolic waste products by creating urine, are responsible for this. The kidneys are located on either side of the spinal column, behind the peritoneum, on the posterior abdominal wall. The right kidney is somewhat smaller than the left, and the functional unit is the neuron. The size of an adult's kidneys is 11–14 cm long, 5–6 cm wide, and 3–4 cm deep. The nephron, which contains about a million cells, is the active part of the kidney. Nephrons are tiny filtering organs that assist the kidneys in removing waste from the blood (Miyoshi *et al*., 2020).

2.2.2. Ureters

The bladder and kidneys are linked by ureters, which are 8 to 10 inches long. The two ureters, which connect the kidney to the back of the bladder through muscular tubes, are

each about 10 inches long (Lopes *et al*., 2019). The kidneys' funnel-shaped renal pelvis, the renal artery, and the renal vein, where the bladder is punctured and twists as it passes the pelvic brim, are all three places where the ureters are constricted along their length.

2.2.3. Urinary bladder

The hollow muscular bladder, an organ, is where urine is deposited (Hickling *et al*., 2017). It is a strong muscle that is situated in the pelvis, just behind the pubic bone and serves as a bladder for storing pee. In adults, it has a maximum capacity of roughly 500 ml. The form of the bladder changes depending on how much pee is present; an empty bladder is pyramidal. Including a neck, a base, an apex, and two inferolateral surfaces.

2.2.4. Urethra

The urethra tube connects the bladder to the outside of the body. It extends from the neck of the bladder to the tip of the penis in men and from the vagina to the pubic bone in women. extend from the male's bladder neck to the tip of the penis, and from the female's vagina to the pubic bone. The urethra measures about 1.5 inches in women and 8 inches in men (Olaniran *et al*., 2016).

2.3. Type of Infection

According to Shariati *et al*. (2019), bacteria from the patient's intestinal flora are the main cause of infection. Bloodstream transfer to the urinary system is a possibility; it can occur directly, through the lymphatics, or most frequently, via the ascending transurethral pathway.

2.3.1. Lower urinary tract infection

Urethritis: Urethritis that affects the urethra and also affects the bladder urethra (Cho, 2017).The most frequent urination throughout the day and at night, painful voiding (dysuria), discomfort and pain in the suprapubic region, hemoturia, and pungent urine are the most common symptoms. *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and urethral infections caused by *Trichomonas vaginalis* are common sexually transmitted causes of urethritis.

Cystitis: Cystitis is an inflammation of the bladder, most usually caused by a bacterial infection that can also affect the kidneys (Hollyer & Ison *et al*., 2018). It's a bladder infection. Cystitis is significantly more common than kidney infections.

2.3.2. Upper urinary tract infection

The most common symptoms of an infection that spreads to the kidney, pelvis, or ureter are loin pain and tenderness, fever, and systemic upset Pyelitis or Pyelonephritis(Nikolla, 2017). However, lower urinary tract infections (UTIs) can also present with less severe symptoms or no symptoms at all, as well as symptoms that are not typical for these infections, such as abdominal pain. Pyelonephritis is brought on by a bacterial infection of the renal collecting system that produces gas (Nikolla, 2017). Inflammation, which typically results from bacterial infection, affects both the pelvis and the renal parenchyma (the upper end of the ureter is located inside the kidney). The fact that 40% of persons with acute pyelonephritis had bacterial infections is significant.

2.4. Pathogenesis

A person is at risk for infection if there are any conditions that make it difficult for the bladder to empty, interfere with normal urine flow, or make it simpler for germs to enter the bladder. In comparison to the male urethra, the shorter female urethra is a less potent barrier to infection (Nitzan *et al*., 2015). Sexual activity and a higher prevalence of urinary tract infections than celibacy are both related to how much simpler it is for bacteria to pass through the urethra during sexual activity, particularly in females. Catheterization significantly increases the risk of UTIs; during catheter placement, bacteria may be transported right into the bladder. The majority of urinary tract pathogens are found in the fecal flora, but only facultative and aerobic species, such as *Escherichia coli*, have the characteristics needed to colonize and infect the urinary tract. Causing organisms' virulence factors include pili that facilitate adhesion, capsules that block phagocytosis, hemolysins that damage kidneys, and urease synthesis that causes pyelonephritis.

2.5. Causal Organisms

According to Ali *et al*. (2018), *Escherichia coli*, which are Gram-negative rods, are the most prevalent cause of ascending UTIs in roughly 60–90% of cases. This is likely because

they are frequently found in the colon and have virulence features such as K antigens and specific fimbriae. Females that are sexually active are associated with *Staphylococcus saprophyticus*. Antibiotic-resistant *Proteus mirabilis* and *Klebsiella* species frequently multiply. Infection is brought on by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, particularly during catheterization or instrumentation (Pachori *et al*., 2019). Acute, simple UTIs are typically caused by a single type of organism, while chronic infections are frequently caused by multiple types of fastidious gram-positive bacteria (such as *lactobacilli*, *streptococci*, and *corynebacteria*), which need to be incubated for 24–48 hours in the presence of CO2 for isolation. Although obligatory anaerobes are extremely infrequently involved, other species, such as *Salmonella typhi*, *Staphylococcus aureus*, and *Mycobacterium TB*, may be discovered.

2.5.1. *Escherichia coli* (*E. coli*)

E. coli is a member of the large class of gram-negative rods known as enterobacteria. They are mainly lactose-fermenting aerobes and facultative anaerobes, non-sporting, and motile, and they cause both primary and secondary infections in humans. According to Ali et al. (2018), 60–90% of urinary tract infections are caused by *E. coli*. Urinary tract infections are particularly common with some *E. coli* serotypes. According to legend, some strains are more invasive than others. A trait linked to virulence is the presence of the K (capsular) antigen, which prevents phagocytosis and the bactericidal effect of typical human serum, as well as the ability to adhere to uro-epithelium due to specialized fimbriae. According to Ljubojevi *et al*. (2016), the acquired tetracycline resistance genes tet(A), tet(B), tet(C), tet(D), and tet(E) are crucial components of tetracycline resistance in *E.coli*. Antimicrobials that have activity against Gram-negative organisms, such as sulphonamides, trimethoprim, cotrimoxazole, nalidixic acid, nitrofurantoin, ciprofloxacin, amoxycillin, cephalosporins, and aminoglycosides, are used to treat urinary *E.coli* infections and other infections.

2.5.2. *Klebsiella species*

According to Riaz *et al*. (2022), hospital-acquired infections of the urinary system and wounds are linked to *Klebsiella aerogenes*. *Klebsiella* are non-motile, gram-negative rods that are typically capsuled. Based on antigenic examination of capsular polysaccharides,

more than 80 serotypes of UTIs in hospital patients have been identified. On common media, they thrive and produce colonies that are frequently, but not always, big and mucoid. Beta-lactamase production by *Klebsiella* is frequent, and it is pencillin-resistant. *Klebsiella* infections are treated with aminoglycosides and cephalosporins.

2.5.3. *Proteus species*

On particular intestinal media, gram-negative, pleomorphic motile rods develop. The principal *proteus* species of medical significance is *Proteus mirabilis*, according to Muhammad *et al*. (2016). Following catheterization or a cystoscopy, it frequently causes urinary infections in young men and the elderly by alkalizing the urine (Cléroux, 2022). Ampicillin, cephalosporins, ciprofloxacin and aminoglycosides are a few antibiotics that are effective against *P. mirabilis*. After incubation at 35–37 ^{o}C for a night, *Proteus* creates distinct non-lactose-fermenting colonies.

2.5.4. *Pseudomonas aeruginosa*

Some strains of gram-negative motile aerobic bacilli are capsuled, have very basic growth requirements, and exhibit little fermentation activity. *Ps. aeruginosa* infections are frequently difficult to treat because of the bacteria's resistance to a number of antibiotics (Al-Khikani and Ayit, 2022). *Pseudomonas aeruginosa* infection, which commonly happens after catheterization, is connected to chronic urinary disease.

2.5.5. *Enterobacter species*

According to Salimiyan *et al*. (2020), *Enterobacter sp*. species can be found in water, sewage, soil, dairy products, and both human and animal intestinal tracts. They are opportunistic bacteria linked to wound infections as well as urine infections. the gram-negative motile rods known as *Enterobacter* species.

2.5.6. *Staphylococcus aureus*

The group includes gram-positive cocci that are non-motile, non-capsulated, catalase, DNase, coagulase-positive, ferment mannitol, and infrequently cause urinary tract infections (Chukwueze *et al*., 2022). Their primary habitat is the human nose, but they can also be found on human skin (Laux *et al*., 2019).

2.5.7. *Staphylococcus saprophyticus*

Gram-positive cocci can be found in pairs, alone, or in groups, and they are all the same size. They lack capsules and motility. According to Ehlers *et al*. (2018), *S. saprophyticus* causes UTIs in sexually active women. According to Onyebueke *et al*. (2019), it is coagulase and DNase-negative and ferments mannitol. As many as one-fourth of symptomatic UTIs in women are brought on by the bacterium. This pathogen's surface agglutinins seem to be a major factor in determining how well it colonizes the urinary system and increases its pathogenicity. Young women can develop community-acquired urethritis due to a fragile bacterium that is resistant to novobiocin in contrast to *S. epidermidis* (Djawadi *et al*., 2023), although *Escherichia coli* is a much more frequent culprit.

2.5.8. *Enterococcus faecalis*

According to Urmi *et al*. (2019), it is a gram-positive cocci that frequently accompany coliform infections. The female vaginal canal and urethra can also be colonized; the human colon is the preferred habitat (Krawczyk *et al*., 2021).

2.5.9. *Streptococcus* species

The *Streptococcaceae* are family of spherical bacteria. The term "*streptococcus*" is derived from the Latin meaning "twisted berry". It refers to how the bacteria typically form strands that resemble a string of beads. can result in uterine and bladder infections in expectant mothers, as well as sepsis (blood poisoning) in young children exposed to the virus.

2.5.10. Other bacteria

Salmonella species, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Chlamydia*, and *mycoplasma* species are just a few examples of bacteria species that can be found in urine but are not typically found in the urinary tract (Mehlhorn *et al*., 2018).

2.5.11. Parasites

UTIs are caused by a very limited number of parasites. Schistosoma hematobium, Micofilaria, and Trichomonas are the most frequent parasites identified in urine (Khurana

et al., 2018). Trichomoniasis is a parasite that can be passed from person to person during sexual activity. It can then travel up the urethra to the bladder and cause an infection. Trichomonas vaginalis, which in both sexes can induce urethritis and cause vaginitis. Schistosomiasis is another example of a parasite that can cause UTIs. It's very rare in the U.S., but it may be a concern if you travel to Africa, South Africa, or Asia.

2.5.12. Fungi

Candida spp. in the urine is called "candiduria," and individuals with renal candidiasis, cystitis, epididymorchitis, prostatis, and pyelonephritis can all have symptoms of this condition (Behzadi *et al*., 2015). On the other hand, asymptomatic candiduria is generally benign and is not considered a distinct illness. One of the most significant fungi that can cause candiduria (20% of nosocomial infections) is *C. albicans*. A wide variety of documented data suggests that C. albicans ranks first for causing candiduria among more than 200 *Candida* species (Behzadi *et al*., 2015). Catheterization is considered the most common risk factor for candida in ICU patients (Behzadi *et al*., 2015). Alfouzan and Dhar (2017) state that bladder infections in immunocompromised and diabetic individuals are usually caused by *Candida albicans*.

