The Prevalence and Antimicrobial Resistance Patterns of The Pathogens Causing Urinary Tract Infection Among Children Under 15 Years in Adama City, Ethiopia

Betelihem Defaru Yami

A Thesis Submitted to The Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Applied Microbiology)

Office of Graduate Studies

Adama Science and Technology University

December,2024

Adama, Ethiopia

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Main Advisor: Zerihun Belay Gemta (Ph. D)

Co -Advisor: Mesfin Bekele (MSc)

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ACKNOWLEDGMENTS

First of all, I thank the Almighty God for his mercy, and grace and for giving me good health throughout my life journey. I am also most grateful to my main research advisor Dr. Zerihun Belay for his continuous and kind support throughout the development of this thesis and for his immense contribution, guidance, and encouragement from the preparation of the research proposal to the write-up of the thesis paper. I would like to also thank my co-advisor Mr. Mesfin Bekele for his support.

My heartfelt gratitude also goes to the all-laboratory experts, inter-doctors, head of the pediatrics department Dr. Esmael Adem in Adama Hospital Medical College, and laboratory staff in Geda Health Center for their enormous contribution during sample collection.

My special gratitude also goes to the management and staff members of Adama Public Health Research and Referral Laboratory for their high-level laboratory for the analysis of samples collected, for providing me with all the required materials, and for their sharing of knowledge.

My special gratitude also goes to the management, staff members and laboratory technicians of the National Animal Health Diagnostic and Investigation Center found in Sebeta for their enormous contribution while doing the MALDI TOF MS analysis.

Finally, my special thanks go to my family and friends for their unfailing support, encouragement, and advice they provided me with throughout my studies.

DECLARATION

I hereby declare that this Master Thesis entitled "**The prevalence and antimicrobial resistance patterns of the pathogens causing urinary tract infection among children under 15 years in Adama city, Ethiopia**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

Betelihem Defaru	_____	_____
Name of the student	Signature	Date

RECOMMENDATION OF ADVISORS

we, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "**The prevalence and antimicrobial resistance patterns of the pathogens causing urinary tract infection among children under 15 years in Adama city, Ethiopia**" prepared under our guidance by **Betelihem Defaru** submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Applied microbiology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Zerihun Belay (Ph. D) _____ _____

Major Advisor Signature Date

Mesfin Bekele (MSc) _____ _____

Co-advisor Signature Date

APPROVAL PAGE FOR THESIS

we, the advisors of the thesis entitled "**The prevalence and antimicrobial resistance patterns of the pathogens causing urinary tract infection among children under 15 years in Adama city, Ethiopia**" and developed by **Betelihem Defaru**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Zerihun Belay (Ph. D) _____ _____

Major Advisor Signature Date

Mesfin Bekele (MSc) _____ _____

Co-advisor Signature Date

APPROVAL OF BOARD OF REVIEWERS

We, the undersigned, members of the Board of Examiners of the thesis by **Betelihem Defaru** have read and evaluated the thesis entitled "**The prevalence and antimicrobial resistance patterns of the pathogens causing urinary tract infection among children under 15 years in Adama city, Ethiopia**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

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LIST OF ABBREVIATION/ACRONYMS

ABU-asymptomatic bacteriuria

AmpC-ampicillin class c beta lactamase

CD14-cluster of differentiation 14

CFU-colony forming unit

CLSI-Clinical and Laboratory Standards Institute

CSF-cerebro spinal fluid

GI-gastrointestinal

IDSA-infectious disease society of America

IgA-immunoglobulin A

IL 6-interleukin 6

IL 8-interleukin 8

kDa- kilodalton

LPS-lipopolysaccharide

MALDI TOF MS- Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

MIC-minimum inhibitory concentration

NICE-national institute for health and care excellence

Spp.- species

TLR4-toll-like receptor 4

UTI-urinary tract infection

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ABSTRACT

After respiratory tract infections, UTIs are the second most common bacterial infection in children with the highest morbidity rate partly due to the pathogens are frequently acquire antimicrobial resistance to different antibiotic drugs globally. The objective of this study was to analyze the microbial profile of UTI bacteria and their antibiotic resistance pattern from outpatients under the age of 15 attending Adama Hospital Medical Collage and Geda Health Center in Adama City, Ethiopia. A total of urine samples were collected from 214 children. The specimens were processed at the Research and Referral Laboratory to isolate bacteria using MacConkey and blood agar. The isolates were characterized based on different cultural, microscopic and biochemical characteristic using standard methods. An antimicrobial susceptibility testing was done using the Kirby-Bauer disc diffusion method. Of the total 214 children 25 children were UTI positive with the prevalence of 11.7%. The prevalence of females is greater than males. The isolates were identified into gram positive (5 isolates) into S. aures, S. saprophyticus, Enterococcus spp. and Corynebacterium spp. The others were gram negative bacteria; E. coli, P. vulgaris, E. cloace and K. oxtyca. E. coli was a common bacteria accounting for 10/25(40%) of all gram-positive and gram-negative isolates. Among gram-positive bacteriuria Corynebacterium spp. were the dominant one accounting for 4/25(14%). The MALDI TOF MS analysis identified K. pneumoniae and E. coli with high confidence. With regard to antibiotic resistance pattern, Gram-positive bacteria were highly sensitive to ciprofloxacin and resistant to sulfa/trim and tetracycline. Gram-negative bacteria were highly sensitive to meropenem and cefazolin and highly resistant to ampicillin-sulbactam and ceftazidime. Gram-negative bacteria were resistance to more than two antimicrobial agents. Generally, in this study, the prevalence of UTI under 15 was 11.7%. The most common causative agent of UTI was E. coli.