2.5.13. Viruses

Viral causes of UTIs seem to be rare, despite a connection to hemorrhagic cystitis and renal syndromes (Goldman and Julian, 2019). Urination pain and difficulty emptying the bladder can result from a urethral infection caused by the herpes simplex virus. Other viral UTIs, like kidney and bladder infections, typically don't appear unless there is a compromised immune system (from cancer, HIV/AIDS, or using drugs that suppress the immune system, for example).

2.6. Epidemiology

According to Pulia *et al*. (2018), urinary tract infections account for the majority of bacterial infections treated in an outpatient setting. Clinicians empirically prescribe antibiotics when patients are diagnosed with these diseases (Lee, 2015). Due to the increasing burden of healthcare costs brought on by antibiotic management, these infections have become a major public health concern on a global scale. UTIs are reported

to affect more than 150 million people annually worldwide. *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* are common agents known to cause UTIs. *Staphylococcus saprophyticus* and *Enterococcus faecalis* (Lagunas-Rangel, 2018). As part of the regular flora, these bacteria typically live in the lower intestines, but when they get into the urinary system, they can cause a UTI. Along with them, *Staphylococcus saprophyticus*, *Klebsiella species*, *Proteus mirabilis*, and *Enterococcus faecalis* are common pathogens found in association. Complicated UTIs brought on by bacteria like *Neisseria gonorrhoea* and *Chlamydia trachomatis* are linked to weakened urinary tract or host defense, including urinary blockade, urinary retention brought on by nervous disorders, low immunity, kidney failure, kidney transplantation, and pregnancy.

2.7. Epidemiology and Risk Factors

According to Keren (2015), characteristics like gender, age, race, whether or not someone has undergone circumcision, general health, and immune status all seem to have an impact on the likelihood of developing an UTI. Age, gender, and colonization all seem to be aspects of the host that encourage more bacterial adherence. It indicates that higher periurethral colonization, whether age-related (such as infancy) or individual (such as the increased colonization connected to persons who have recurrent UTI or uncircumcised newborns), increases the risk for UTI. Structure abnormalities, neurologic impairments, or behavioral voiding dysfunction that leaves left over urine in any part of the urinary tract may also affect the durability of bacteriuria once it has occurred.

2.8. Antibiotic Resistance Pattern

Antibiotic resistance mechanisms are clearly emerging widely around the globe. Inappropriate antibiotic use, along with other related issues such as lax adherence to infection prevention and control measures, is a contributing cause to the increased cases of drug resistance (Tang, 2023). These behaviors have been linked to the overuse of antimicrobials (Seifu and Gebissa, 2018). Treatment with broad-spectrum antibiotics rather than narrow-spectrum antibiotics is now preferred due to concerns about multidrug resistance. Extended-spectrum-lactamases (ESBLs), which have been created plasmids, are what cause the resistance seen in *E. coli* and *K. pneumoniae*, according to literature. It is known that the most frequent method by which bacteria acquire resistance to a wide range

of antibiotics is horizontal gene transfer, or transfer via plasmids. As a result, employing one antibiotic can result in resistance to numerous other antibiotics. One of the risk factors for antimicrobial resistance is previous, extended usage of a specific antibiotic. The emergence of *E. coli* with an expanded spectrum beta-lactamase enzyme that causes penicillin resistance is a significant problem. This is consistent with hospital research whose findings showed a significant percentage of isolates of gram-negative bacteria that were resistant to vancomycin, tetracycline, and pencillin. Additionally resistant to vancomycin were the isolated gram-positive bacteria. Comparably, a study carried out in Bangladesh by Sarjana in 2022 showed the highest resistance to ampicillin (98.5%). *E. coli* accounted for 70% of the isolates in that investigation. To manage UTI in light of this presented a challenge.

2.9. Antibiotics Resistance Genes

Drug resistance Genes are genes that, when expressed, provide the host with antibiotic resistance. The formation and spread of antibiotic resistance in microbial populations is inescapable due to biological and evolutionary factors. Resistance can be acquired naturally (transformation, transposition, and conjugation), by spontaneous mutations (de novo), or through horizontal gene transfer from donor bacteria, phages, or free DNA(Sharma *et al*., 2016). The main mechanisms of resistance are drug uptake limitation, drug target alteration, drug inactivation, and active efflux of a drug (Reygaert, 2018). inherent variation in bacteria's capacity to inhibit antimicrobial agent absorption. *Staphylococcus aureus* recently developed vancomycin resistance. The method permits the bacteria to create a thicker cell wall, making it difficult for the medication to enter the cell and providing intermediate resistance to vancomycin. The β -lactamases (penicillinases) inactivate -lactam medicines by hydrolyzing a specific spot in the -lactam ring structure, causing the ring to open. These enzymes may be recognized by their enzyme family, such as the SHV (sulphydryl variable) family, which is present on the bacterial chromosome, or they may be obtained via a plasmid in the Enterobacteriaceae family, such as *Klebsiella pneumonia*, which has chromosomal-lactamase genes (Reygaert, 2018). More than forty acquired tetracycline-resistant genes encode for efflux pumps, enzymatic inactivation, or ribosomal protection. The tetA gene, for example, encodes the tetA efflux pump, one of the

more commonly characterized mediators of tetracycline resistance in Enterobacteria, including *E. coli* (Perewari *et al*., 2022).

2.10. Emerging Therapies

Researchers must come up with alternate therapies that would help stop this trend in order to address the problem of resistance. The development of alternative therapies can include a variety of techniques, ranging from focusing on bacterial virulence pathways to studying UTI pathogenesis in general (Dickey, 2017). By using these alternate pathways, it is hoped that the bacteria's capacity to cause disease can be effectively neutralized without upsetting the flora that normally resides in the digestive system.

2.11. Diagnosis

According to Kranz *et al*. (2017), a clinical symptom and the pathogen must both be present to make a diagnosis of a urinary tract infection. The bacteria are detected by urine culture using midstream pee collected in the morning. One can estimate the bacteria with this test. More than 10^5 colony-forming units CFU/mL of bacteria are commonly used as the cutoff in laboratories. This criterion, it should be stressed, excludes important infections. In contrast to women, a single finding of bacteriuria greater than 10^5 (CFU)/mL in males is typically enough to confirm a UTI diagnosis. According to Swerkersson et al. (2016), a substantial bacterium count is one that is equivalent to or more than 100,000 CFU/ml.

2.12. National Guidelines on UTI Treatment

If it is determined that it is positive for leucocytes and nitrite, a midstream urine sample is taken for culture and sensitivity testing before any antibiotics are prescribed. After the antibiotic sensitivity data are obtained, review the prescribed drug. The doctor may change the antibiotic being given if the patient's symptoms do not get better, but they should always choose a narrow-spectrum drug (Garbi *et al*., 2021). Additionally, people who have prostate issues shouldn't receive nitrofurantoin because it is unlikely that the drug will reach therapeutic levels in that organ. Trimethoprim is still advised for those patients, but the dosage should be based on the baby's month and weight.

3. MATERIALS AND METHODS

3.1. Study Area

The Attat Specialized Hospital, located 175 kilometers southwest of Addis Ababa in Cheaha Woreda, Gurage Zone, is where the current study was conducted. Medical mission sisters from the Eparchy of Emdibir were in charge of running the Hospital since it was first established in 1969 (Sahiele, 2021). The Attat Hospital and its extensive array of integrated health services, according to the medical missionaries, provide care to over 800,000 people both inside and outside the operation area (Figure 1).

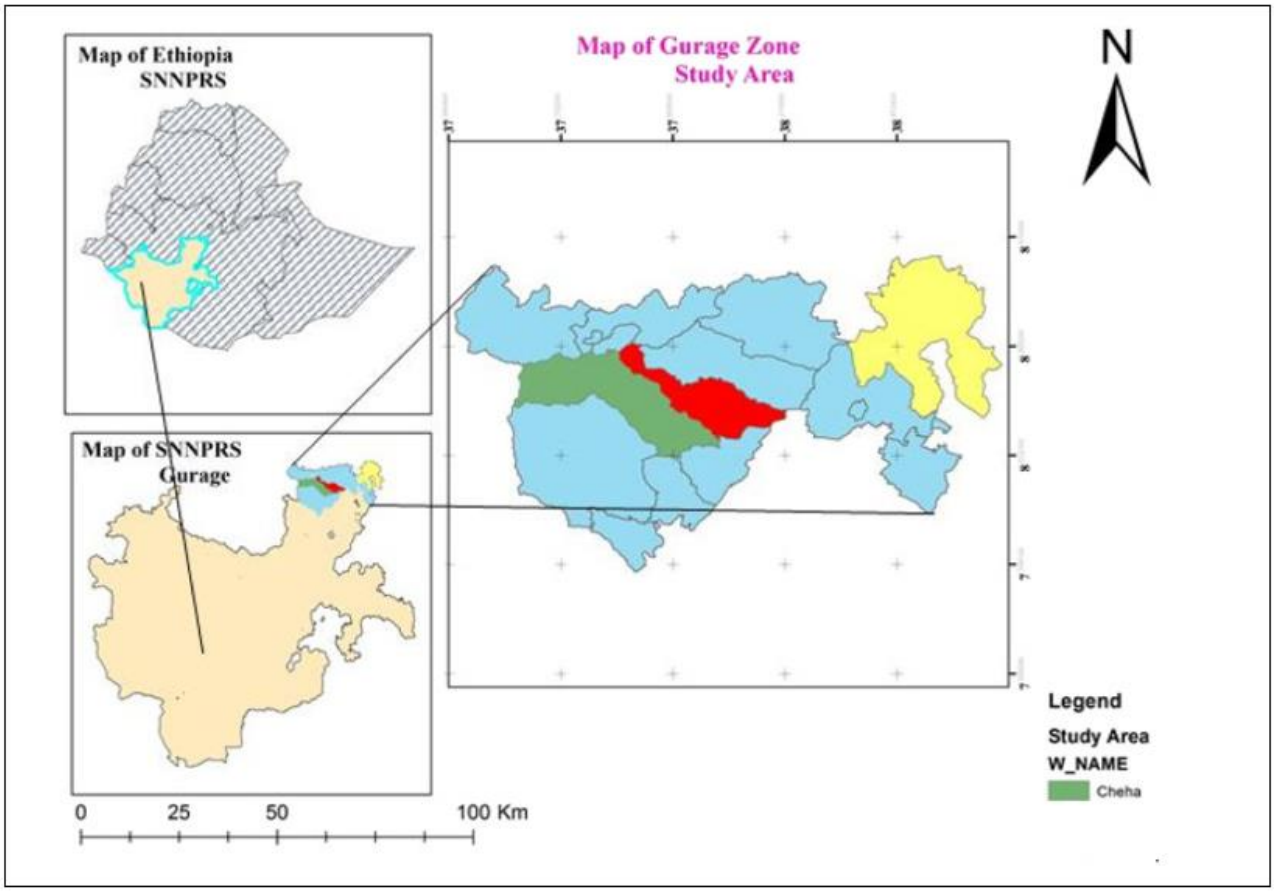

Figure 1. Map of the study area

3.2 Study Design

At Wolkite University's Department of Biotechnology and Molecular Biology Laboratory, a cross-sectional study was carried out between June 2022 and September 2023. The research design involved urine sample collection from patients who were exhibiting symptoms of urinary tract infections during the study period.

3.3 Sample Size Determination

The following formula was used to determine the required sample size (Ondari, 2020). The study utilized a prevalence of 50% (the most conservative estimate) due to the absence of data on the prevalence of UTI among patients at Attat Hospital. The following formula was used to calculate the sample size(n): where as: p = proportion or prevalence of occurance is 50%, d = acceptance of error is 5%, and z = the z-score 95% of the confidence interval is 1.96

$$n = \frac{(Z_{\propto/2})^2 \times P(1-P)}{d^2} = \frac{(1.96)^2 \times 0.5 \times (1-0.5)}{(0.05)^2} = 384$$

After calculating, 384 samples were taken for analysis in the department of biotechnology laboratory, College of Natural and Computational Science, Wolkite University.

3.4. Sampling Technique

A systematic random sampling technique was used to select the eligible study participants. Instructed on how to calculate urine and the need for prompt delivery to the laboratory.

3.5. Data Collection

3.5.1. Demographic and clinical information

Demographic and clinical information about the subjects was obtained by chart abstraction and recorded on a prepared data collection form. Information was collected on the patient's age, gender, residence, and health-seeking behavior.

3.5.2 Sample Collection and transportation

Early morning clean catch urine samples were collected in sterile disposable universal sample containers(Appendix 1) as described by (Shu'aibu and Rashida, 2022). ID, age, sex, and time collection were indicated on the sample bottles. The samples were placed in the ice box and transported to the Wolkite University Department of Biotechnology laboratory work area within an hour from the collection time.

Inclusion Criteria: Patients who reported symptoms such as fever, dysuria, frequency, urgency, and suprapubic pain were included in the study.

Exclusion Criteria*:* Patients with a week's history of hospital admission should rule out hospital-acquired infections.

3.5.3 Laboratory procedures

Urine dipstick

urine sample analysis were preformed according to modification of lockwood (2022). As the color changed, it was compared to the specified color chart, and tapped from the side to eliminate any extra pee. The urine was cultured and the leukocytes, nitrites, and color changes were identified.

Nitrite strip test

The procedure involved collecting midstream urine first and then submerging the strips in the urine. According to Fernandos *et al*. (2018), the strips were calibrated to turn pink in 60 seconds to indicate the presence of bacteria in urine.

Leucocyte strip test

A pink-red color was created on the strip as a result of the pyrazole's subsequent reaction with diazonium salt. Using the technique, successive drops of pee were put onto the strip. the procedure involved inserting the strip for one second into the samples of freshly urinated pee and removed any extra urine from the container's rim, and finally, 60 seconds later, the test was analyzed by comparing it to the color scale on the manufacturer's label.

3.5.4 Isolation bacteria of pathogens

For the semi-quantitative procedure, the plating was carried out using a calibrated, sterile wire loop with a diameter of 4.0 mm that is designed to dispense 0.1 ml. Two blood and macconkey agar plates were inoculated with the thoroughly mixed urine sample. All plates were then incubated aerobically for 24 hours at 37°C. The culture plate was examined for the presence of bacteria, and mixed colonies on the plate were reinoculated with additional blood agar and nutrient agar media to support the growth of the pure colony. The bacterial colonies were counted and multiplied by 100 to estimate the general number of bacteria present per milliliter of urine.

3.5.5 Identification of bacteria isolate

According to reported colony features (appearance, size, and color), cell morphology, gram responses, and biochemical tests, the identification of bacteria isolates was carried out using conventional bacteriological procedures (Oros *et al*., 2020). The plates could be incubated 24 hours at 37 °C (Agegnehu *et al*., 2020). Through morphological and gram-stain analyses, followed by biochemical assays, uropathogenic bacteria are identified and described. The gram-negative bacteria pathogens' presumptive identification was reported based on a number of biochemical tests, colony morphology, and the standard identification process of gram staining. Phenotypic factors, including mannitol salt agar growth, colony shape, and gram staining, were assessed and then submitted to the proper biochemical test for the correct identification of the gram-positive isolate. By placing antibiotic discs on agar surfaces and measuring the zones of inhibition, the disc diffusion method is used to investigate the susceptibility of microbes to antibiotics.

Colonial morphology

For size, color, and lactose fermentation, the infected media underwent morphological analysis. Blood agar and macconkey agar plates were used for direct monitoring of the colony growth at 24 hours (Mahon, 2022).

Gram staining

As stated (Mallik, 2015), this was done using normal techniques and a step-by-step application of crystal violet solution, iodine solution, ethanol (95%), and safranin solution. A drop of regular saline was added to a slide. The alleged colonies were smeared and emulsified. Dry heat was used to cure the streaks, and after that, a crystal violet stain was applied for 60 seconds. With tap water, the stain was immediately cleaned, then left aside. Lugos' iodine was then applied to the discolored smear for 60 seconds. Iodine was promptly removed by washing, and ethanol was used to decolorize the smear for 30 seconds. A minute is spent adding succinic acid to the stain. After using tap water to remove the red stain and performing a smear preparation, the sample was allowed to air dry before being examined under a microscope with 100x oil immersion high-resolution objective power (Appendix 2).

3.5.6 Identification of gram-positive cocci

Catalase test

An isolated loop was put on a glass slide with two to three drops of 3% H_2O_2. A positive catalase test was identified by the development of bubbles. A bubble appearance appeared when the isolated colony was touched. The classification of bacteria as catalase-positive or catalase-negative was based on the creation of bubbles (Mwajita, 2017).

Coagulase test

The isolates were delivered with brain-heart infusion broth and allowed to develop densely for 24 hours at 37°C. 0.5 ml of human plasma was placed in a sterile, dry tube. 50 µl of the test isolates' overnight broth culture was then added. Once the materials had been well mixed, they were incubated for up to four hours at 37°C in an incubator. For up to four hours, tubes were checked for the development of a clot or coagulation at regular intervals.

Mannitol salt agar (MSA)

Mannitol salt agar plates were made, the test bacteria were added, and the plate was incubated at 37 degrees Celsius for 24 hours. The color was then evaluated to determine whether it had altered (Arjyal and Neupane, 2020).

3.5.7 Identification of gram-negative rods

Indole test

About 5 milliliters of isolate-inoculated tryptophan broth were incubated for a full day at 37°C. A few drops of Kovac's reagent were added following incubation. saw the creation of rings with a dark red color. Bacteria were classified as indole-positive or negative based on the production of red rings. Pure cultures of *Enterobacter aerogenes*, an indole-negative bacterium, and *E. coli*, an indole-positive bacterium, are considered positive.

Methyl Red (MR) test

Incubated at 37°C for 24 hours after 10 mL of prepared MR-VP broth was inoculated with the isolates. The tubes were then filled with a few drops of methyl red indicator solution following the incubation period. Methyl Red (MR) positive and negative bacteria were identified based on the color alteration of the MR-VP broth to apple red.

Voges-Proskauer (VP)

The isolates were added to 10 milliliters of prepared MR-VP broth in test tubes according to the appropriate amount for twenty-four hours at 37°C. Following the incubation period, 0.5 ml (8–10 drops) of the α-naphthol (Baritt A reagent, Himedia) solution and 0.5 ml of the 40% KOH solution with 0.3% creatine (Baritt B reagent, Himedia) were put into the test tubes. Allow it to stand for five to thirty minutes after giving it an adequate shake. The bacteria in MR-VP broth were classified as either VP-positive or negative based on their color change to red.

Citrate utilization test

Slants of Simmon's Citrate Agar were streaked with the isolates and incubated at 37°C for 48 hours. Growth on the medium was accompanied by a rise in pH and a change in the medium, from its initial green color to deep blue. Based on the color change to deep blue, the results were reported as citrate-positive or negative. As a positive control, pure cultures of the citrate-positive bacteria *Enterobacter aerogenes* and the citrate-negative bacteria *E. coli* were taken.

Motility test

After being streaked over nutritional broth, the bacterial culture was incubated for 24 hours at 37^{o}C. The center of the cover slip was filled with a loopful of culture. Pressing the inclined part of a depression slide over the cover slip, the slide was placed over the drop. In order to avoid disturbing the drop, the slide was immediately recovered. The cell motility on the slide was examined under a microscope. Working gradually up to the oil immersion target, start with the lowest power objective and concentrate.

Urease test

The isolates were streaked onto the prepared urea broth slants. Results were recorded as either urease-positive or negative after being incubated for 24 hours at 37°C based on a color shift to red. Pure cultures of the urease-negative bacteria *E. coli* and the urease-positive bacterium *Proteus vulgaris* were used as positive controls.

Oxidase test

A fresh culture of isolated bacteria was cultivated for 24 hours in 5 ml of nutrient broth. Following the addition of 0.2 ml of 1% naphthol, 0.3 ml of 1% paminodimethylaniline oxalate (Gaby and Hadley reagents) was added. Lastly, check to see if the color has changed. They were detected as soon as the color changed to blue within 15–30 seconds, and bacteria were delayed oxidase positive and the color changed to purple after 2–3 minutes (Shields and Cathcart, 2010).

3.5.8 Antibiotic susceptibility and resistance test

Use a sterile wire loop to touch three to five colonies of isolated, cultivated organisms. Emulsify the suspension in three milliliters of normal saline, and then compared the suspension's turbidity to the standard. Employing a sterile swab, the bacterial solution was applied. Any surplus fluid was eliminated by pressing the swab against the tube's wall. Muller-Hinton agar was then streaked over the three directions, with the plates being rotated by about 60 degrees to guarantee uniform dispersion. After three to five minutes, insert the suitable antimicrobial discs using sterile forceps; the discs was placed 25 mm from the next disk and 15 mm from the edge (Ruckmani, 2021). The plate was incubated aerobically for 18 hours at 37 degrees Celsius. Following this time, the zone of inhibition was measured using a ruler, and each disc's zone was assessed using an interpretive chart. The results were described as sensitive, resistant, or intermediate (Alabaid, 2022).

Table 1. Antibiotics drugs

Antibiotics	Abbreviation	Disk content(µg)	Diameter of zone inhibition(mm)		
			S	I	R
Ciprofloxacin	CIP	5	>21	16-20	<15
Cefepime	CP	30	>18	17-15	<14
Tetracycline	TET	30	>19	15-18	<14
Pencilin	PEN	10	>28	20-27	<19
Vancomycin	VAN	30	>17	16-15	<14
Erythromycin	E	30	>23	14-22	<13
Clarithromycin	CLA	15	>18	17-14	<13

3.6. Identification of Antiobiotics Resistance Genes

3.6.1. Genomic extraction

One colony from each bacterial culture was placed in 5 ml of LB broth and shaken at 250 rpm for the entire overnight period at 37 °C. After 24 hours, 2 ml of the culture was transferred to an Eppendorf tube and centrifuged at 10000 rpm for 5 minutes. The supernatant was then decanted and thrown away. To keep the pellets suspended, 500 µl of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 7.4) was added. Then, 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K were added, well mixed by vortexing, and incubated for 1 hour at 37 °C (Wright *et al*., 2017). once more centrifuged for five minutes at 10,000 rpm. 500µl each of phenol and chloroform: 24:1 isoamyl alcohol After thoroughly blending by inversion, the mixture was allowed to sit at room temperature for five minutes. Again, 500µl of chloroform (not chloroform or isoamyl alcohol) was added, and the mixture was centrifuged at 10,000 rpm for 10 minutes at 4^{o}C. 100 µl of 5M sodium acetate and 700µl isopropanol were added and mixed gently by inversion till white strands of DNA precipitated out. The mixture was centrifuged at 5,000 rpm for 10 minutes, after which the aqueous phase was transferred to a 2 ml Eppendorf tube. The DNA pellet was washed with 500 µl of 70% ethanol for 30 seconds and centrifuged at 5,000 rpm for 10 minutes after the highly viscous jelly-like supernatant was collected using a sterile pipette tip. 50 µl of distilled water were added after the product had air-dried for 5 minutes. Spectrophotometric analysis was carried out using a spectrophotometer at 260/280 nm and agarose gel electrophoresis to evaluate the concentration and purity of the isolated genomic DNA.