Keywords: antimicrobial resistance, children, prevalence, urine culture, urinary tract infection

CHAPTER ONE: INTRODUCTION

1.1 Background

Upper Respiratory Infection (UTI) is the process by which microorganisms invade and constantly proliferate the urinary tract affecting the urethra, ureters, and bladder (Rahman et al., 2019). It is one of the most prevalent bacterial illnesses in humansand affecting people worldwide. It is 14 times more common in women than in men due to the female urethra's smaller size and proximity to the anus. As a result, up to 40% of women will encounter a UTI at some point in their lives, with most of these women experiencing recurrent UTIs. It is also linked to renal scarring from a kidney UTI resulting in secondary hypertension and chronic kidney disease (Biondo, 2023).

E. coli, S. marcescens, *E. faecalis*, S. saprophyticus, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus* are the most frequently found bacteria that cause UTIs in humans of which E. *coli* bacteria are the primary cause of 85% of UTIs in the community and 50% of UTIs acquired in hospitals. The frequency of urinary tract infections is influenced by several variables, including immunosuppression, urological equipment, age, and gender (Rahman et al., 2019).

Broad-spectrum antibiotics are often prescribed as an empirical treatment for UTI. However, due to Antibiotic misuse and the availability of over-the-counter medications have led to the emergence of antibiotic resistance worldwide against common pathogens. Multidrug resistance uropathogens have been more common in the past few years, with an increase seen in both community-dwelling and hospitalized patients (Muhammad et al., 2020).

The empirical use of cephalosporins and ciprofloxacin has been threatened by the emergence of extended-spectrum beta-lactamases. This is due different mechanisms, employed by microorganisms, including recombination of foreign DNA in bacterial chromosomes, horizontal gene transfer, and genetic material alteration, Microorganisms' resistance patterns differ between nations, states, big and small hospitals, and hospitals and communities(Sabir et al., 2014).

A study conducted by the European Survey of Antibiotic Consumption found that the mortality rate from complex UTIs is caused by Multidrug resistance bacterial strains, accounting for about 25,000 deaths in Europe annually(Thesis & Andr, 2020).

The prevalence of UTIs in Africa varies from country to country and geographical location. Personal hygiene, prostate problems, compromised immunity, sex, diabetes, and use of spermicidal contraception are some of the factors for UTIs. For instance, in Ghana, the prevalence rate is 15.9%, in Senegal 4.5%, and 12.3% in Nigeria. On average, the overall prevalence of UTIs in the nine countries of sub-Saharan Africa was 32.12% with South Africa ranking high (67.6%); followed by Nigeria (43.65%); Zambia (38.25); Uganda (35.66%); Ethiopia (37.47%); Tanzania (23.7%); Ghana (19.2%); Kenya (18.53%); and Senegal (5.1%) (Mchami, 2022).

In Ethiopia, several research were conducted. Adugna Fenta et al (2020) surveyed UTI in northwest Ethiopia and showed a prevalence of 28.6% with bacterial pathogens accounting for 75.4% (49/65) and fungal pathogens accounting for 24.6% (16/65) of the total. *E. coli* and *K. pneumoniae* accounted for approximately 79.6% of bacterial etiology. The study also showed the highest resistance was observed among symptomatic pediatrics patients attending St. Paul Hospital Millennium Medical College, Addis Abeba against ampicillin (100%), cefazolin (92.1%), and trimethoprim-sulfamethoxazole (84.1%) (Bitew et al., 2022).

Another study conducted in Adiss Abeba City (Fenta et al., 2020) showed that the overall prevalence of urinary tract infections was 16.7%. Gram-negative and Gram-positive bacterial isolates were recovered at rates of 44/50 (88%) and 6/50 (12%), respectively. *E. coli* was predominant among Gram-negative isolates (28/44, 63.6%), while *S. saprophyticus* was prevalent among Gram-positive isolates (2/6, 33.3%).