3.6.2 Detection of tetrA , vanA and blaSHV antibiotics resistance gene

The conventional PCR amplification method was used for the amplification of antibiotic resistance genes such as tetrA, vanA, and blaSHV (Table 2). The previously designed forward and reverse primers for tetrA, vanA, and blaSHV genes were synthesized at Sigma-Aldrich, France (Appendix 4). The PCR amplification was performed with the following PCR reaction conditions: a total of 25 µl of PCR reaction mixture, 1.3 µl of forward and reverse primer, 2 µl of DNA template, 0.2 µl of Taq polymerase, 2 µl of cofactors, 2.5 µl of 10*PCR buffer, and the remaining 14.5 µl of distilled water fill up to 25 µl are needed. The PCR amplification procedure was as follows: a 2-minute preheating

stage at 95°C, 35 cycles (1 minute at 94°C for denaturation, 1 minute at 52°C for annealing, and 1 minute at 72°C for extension), and a 10-minute extension step at 72°C. The PCR products were separated using 1.5% agarose gel electrophoresis and stained with 0.3 g/mL ethidium bromide before being seen under UV light. A DNA ladder of 100 bps is used to estimate the molecular weights of the amplified product.

Table 2. The designed primers used to amplify genes encoding antibiotics resistance in three bacteria

No	Genes-marker	Fragment size	Primer sequence (5'-3')	Annealing temperature	References
1	*TetrA*	501bps	F: 5'GGC GGT CTT CTT CTT CAT CAT GC3'	62.0 °C	Olowe *et al*., 2013
			R: 5'CGG CAG GCA GAG CAA GTA GA3'		
2	*vanA*	713bps	*F: 5'GGC AAG TCA GGT GAA GAT G3'*	61.0 °C	Maharjan *et al*., 2021
			R: 5'ATC AAG CGG TCA ATC AGT TC3'		
3	*blaSHV*	747bps	F: 5'ATG CGT TATATT CGC CTG TG3'	60.0 °C	Paterson *et al*., 2003
			R: 5'TGC TTT GTT ATT CGG GCC AA3'		

3.7 Data Analysis

The gathered data was divided up according to the format that had been planned. Age groups and gender were used to categorize the study's laboratory findings. SPSS version 27 statistical analysis program, data could be entered, summarized in frequencies and percentages, shown in tables and graphs, and other descriptive statistics could be produced and examined. P-values less than 0.05 were regarded as statistically significant.

4. RESULTS

4.1 Prevalence of UTIs among Patients visiting Attat Hospital

Among 384 collected urine samples, 288 cases of bacterial infection were detected. The overall prevalence of UTI, indicated by a positive urine culture, among the participants in the study was 75%. This prevalence was calculated based on the proportion of patients with positive urine cultures during the data collection period. Females with 45.3 % shows higher infection rate than males 29.7% (Table 3).

Table 3: Prevalence of UTIs among patients visiting with their gender at Atat Hospital

		Prevalence of UTIs Count (%)		Total
		Positive	Negative	
Sex	Male	114(29.7)	32(8.3)	154(40.1)
	Female	174(45.3)	64(16.7)	230(59.9)
Total		288(75.0)	96(25.0)	384(100.0)

4.1.1 Age distribution of UTI patients

The findings showed that among the people with high prevalence UTIs, 98 (34.0%) were between the ages of 21 and 30, and 44 (15.3%) were older than 60. Comparable results were found for ages 31–40 and 11–20, with 36 (or 12.5%) and 35 (or 12.2%), respectively. The age group with the lowest prevalence of UTIs under 11 years old was 18 (6.3%).

Table 4: Number of outpatients booked in the study and their corresponding age groups

			Sex of patients		Total
			Male	Female	
Age of patients	<11	Count (%)	11(3.8)	7(2.4)	18(6.3)
	11-20	Count (%)	10(3.5)	25(8.7)	35(12.2)
	21-30	Count (%)	28(9.7)	70(24.3)	98(34.0)
	31-40	Count (%)	12(4.2)	24(8.3)	36(12.5)
	41-50	Coun(%)	13(4.5)	19(6.6)	32(11.1)
	51-60	Coun(%)	13(4.5)	12(4.2)	25(8.7)
	>60	Coun(%)	27(9.4)	17(5.9)	44(15.3)
Total		Count (%)	114(39.6)	174(60.4)	288(100)

4.1.2 Bacterial isolates among patients visiting Attat Hospital

Out of the 288 samples analyzed, gram-negative bacteria were more prevalent than gram-positive bacteria. Gram-negative bacteria accounted for 262 (91.0%) of the cases, with *E. coli* being the most commonly identified strain, occurring in 142 cases (49.3%). *K. pneumoniae* was the second most prevalent strain, identified in 43 cases (14.9%), followed by *Enterobactor aerogenes* at 30 (10.4%), *Protus vulgaris* at 16 (5.6%), *P. mirabilis* at 23 (8.0%), and *P. aeuruginosa* at 8 (2.8%). Among the gram-positive bacteria, *Staphylococcus aureus of 26 (9.0%)* was the only species implicated in this study (Table 5).

Table 5: Distribution of bacterial isolates

Bacterial isolates		Frequency	Percent
Gram-negative	E. coli	142	49.3
	K. pneumonia	43	14.9
	E. aerogenes	30	10.4
	P. mirabilis	23	8.0
	P. vilguris	16	5.6
	P. aeruginosa	8	2.8
Total		262	91.0
Gram-positive	S. aureus	26	9.0
Grand Total		288	100.0

4.2 Morphological and Biochemical Characteristics of the selected Bacterial Isolates

4.2.1 Morphological characteristics of the selected bacterial isolates

Enterobactor aerogenes (Figure 4A), *Klebsiella pnumoniae* (Figure 3A), and *Escherichia coli* (Figure 2A). The non-lactose fermentors were *Protus mirabilis*, *Protus valgaris*, and *Psuedomonas aeruginosa*.

Figure 2: *Escherichia coli* grown on Macconkey media (A) and nutrient agar (B)

Klebsiella pneumonia(e produced lactose-fermenting colonies on MacConkey agar.

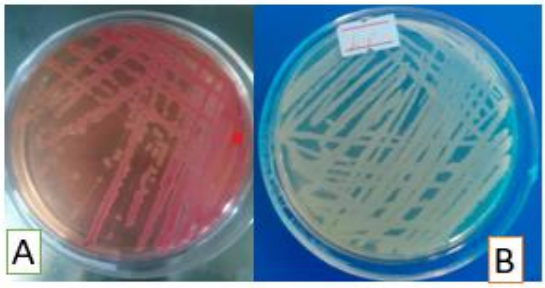

Figure 3: *Klebsiella pneumoniae* grown on Macconkey media (A) and nutrient agar (B)

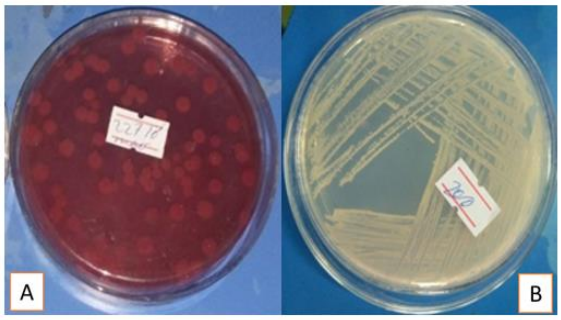

Figure 4: *Enterobactor aerogenes* grown on macconkey (A) and nutrient agar (B)

Pseudomonas aeruginosa fermented lactose (Figure 5A).

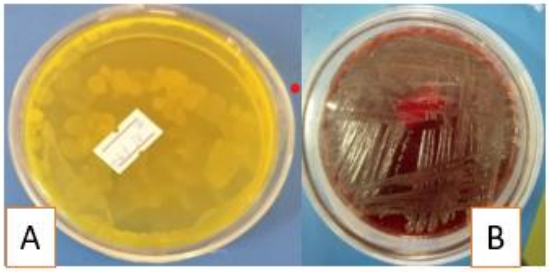

Figure 5: *Pseudomonas aeruginosa* grown on macconkey (A), and blood agar (B)

Proteus mirabilis on the media indicated that lactose was not fermented (Figure 6A).

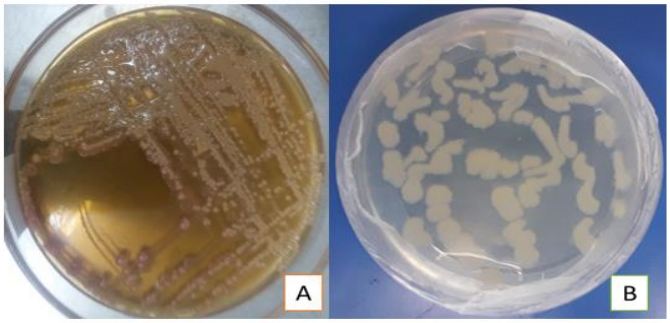

Figure 6: *Protus mirabilis* grows on Macconkey media (A) and nutrient agar (B).

Proteus vulgaris showed neutral red on the medium (Figure 7A).

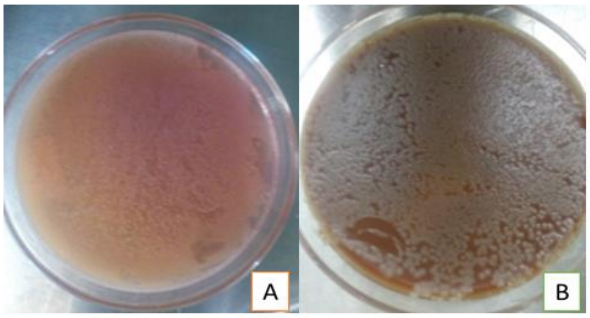

Figure 7: *Protus vulgaris* grows on macconkey (A) and blood agar (B).

On blood agar plates, *Staphylococcus aureus* colonies grew to a golden color (Figure 8A), while on MSA(Figure 8B).

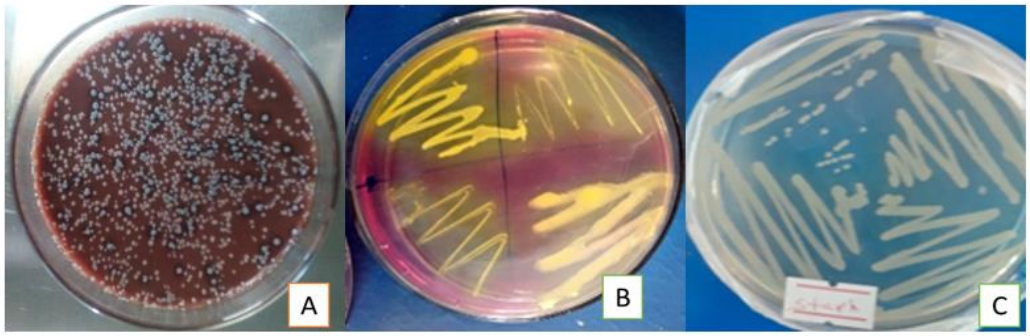

Figure 8: *Staphylococcus aureus* grown on blood (A), MSA (B), and nutrient agar (C)

Table 6: Cultural and morphological characteristics of the bacterial isolates from UTI suspected patients on Macconkey, blood agar, and nutrient media

No	No Isolated	Growth media			Morphological characterization		Urine dipstick	
							LEU	NIT
1	215	+	Lac+	+	-	Short rods	+	+
2	47	+	Lac-	+	-	Short rods	+	+
3	26	+	-	+	+	Cocci in cluster	+	+

Note: Lac+ = lactose ferment bacteria , Lac- non lactose ferment bacteria and, - = bacteria not grown MAC, BA=blood agar, MAC= macconkey, NA= nutrient agar, LEU= Leucocyte strip, NIT= Nitrite strip test.