Adugna Fenta et al (2020) surveyed UTI in northwest Ethiopia and showed Gram-negative bacteria had high level of resistance to ampicillin, augmentin, and tetracycline at rates of 44/44 (100%), 39/44 (88.6%), and 36/44 (81.8%), respectively. Approximately 33/50 (66%) of total multidrug resistance was observed (95% CI 52-78). About six Gram-negative bacterial isolates produced extended-spectrum beta-lactamases (Fenta et al., 2020).

Therefore, it is critical to prevent the overuse and inappropriate use of antibiotics, which can result in multidrug resistance. To this end, the IDSA advises conducting regional surveillance

in a particular area to track changes in patterns of antibiotic susceptibility(Muhammad et al., 2020).

1.2 Statement of the problem

Approximately 150 million individuals worldwide experience urinary tract infections each year. It is predicted that approximately six million patients worldwide visit outpatient departments and that approximately 300,000 receive ward treatment for UTIs annually. 10% of people will experience a UTI at some point in their lives(Pant et al., 2015). In roughly 95% of patients with acute pyelonephritis, the most common causative bacteria are usually Gram-negative isolates like *P. mirabilis* and *K. pneumoniae*, with some Gram-positive organisms found to be *S. agalacticus* and Coagulase negative Staphylococci(Rakesh et al., 2014). Renal calculi, renal failure, indwelling catheters, renal transplantation, immunosuppression, blockage, and pregnancy are risk factors for complex UTIs. More than 95% of UTI cases are typically caused by bacteria, but other microorganisms like fungi, parasites, and viruses can also cause UTIs(Muhammad et al., 2020). Roughly 9% of children seeking medical attention for any reason in developed countries have a UTI, whereas 45% of children in developing countries have one(Article, 2017).

Some studies were conducted to determine the prevalence of UTI among pediatric patients in different regions of Ethiopia: the study at Hawassa referral hospital, Yekatit 12 hospital, Felege-Hiwot specialized, and Gondar University hospitals revealed 27.5%,15.9%, 16.7%, and 26.45% respectively (Bitew et al., 2022). Although UTI is the most prevalent and reported infection among children in other parts of the country, there is an information gap among the pediatric population in Adama. Moreover, the patterns of prevalence differ across regions and there is a data gap in many parts of the cities. The global spread of multidrug-resistant organisms has also led to an increase in UTIs in children that are difficult to treat (In & Town, 2018). At present, antibiotic resistance in pediatrics is a big problem both globally and in Ethiopia(Reta et al., 2019). Therefore, it is it is important to know, the prevalence and the antimicrobial resistance pattern of the isolates associated with UTI from Adama City.

1.3 Significance of the study

The significance of studying the prevalence and antimicrobial resistance patterns of pathogens causing urinary tract infections (UTIs) in children lies in several key areas for instance by

understanding the prevalence of UTIs in children helps identify how common these infections are in the population. This information is vital for public health strategies and resource allocation. Also, by examining antimicrobial resistance patterns, we can identify which antibiotics are becoming less effective against common pathogens. This knowledge is crucial for informing treatment guidelines and ensuring that healthcare providers use the most effective medications. Knowing which pathogens are prevalent and their resistance patterns can lead to more targeted and effective treatment approaches, ultimately reducing complications and improving recovery rates in children. The study can provide healthcare professionals, parents, and policymakers with important information to promote better practices in managing UTIs in children, including the responsible use of antibiotics. Generally, studying the prevalence of UTIs and antimicrobial resistance patterns in urine specimens of children is crucial to enhancing public health measures, improving diagnostics, promoting antimicrobial stewardship, guide treatment guidelines, and implementing effective prevention strategies to safeguard the health of young children.

1.4 Objectives

1.4.1 General objective

- To determine the prevalence and antimicrobial resistance patterns of the bacterial pathogens causing urinary tract infection among outpatient children under 15 years old in, Adama, Ethiopia.

1.4.2 Specific objectives

- To identify and characterize the isolated bacteria using microscopic, biochemical, and MALDI TOF MS
- To determine the prevalence of UTI by host factor (age and gender)
- To determine the antibiotic susceptibility profiles of the bacterial isolates using standard susceptibility testing method

CHAPTER TWO: LITERATURE REVIEW

2.1 Urinary tract infection

Bacteria are the most important causative agents of UTIs(Gebremedhin et al., 2024). Moreover, more than 30% of all infectious complications in patients undergoing kidney transplantation are UTIs. Due to their frequent occurrence, recurrence, and global rise in antibiotic resistance, both lower and upper urinary tract infections continue to pose serious challenges in terms of diagnosis and effective treatment. Unusual symptoms from UTIs can include atypical symptoms, asymptomatic kidney abscess and urosepsis symptoms, and associated kidney failure that can even be fatal(Biondo, 2023).