4.2.2 Biochemical characteristics of the selected bacterial isolates

In this investigation biochemical tests were then carried out to differentiate specific bacteria species. *Escerchia coli* biochemical tests revealed that motility, indole, and methyl red catalase were positive, but oxidase, citrate, urease, and voges proskauer were negative (Figure 9 and Table 7). Citrate, voges proskauer, and catalase were positive for *Klebsiella pneumoniae* and indole, oxidase, motility, urease, and methyl tests were negative test (Figure 10 and Table 7) . *Enterobacter aerogenes* were negative for methyl red, oxidase, indole, urea, whereas the tests for citrate, motility, voges proskauer and catalase were positive (Figure 11 and Table 7). *Proteus mirabilis* were negative for voges proskauer and indole, while positive results were found for citrate, methyl red, and urease (Figure 12 and Table 7). The sole distinction between *Protus mirabilus* and *Protus valigaris* in the biochemical test was that Indole tested positive for Protus valigaris (Figure 14 and Table 7). For *Staphylococcus aureus* Catalase, citrate, urea, methyl red, voges proskauer, catalase, and coagulase tests were positive and oxidase, indole, and motility tests were negative (Figure 15 and Table 7). However, the tests for oxidase, citrate, motility, and catalase in *Psuedomonas aeruginosa* were positive, whereas those for indole, urease, coagulase, methyal red, and voges proskauer were negative (Figure 13 and Table 7).

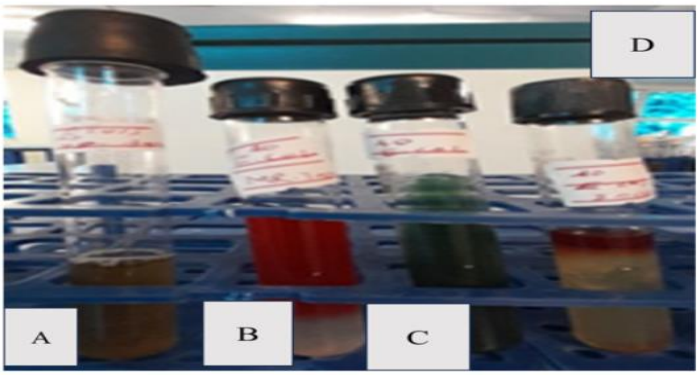

Figure 9: Biochemical tests for *E. coli* isolates: voges proskauer (A), methyl red (B), citrate test (C), and indole test (D).

Klebsiella pneumoniae had identified indole, and the methyl red test was negative, while both citrate and voges proskauer were positive (Figure 10).

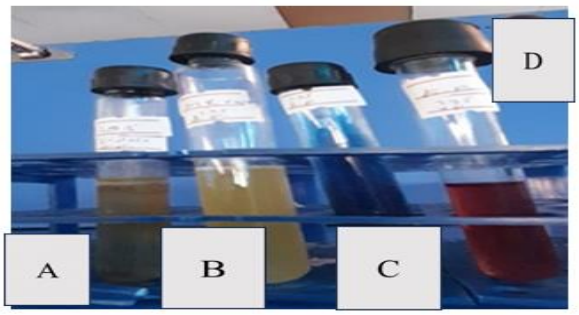

Figure 10: Biochemical test of *Klebssiella pneumoniae*: indole (A), methyl red (B), citrate(C) and voges proskauer (D).

Indole and methyl red were negative for *Enterobactor aerogenes*, whereas voges proskauer and citrate were positive (Figure 11).

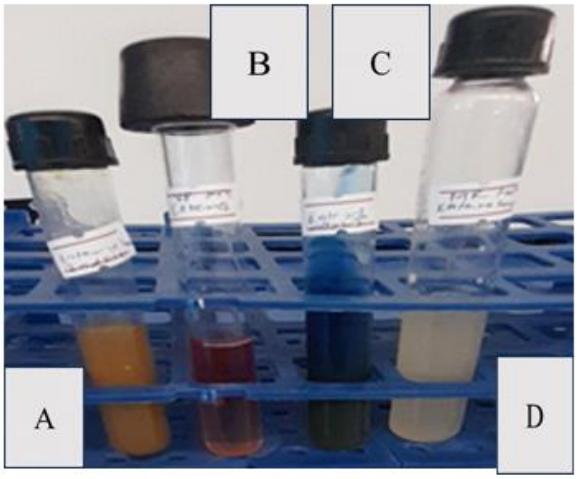

Figure 11: *Enterobactor aerogenes* biochemical test: indole (A), voges proskauer (B), citrate(C), methyl red (D)

Tests on *Proteus mirabillis* for indole and voges proskauer were negative; however, they were positive for citrate and methyl red (Figure 12).

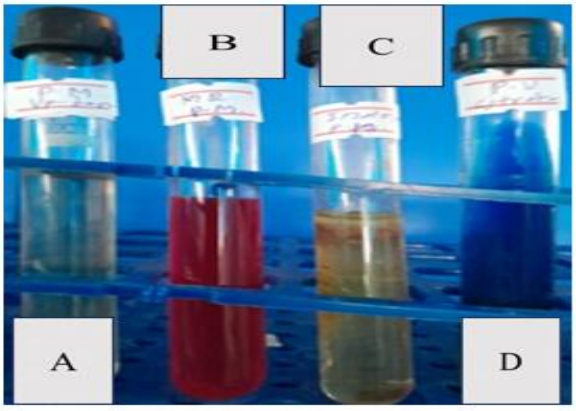

Figure 12: Biochemical results from the *Proteus mirabillis* test showed: vagos prokaeur (A), methyl red (B), indole (C), and citrate (D).

Indole, methyl red, and voges proskauer were all negative tests for *Pseudomonas aeruginosa*, while citrate was a positive test.

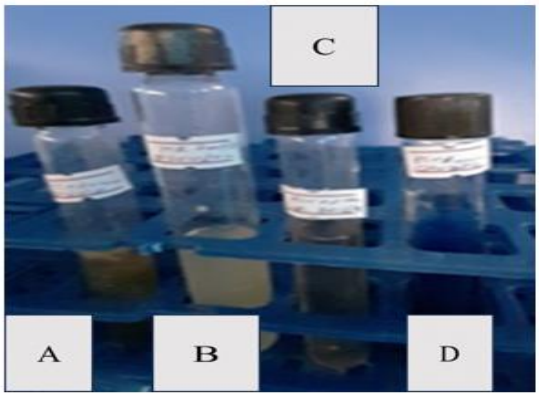

Figure 13: Biochemical test for *Pseudomonas aeruginosa*: indole (A), methyl red (B), vacose prokauer (C), and citrate (D)

Proteus vulgaris tested voges proskauer negative, whereas tests for citrate, methyl red, and indole were positive (Figure 14).

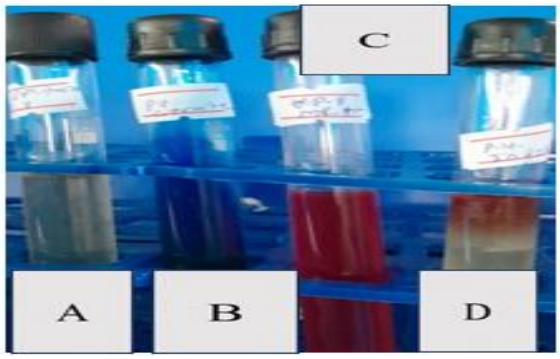

Figure 14: Biochemical analysis of *Proteus vulgaris*: voges proskauer (A), citrate (B), methyl red (C), indole (D).

The indole test for *Staphlococcus aerues* was negative, but the tests for methyl red, citrate, voges proskauer, catalase, urease, and coagulase were all positive (Figure 15).

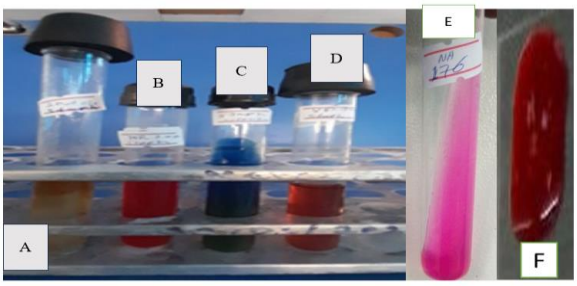

Figure 15: The following biochemical assays were performed on *Staphlococus aureus*: indole (A), methyl red (B), citrate (C), voges proskauer (D), urease (E), and coagulase (F).

Table 7: Bacteria identified with biochemical characterization

No	No Isolates	Biochemical test								Bacteria species
		CIT	OXI	URE	IND	MR	MOT	VP	CAT	
1	142	-	-	-	+	+	+	-	+	*E. coli*
2	43	+	-	-	-	-	-	+	+	*K. pneumonia*
3	30	+	-	-	-	-	+	+	+	*E. aerogenes*
4	26	+	+	+	-	+	-	+	+	*S. aureus*
5	23	+	-	+	-	+	+	-	+	*P. mirabilis*
6	16	+	-	+	+	+	+	-	+	*P. valigaris*
7	8	+	-	+	+	+	+	-	+	*p. aeruginosa*

Note: CAT=catalase, CIT =citrate, OXI= oxidase, URE=urease, IND= indole, MR=methyle red, MOT=motility, VP=voges proskauer tests

4.3 Distribution of Bacteria against different Age Groups

Escherichia coli was the most often isolated bacterium overall, accounting for 142 cases (49.4%). *Pseudomonas aeruginosa* was the least isolated bacterium, with 8 (2.8%) cases and no isolations from patients in the 11–20 and 31–40 age groups. Furthermore, no *Enterobactor aerogenes* were found in patients between the ages of 11 and 20 or 31 and 40. All detected bacteria were found in relatively low prevalence in individuals under the age of 11.

Table 8: The distribution of bacteria isolated by age groups

Isolated of bacteria		Age of patients							Total
		<11	11-20	21-30	31-40	41-50	51-60	>60	
E. coli	Count (%)	8(2.8)	14(4.9)	47(16.3)	20(6.9)	19(6.6)	12(4.2)	22(7.6)	142(49.3)
K.pneumonia	Count (%)	2(0.7)	8(2.8)	15(5.2)	6(2.1)	3(1.0)	1(0.3)	8(2.8)	43(14.9)
E.aerogenes	Count (%)	0(0.0)	4(1.4)	16(5.6)	3(1.0)	0(0.0)	3(1.0)	4(1.4)	30(10.4)
S. aureus	Count (%)	2(0.7)	4(1.4)	8(2.8)	1(0.3)	4(1.4)	3(1.0)	4(1.4)	26(9.0)
P.mirabilis	Count (%)	2(0.7)	3(1.0)	7(2.4)	2(0.7)	3(1.0)	4(1.4)	2(0.7)	23(8.0)
p.valigaris	Count (%)	2(0.7)	2(0.7)	3(1.0)	4(1.4)	2(0.7)	1(0.3)	2(0.7)	16(5.6)
P.aeruginosa	Count (%)	2(0.7)	0(0.0)	2(0.7)	0(0.0)	1(0.3)	1(0.3)	2(0.7)	8(2.8)
Total	Count (%)	18(6.3)	35(12.2)	98(34.0)	36(12.5)	32(11.1)	25(8.7)	44(15.3)	288(100)

4.4 Antibiotic Resistance and Sensitivity Pattern of the Identified Bacteria

The isolated bacteria showed resistance to Vancomycin, Tetracycline, Pencilin, Cefepime, Clarithromycin, and Erythromycin, whereas Ciprofloxacin (CPR) was the most effective antibiotic against all bacteria. 100% of *P. mirabilis* were ciprofloxacin-sensitive, while 100% were clarithromycin-resistant (Table11). The following tables display how each individual bacterium reacted to the different antibiotics employed in the study.

4.4.1 Antibiotic susceptibility and resistance of *Escherichia coli*

Ciprofloxacin sensitivity was observed in *Escherichia coli* (85.92%). The following table 9 shown the resistance to Tetracycline (91.54%), Pencillin (85.92%), Vancomycin (82.39%), Cefepime (76.05%), Erythromycin (72.54%), and Clarithromycin (63.38%).

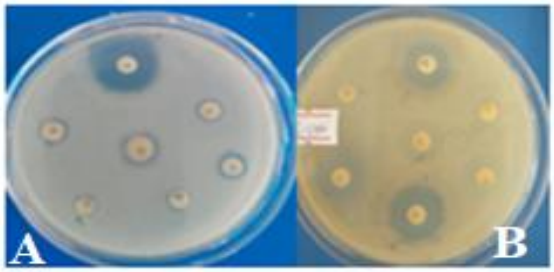

Figure 16: *E. coli* is susceptible and resistance to antibiotics: (A) Isolate-5, and (B) Isolate-13.

Table 9: Antibiotic sensitivity and resistance of *Escherichia coli*

Escherichia coli	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	122(85.92)	20(14.08)	Nil
Vancomycin	5(3.53)	20(14.08)	117(82.39)
Tetracycline	Nil	12(8.45)	130(91.55)
Cefepime	6(4.23)	28(19.72)	108(76.05)
Pencillin	Nil	20(14.08)	122(85.92)
Clarithromycin	12(8.45)	40(28.17)	90(63.38)
Erythromycin	2(1.41)	37(26.05)	103(72.54)

4.4.2 Antibiotic susceptibility and resistance of *Klebsiella pneumonia*

Ciprofloxacin has shown high sensitivity against *Klebsiella pneumonia* (97.67%). Both Erythromycin (76.74%) and Pencillin (100%) demonstrated high resistance.

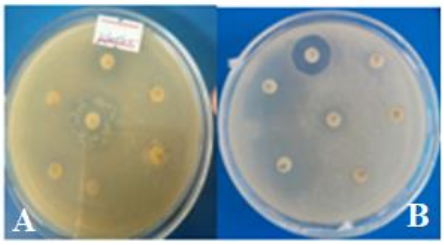

Figure 17: Susceptibility and resistance to antibiotics in *Klebsiella pneumonia:* (A) Isolate-15, and (B) Isolate-33.

Table 10: *Klebsiella pneumonia* antibiotic susceptibility and resistance

Klebsella pneumonia	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	42(97.67)	1(2.33)	Nil
Vancomycin	4(9.30)	6(13.95)	33(76.75)
Tetracycline	Nil	15(34.88)	28(65.12)
Cefepime	5(11.63)	10(23.25)	28(65.12)
Pencillin	Nil	Nil	43(100)
Clarithromycin	3(6.98)	7 (16.28)	33(76.74)
Erythromycin	2(4.65)	8(18.61)	33(76.74)

4.4.3 Antibiotic susceptibility and resistance of *Proteus mirabilis*

Ciprofloxacin demonstrated great sensitivity in *Proteus mirabilis* (100%) but lower sensitivity in Cefepime (8.70%). Tetracycline (78.26%), and Pencillin (73.91%) demonstrated substantial levels of resistance. Vancomycin demonstrated the lowest level of sensitivity (47.82%).

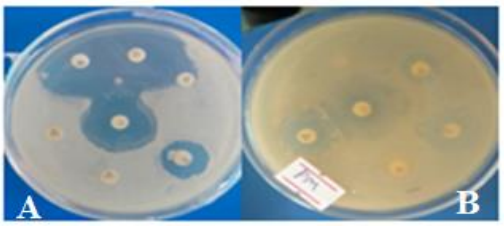

Figure 18: Antibiotic resistance and susceptibility tests for *Proteus mirabilis* isolates: (A) Isolate-2, and (B) Isolate-36.

Table 11: *Proteus mirabillis* antibiotic susceptibility and resistance

Proteus mirabillis	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	23(100)	Nil	Nil
Vancomycin	10(43.48)	2(8.70)	11(47.82)
Tetracycline	Nil	5(21.74)	18(78.26)
Cefepime	2(8.70)	8(34.78)	13(56.52)
Pencillin	Nil	6(26.09)	17(73.91)
Clarithromycin	Nil	Nil	23(100)
Erythromycin	13(56.52)	2(8.70)	8(34.78)

4.4.4 Antibiotic susceptibility and resistance of *Pseudomonas aeruginosa*

High sensitivity to Ciprofloxacin was demonstrated by *Pseudomonas aeruginosa* (100%). Tetracycline, Pencillin, Vancomycin, and Clarithromycin all demonstrated high resistance (62.5%).

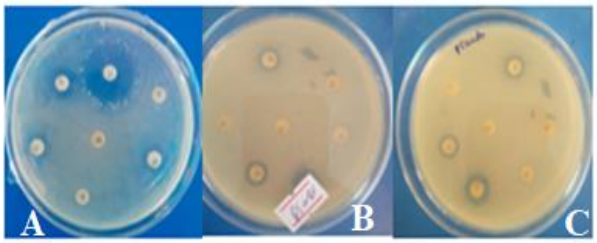

Figure 19: The *Pseudomonas aeruginosa* bacteria susceptible and resistance to antibiotics: *(A) Isolate-51, (B) Isolate-85, and (B) Isolate-162.*

Table 12: Antibiotic susceptibility and resistance of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	8(100)	Nil	Nil
Vancomycin	Nil	3(37.5)	5(62.5)
Tetracycline	1(12.5)	2(25)	5(62.5)
Cefepime	2(25)	3(37.5)	3(37.5)
Pencillin	1(12.5)	2(25)	5(62.5)
Clarithromycin	2(25)	1(12.5)	5(62.5)
Erythromycin	3(37.5)	2(25)	3(37.5)

4.4.5 Antibiotic susceptibility and resistance of *Staphylococcus aureus*

With a Ciprofloxacin sensitivity of 76.92%, *Staphylococcus aureus* and Vancomycin (81.76%,) was showed the highest resistance.

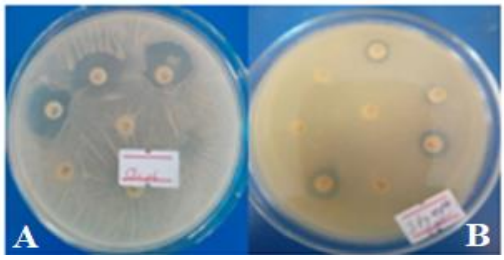

Figure 20: *Staphylococcus aureus's* susceptibility and resistance to antibiotics: *(A) Isolate-3, and (B) Isolate-51.*

Table 13: Antibiotic susceptibility and resistance of *Staphylococcus aureus*

Staphylococcus aureus	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	20(76.92)	6(23.08)	Nil
Vancomycin	2(7.69)	3(11.54)	21(80.77)
Tetracycline	5(19.23)	5(19.23)	16(61.54)
Cefepime	5(19.23)	7(26.92)	14(53.85)
Pencillin	2(7.70)	6(23.07)	18(69.23)
Clarithromycin	10(38.46)	5(19.23)	11(42.31)
Erythromycin	10(38.46)	5(19.23)	11(42.31)

4.4.6 Antibiotic susceptibility and resistance of *Protus vulgaris*

Protus vulgaris had a high susceptibility to the antibiotics Ciprofloxacin (100%). Pencillin (87.5%), Clarithromycin (75%), and Cefepime (68.75%) all demonstrated high levels of resistance, as did Tetracycline (62.5%) and Erythromycin (62.5%).

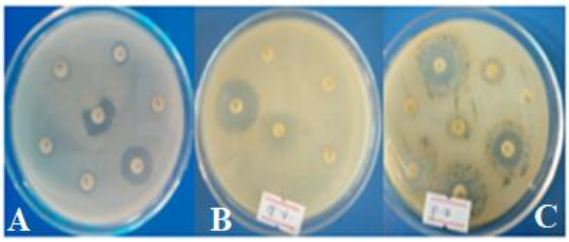

Figure 21: Antibiotic susceptibility and resistance of *Protus vulgaris: (A) Isolate-30, (B) Isolate-45, and (C) Isolate-96.*

Table 14: Antibiotic susceptibility and resistance of *Protus vulgaris*

Protus vulgaris	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	16(100)	Nil	Nil
Vancomycin	2(12.5)	5(31.25)	9(56.25)
Tetracycline	1(6.25)	5(31.25)	10(62.5)
Cefepime	Nil	5(31.25)	11(68.75)
Pencillin	Nil	2(12.5)	14(87.5)
Clarithromycin	2(12.5)	2(12.5)	12(75)
Erythromycin	3(18.75)	3(18.75)	10(62.5)

4.4.7 Antibiotic susceptibility and resistance of *Enterobactor aerogenes*

High sensitivity to ciprofloxacin (100%) was demonstrated by *Enterobactor aerogenes*. Erythromycin (83.34%) , Tetracycline (70%) and Cefepime (70%) have shown strong resistance.

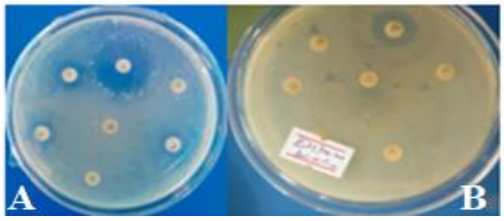

Figure 22: Antibiotic susceptibility and resistance of *Enterobactor aerogenes:*
(A) Isolate-14, and (B) Isolate-23.

Table 15: Antibiotic susceptibility and resistance of *Enterobactor aerogenes*

Enterobactor aerogenes	Sensitivity n (%)	Intermediate n (%)	Resistant n (%)
Ciprofloxacin	30(100)	Nil	Nil
Vancomycin	10(33.33)	5(16.67)	15(50)
Tetracycline	1(3.33)	8(26.67)	21(70)
Cefepime	4(13.33)	5(16.67)	21(70)
Pencillin	2(6.67)	8(26.67)	20(66.66)
Clarithromycin	2(6.67)	8(26.67)	20(66.66)
Erythromycin	1(3.33)	4(13.33)	25(83.34)

4.4.8 Multiple drug resistance patterns of bacterial isolates from urine samples

Both gram-positive and gram-negative bacteria isolated have shown resistance to two or more medications. *Staphlococcus aureus* was found to have drug resistance to two different medicines (Pencillin and Vancomycin), and (Tetracycline and Cefepime). *P. mirabilis* had drug resistance to just one different drug (Clarithromycin and Pencillin) (Table 16).

Table 16: Multi-drug resistance patterns of bacterial isolates

Organism	Total	R0	R1	R2	R3
Gram negative	262(90.97%)				
E.coli		Nil	Nil	Nil	142(100%)
K. pneumonia		Nil	Nil	Nil	43(100%)
P. mirabilis		Nil	Nil	1(4.35)	22(95.65%)
P. aeruginosa		Nil	Nil	Nil	8(100%)
P.vulgaris		Nil	Nil	Nil	16(100%)
Enterobactor aerogenes		Nil	Nil	Nil	30(100%)
Gram positive	26(9.03%)				
S. aureus		Nil	Nil	2(7.69%)	24(92.31%)
Total	288(100%)				

R0-sensitive to all antibiotics tested; R1 (resistant to one) : R2 (resistant to two antibiotics), and R3 (resistant to more than three antibiotics).

4.5 Detection of the tetA, blaSHV and vanA

Based on PCR amplification, only 26 of 43 *Klebsiella pneumonia* isolates were carried out for blaSHV gene detection (747 bps), 14 of 26 *Staphylococcus aureus* isolates were detected for vanA genes (713 bps), and only 59 of 142 *E. coli* isolates were carried out for tetA gene detection (501 bps).

4.5.1 Detection of tetA antibiotics resistance gene of *E.coli* isolates

Only 32 of the 59 *E. coli* isolates tested positive for the tetA gene after PCR amplification. This was demonstrated by the presence of DNA with an expected size of approximately 501 bp in the gel electrophoresis (Figure 23). The tetA gene, in contrast, was not found in the remaining *E. coli* samples

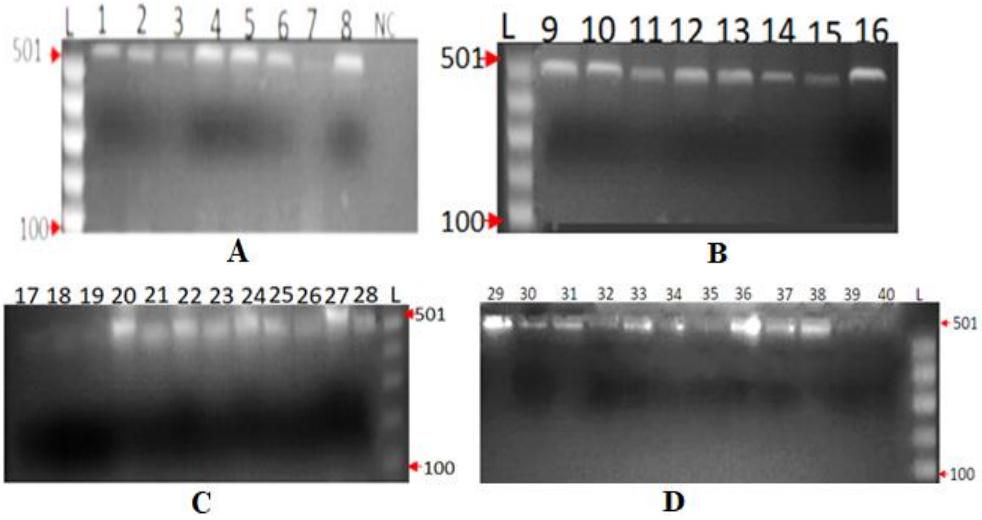

Figure 23: Gel image of amplified tetA gene from *E. coli* isolates by conventional PCR, out of 59 *E. coli* isolates, 54.2% were positive for the tetA gene).

L- molecular ladder, NC-negative control, positive tetA gene(1- 6, 8, 9, 11-16, 20- 29, 32-34, 36- 38, and 50), negative tetA gene (7, 17- 19, 26, 35, 39, 40, 41- 49, 51-58 and 59).

4.5.2 Detection of blaSHV antibiotics resistance gene of *Klebsiella pneumonia*

Using traditional PCR detection, *K. pneumoniae* isolates that tested positive for the blaSHV gene Only 26 *Klebsiella pneumonia* isolates were tested for the presence of the blaSHV gene, which has a size of 747 bps. In total, 14/26 *K. pneumoniae* isolates (53.8%) tested positive for the blaSHV gene, and the remaining 46.2% were blaSHV gene negative.

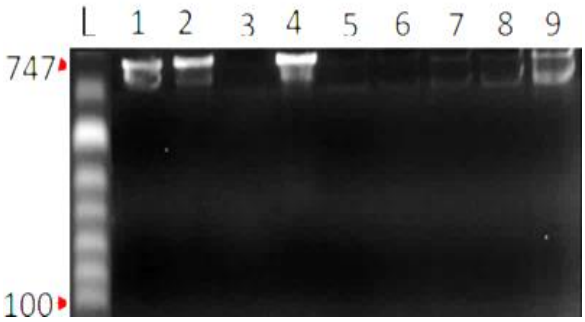

Figure 24: Gel image of the amplified blaSHV gene from *Klebsiella pnumonia* isolates by conventional PCR, 26 isolates were 53.8% positive for the blaSHV gene).

L-ladder, NC- negative control, blaSHV gene positive (1,2,4,9,) and blaSNV gene negative(3,5,6,7,8,).

4.5.3 Detection of vanA antibiotics resistance gene of *Staphloccus auerus* isolates

Using a conventional PCR method, 14 isolates of *Staphlococcus aeurus* were examined, and the isolate contained vanA genes with a gene size of nearly 713bps. 78.6% of these (11/14) had positive vanA genes, while the rest had negative results.

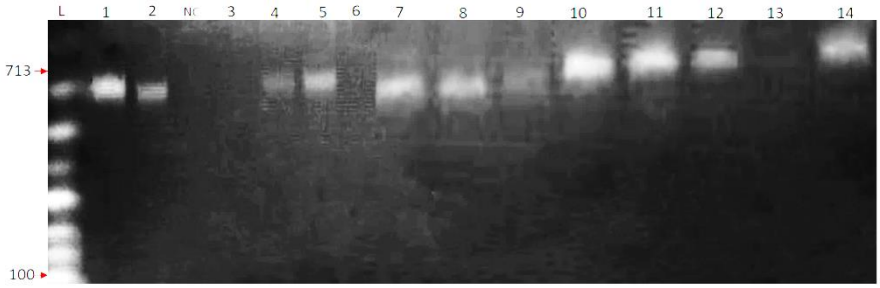

Figure 25: Gel image of the amplified vanA gene from *Staphyloccus auerus* isolates by conventional PCR (and 14 isolates were 78.6% positive for the vanA gene)

L–(100 bp leader), NC-(negative control), positive vanA gene(1,2,4,5,7,8,9,10,11,12,14), and negative vanA gene (3,6,14).

5. DISCUSSION

The study examined 384 samples from members with urinary tract contamination going to the Atat Hospital, disseminated over all age groups. The study looked to discover the predominance of urinary tract diseases in members, microscopic organisms causing UTI, and the microscopic organisms' resistance to antibiotics and the medications they are prescribed. During the data collection period, the prevalence of UTIs among patients visiting Atat Hospital was found to be 75% (Table 3). This prevalence rate was higher than consistent with previous studies by Islam *et al*. (2022), which reported a prevalence rate of 71%, and a similar study conducted in Kirkuk city, Iraq (Fakhraddin and Ahmed, 2022). But, it was lower than similar study conducted in shashemene city, Ethiopia, which reported prevalence rate 90.1% (Seifu and Gebissa, 2018). The high predominance of urinary tract contamination may be credited to the abuse of anti-microbials coming about from self-diagnosis and treatment with effortlessly open, over-the-counter anti-microbials accessible locally (Tilakaratne and Kallarakkal, 2023).

The distribution of urinary tract diseases varied among different age groups (Table 8). Participants between the ages of 21 and 30 had the highest rates of urinary tract infections 94(34%), followed by the age group of above 60 years (15.3%). The lowest infection rate was observed among participants aged below 11 years (6.3%). This result corresponds to a study carried out in medicare at Kiambu Level 5 hospital, Kenya, which also revealed that patients between the ages of 21 and 30 had the largest occurrence of UTI (29.2%) (Wanja *et al.,* 2021). The high prevalence among participants between the ages of 21 and 30 can likely be attributed to their reproductive age and sexual activity, as well as their inclination towards self-diagnosis and treatment based on information found online. Inappropriate use of antibiotics without a proper prescription can lead to a greater risk of incorrect, unwarranted treatment, incorrect diagnosis, delays in proper treatment, and an increase in disease (Mehmood *et al.*, 2019).

It has shown that the bacteria that cause urinary tract infections are gram-negative bacteria (91.0%) and are the most common cause of urinary tract infections in patients compared to gram-positive bacteria (9.0%) (Table 6). Among the gram-negative bacteria, *E. coli* (49.30%) was the most common cause of infections. This trend was comparable to or

lower than in many other studies in which *E. coli* (51.6%) was the main cause of urinary tract infections (Islam *et al.*, 2022). This predominance was attributed to the fact that *E. coli* naturally colonizes the urinary tract and evades the immune system. The second most common gram negative was *Klebsiella pneumonia* (14.93%), followed by *enterobactor aerogenes* (10.41%) (Table 5). This finding is similar to the findings of Seifu and Gebissa (2018) Shashemene, Ethiopia.

Biochemical assays for microbiological identification use distinct metabolic activities exhibited by various kinds of bacteria (AL-Joda and Jasim, 2021). *Escherchia coli* biochemical tests revealed that motility, indole, and methyl red catalase were positive, but oxidase, citrate, urease, and vagos proskuer were negative (Table 7). This biochemical test *E.coli* similar test done (Rai et al., 2017). The assays for citrate, vagos prokauers, and catalase tests were positive, and indole and methyl tests were negative suggesting that *Klebsiella pneumonia* (figure 10). The biochemical tests for *Proteus mirabilis* were negative for vagos prokauer and indole, while positive results were found for citrate, methyl red, and urease. Since the indole test was positive for *P. vulgaris* and negative for *P. mirabilis*, it was utilized to distinguish between the two species *(*El-Tarabiliet *et al*., 2022*)*. Catalase, citrate, urea, methyl red, vagos proskuer, catalase, and coagulase tests were positive for *Staphylococcus aureus*; indole tests, on the other hand, was negative (figure 15).

Antibiotic susceptibility patterns seven antibiotics were tested *in vitro* against all bacteria isolated in the study to determine their antibiotic susceptibility patterns. These are the medications that the Ministry of Health often prescribes to treat urinary tract infections. Antibiotic testing revealed varying resistance patterns among the isolated bacteria. *S. aureus* showed high resistance to tetracycline (61.5%), penicillin (69.2%), and vancomycin (81.56%) (Table 13). Similar resistance patterns were observed in a survey conducted in China (Liu *et al*., 2022). The research is also comparable to a study on gram-positive urinary tract infection bacteria conducted by Urmi *et al*. (2019). The highest antibiotic resistance of *S. aureus* was associated with the abundance of substandard and counterfeit drugs on the market as well as the indiscriminate use of these drugs by patients (Acharya and Wilson, 2019).

Gram-positive bacteria, such as *Staphylococcus aureus* (9.0%) (Table 5), were less commonly compare to gram-negative bacteria implicated in urinary tract infections from this study. VanA vancomycin-resistant genes were found in the isolates of vancomycin-resistant *S. aureus* (VRSA). The vanA gene was identified using the common polymerase chain reaction (PCR) method in all phenotypically verified VRSA. 78.6% of the vanA gene was discovered in this study. The similar study done Maharjan and others, (2021) study showed that 40% VRSA-positive isolates showed the vanA gene, which was less prevalent than the current investigation. However, it was less than a study that assessed the prevalence of 100% of the vanA genes that code for vancomycin resistance (Basil *et al*., 2022). The study highlighted the importance of antibiotic susceptibility testing in identifying resistance patterns and developing precise treatment plans for UTIs. It also emphasized the need to address the misuse and overuse of antibiotics, as well as the emergence of multidrug-resistant bacteria (Rusu *et al*., 2023).

In the current study, *Escherichia coli* showed considerable resistance to tetracycline (91.5%) and pencilin (85.9%), while Ciprofloxacin was effective against them (85.9%) (Table 8). Furthermore, the tetA gene was present in 54.2% (32 of 59) of the molecular samples tested by coventiomal PCR (Fig. 23). According to a related study conducted at Ladoke Akintola University of Technology in Osun State, Nigeria (Olowe *et al*., 2013), the TetA gene was discovered in isolates of *E. coli* in 43.8% of cases. On the other hand, a recent study discovered that 89.9% of *E. coli* isolates from the Southwest region of Nigeria had the tetA gene present, indicating a relatively high level of gene-mediated antibiotic resistance to tetracycline and other antibiotics (Perewari *et al*,. 2022).

53.8% (14 of 26) of the *Klebsiella pneumoniae* genotypes examined possess the blaSHV gene (fig. 24). According to research conducted at Tehran's Shahid Beheshti University of Medical Science, the prevalence of the blaSHV gene was shown to be 45% less common (Seyedjavadi et al., 2016). Another study done by Pishtiwan and Khadija (2019) in the isolates of *K. pneumoniae* from the University of Salahaddin-Erbil in Iraq, 35.2% possessed blaSHV genes.

In this investigation, *Proteus mirabilis* was susceptible to ciprofloxacin (Table 11). However, *Proteus mirabilis* is known to display multidrug resistance to numerous types of antibiotics, making the treatment of UTIs more challenging (Schaffer and Pearson et al., 2017). Numerous studies have also connected the creation of additional *Proteus mirabilis*-resistant strains to the indiscriminate prescription of drugs, which results in an unsuccessful attempt at empirical therapy. Many of the frequently prescribed antibiotics in the study exhibit a significant proportion of antibiotic resistance, which may be related to the drugs' accessibility in pharmacies and their affordable, over-the-counter sale.

6. CONCLUSIONS AND RECOMENDATION

During June and September of 2022–2023, a large percentage of outpatients with urinary tract infections (75% of patients) visited Atat Hospital. *E. coli* had the highest prevalence (49.30%), and *P. auroginosa* had the lowest (2.78%) among the gut bacteria that were isolated. Between the ages of 21 and 30, UTI cases were most common, while those below the age of 11 were less common. The specific antibiotic efficacy that the isolated bacteria exhibited varied greatly. Overall, the current investigation found that ciprofloxacin was effective against both gram-positive and gram-negative bacterial uropathogens. Whereas pencillin was the least effective. Furthermore, this investigation showed that a high prevalence of antibiotic resistance determinants, including the vanA, terA, and blaSHV genes, was observed.

As a result, patients at Atat Hospital must receive prompt treatment for urinary tract infections and use antibiotics with caution. To help with optimal UTI management, a hospital should routinely survey UTIs caused by enteric bacteria, particularly *E. coli*. Effective antibiotics like ciprofloxacin should be used to treat UTIs because the majority of isolates are resistant. It would also be helpful to handle cases of unsuccessful therapy and stop the spread of antibiotic resistance genes in order to monitor the progress of those who have received treatment. Creating strategies and processes for drug utilization management is the most efficient way to lower antibiotic resistance. The future of our healthcare is extremely complicated and challenging due to the developing trend of antibiotic resistance against the majority of popular antibiotics. Additionally, the growth of multi-drug-resistant (MDR) bacteria, which renders the majority of antibiotics ineffective through the development of newer and extremely 50 complicated resistance mechanisms, is quite concerning. To ascertain the relationship between phenotypic and genotypic resistance features in uropathogenic *Escherichia coli, Klebsiella pneumonia,* and *Staphylococcus* aureus, cross-sectional studies were done. Due to the antibiotic sensitivity biogram's clear reflection of the medication trend, this study was able to offer evidence-based guidance on the best antibiotic therapy for treating bacteria in patients with urinary tract infections

To assess this dire situation countrywide and establish control solutions, a national anti-biotical resistance survey and research are finally required. Antibiotic usage should be

limited and administered only after performing a culture and sensitivity test in order to control drug resistance. It is advised to perform additional scanning sensitivity testing and antibiotic resistance gene.

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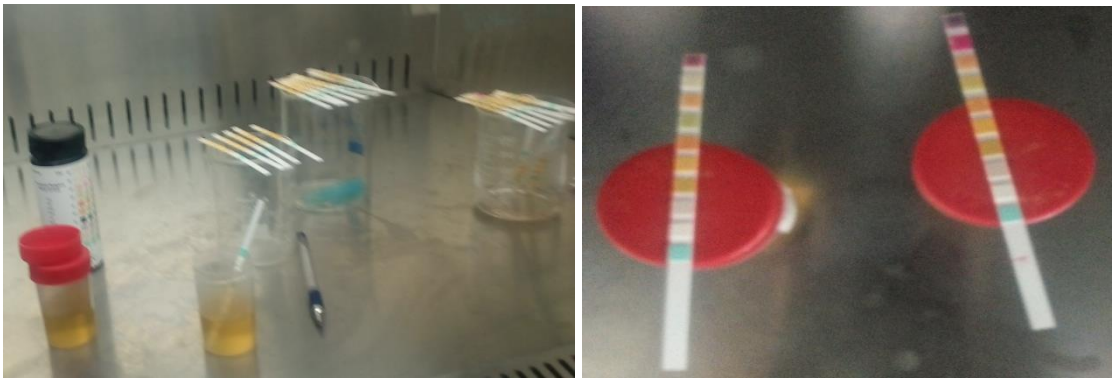
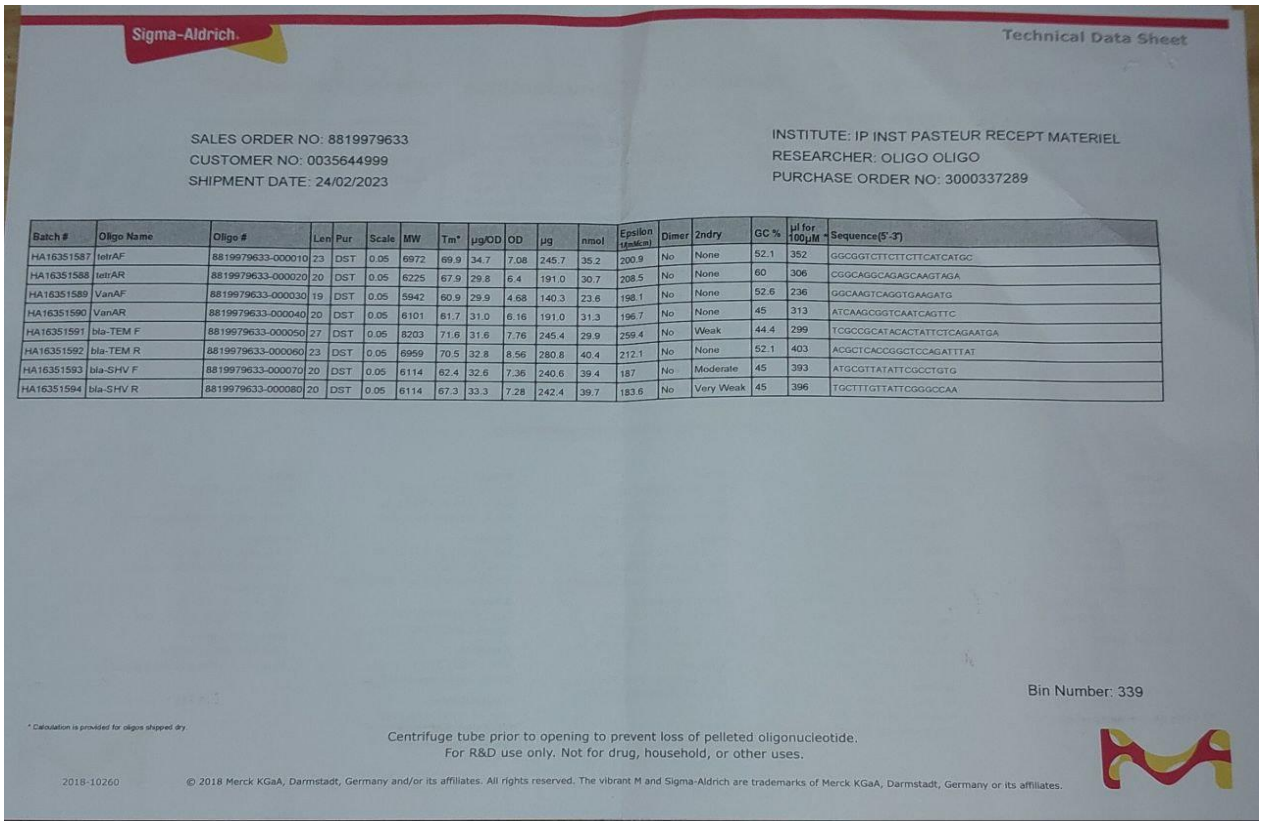
8. APPENDICES

Appendix 3.Collection midstream urine in Attat Hospital



Appendix 4. Urinalysis using dipstick test

Appendix 5.Gram stain and microscope examine



Sigma-Aldrich.

Technical Data Sheet

SALES ORDER NO: 8819979633
CUSTOMER NO: 0035644999
SHIPMENT DATE: 24/02/2023

INSTITUTE: IP INST PASTEUR RECEPT MATERIEL
RESEARCHER: OLIGO OLIGO
PURCHASE ORDER NO: 3000337289

Batch #	Oligo Name	Oligo #	Len	Pur	Scale	MW	Tm*	µg/OD	OD	µg	nmol	Epsilon (dm³/cm)	Dimer	2ndry	GC %	µl for 100µM	Sequence(5'-3')
HA16351587	tetrAF	8819979633-000010	23	DST	0.05	6972	69.9	34.7	7.08	245.7	35.2	200.9	No	None	52.1	352	GGCGGTCTTCTTCTTCATCATGC
HA16351588	tetrAR	8819979633-000020	20	DST	0.05	6225	67.9	29.8	6.4	191.0	30.7	208.5	No	None	60	306	CGGCAGGCAGAGCAAGTAGA
HA16351589	VanAF	8819979633-000030	19	DST	0.05	5942	60.9	29.9	4.68	140.3	23.6	198.1	No	None	52.6	236	GGCAAGTCAGGTGAAGATG
HA16351590	VanAR	8819979633-000040	20	DST	0.05	6101	61.7	31.0	6.16	191.0	31.3	196.7	No	None	45	313	ATCAAGCGGTCAATCAGTTC
HA16351591	bla-TEM F	8819979633-000050	27	DST	0.05	8203	71.6	31.6	7.76	245.4	29.9	259.4	No	Weak	44.4	299	TCGCCGCATACACTATTCTCAGAATGA
HA16351592	bla-TEM R	8819979633-000060	23	DST	0.05	6959	70.5	32.8	8.56	280.8	40.4	212.1	No	None	52.1	403	ACGCTCACCGGCTCCAGATTTAT
HA16351593	bla-SHV F	8819979633-000070	20	DST	0.05	6114	62.4	32.6	7.36	240.6	39.4	187	No	Moderate	45	393	ATGCGTTATATTCGCCTGTG
HA16351594	bla-SHV R	8819979633-000080	20	DST	0.05	6114	67.3	33.3	7.28	242.4	39.7	183.6	No	Very Weak	45	396	TGCTTTGTTATTCGGGCCAA

Bin Number: 339

* Calculation is provided for oligos shipped dry.

Centrifuge tube prior to opening to prevent loss of pelleted oligonucleotide.
For R&D use only. Not for drug, household, or other uses.

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Appendix 6. PCR primers used in this study