About 50% of children with feverish urinary tract infections develop pyelonephritis and kidney scarring. Immunocompromised patients often have the worst clinical course. However, the prognosis is good if a suitable therapy is started at an early age. A precise diagnosis and prompt treatment are also essential because there is a chance of long-term effects, such as chronic kidney disease(Masajtis-Zagajewska & Nowicki, 2017).

Most UTIs are caused by bacteria, although they can also be caused by fungi, viruses, or parasites(Kahsay et al., 2024). The most common UTI causes cystitis, although other UTIs can result in pyelonephritis, urethritis, and prostatitis(Biondo, 2023). Urinary tract bacterial colonization is not always accompanied by symptoms, and women and older people are more likely to have bacteriuria or asymptomatic bacterial colonization. There are two types of UTIs: complicated and uncomplicated. The most common type of infection is an uncomplicated UTI, which happens when there is no anatomical or functional abnormality within the urinary tract. An abnormal urinary tract or other factor that increases susceptibility to infection is the cause of a complicated UTI(Huang et al., 2022).

Childhood UTIs are common before the age of ten, two percent of kids get at least one UTI. An infection can be hard to diagnose because it usually manifests as a feverish sickness without any distinguishing symptoms. Getting a urine sample for culture adds another layer of difficulty. It is necessary to diagnose, investigate, and treat UTIs as soon as possible to reduce the risk of scarring and progressive renal damage. Reflux nephropathy, or kidney damage caused by urine reflux from the bladder into the ureter, is especially dangerous for children who also have an infection. Reflux nephropathy, also known as chronic pyelonephritis, and

dysplastic kidneys are among the diseases that cause 20% of pediatric renal failure and adult hypertension and renal impairment. Even though it is not always significant, kidney damage can still happen without an infection. Age determines how best to treat and screen children for reflux and other abnormalities of the renal tract; NICE has recently released comprehensive guidelines in this regard. This is especially important for young male children(Biondo, 2023).

2.2 Etiological agents

The consensus is that urine is sterile and germ-free. Any potential source of infection enters through the urethra, which starts the infection's occurrence(Dm et al., 2020). Although other pathogens (fungi, mycobacteria, and viruses) are also encountered, enteric bacteria are the most common pathogenic source of UTIs(Sahu et al., 2019). Group B streptococci urinary infections are more common in newborns than in older kids and adults, which may be related to the mothers' colonization status and the possibility that infants are more vulnerable to systemic seeding infections(Cano et al., 2021).

Most urinary tract infections are caused by gram-negative aerobic bacteria, which enter the urinary system through the urethra. More than 60% of UTIs are caused by *E. coli*, which is now known to be a diverse genetic group. *Proteus* spp. (common in boys and the presence of renal stones), *Klebsiella* spp., *Pseudomonas* spp., and *Enterococcu*s spp. are other frequently found bacteria. *S. aureus* and *S. epidennidis* are less frequent microorganisms. Other viruses that can cause UTIs include adenoviruses, vaccinia, herpes simplex, and vaccinia (Biondo, 2023). Fungal UTIs are also becoming more common; these are mostly brought on by *Candida* species, with *Aspergillus* species and *C. neoformans* contributing less (Dm et al., 2020).

When pathogens enter a healthy child's urinary tract, they typically do so through retrograde migration of enteric bacteria that have colonized the periurethral area, reflecting this flora. However, in an immunocompromised person, other uncommon and opportunistic pathogens might also be at play(Cano et al., 2021) . Following birth, both aerobic and anaerobic microorganisms colonize the periurethral region, including the distal urethra. These microbes appear to serve as a barrier against uropathogen colonization. Enterobacteria and enterococci are typical members of the periurethral flora in early childhood. In young girls, *E. coli* is the predominant gram-negative species, while in boys, *E. coli* and *Proteus* species are more prevalent(Larkins & Hewitt, 2018).

P. mirabilis (common in boys), *K. pneumoniae, P. aeruginosa*, Enterobacter species, *S. aureus* (especially in older children), *S. viridans*, enterococci, and *C. albicans* are other important organisms, especially in neonates and in complicated cases. A single organism typically causes an uncomplicated UTI, but multiple organisms may be present in patients with complicated UTIs. Urease-producing bacteria like *P. mirabilis, K. pneumoniae,* and *P. aerogenosa* can cause urinary tract infections that lead to the alkalinization of the urine and the development of calculous (struvite) deposits. Both bacteria and adenoviruses can cause acute hemorrhagic cystitis. The presence of urethral symptoms and low colony counts (less than 140 per milliliter) are diagnostic indicators of urethritis(Dm et al., 2020).

2.3 Epidemiology

Since young children with UTIs may only experience fever and lack specific urinary tract symptoms (dysuria, urgency, frequency of urination), it is challenging to determine the actual incidence of UTI in children (costovertebral angle tenderness, for example). The definition of infection, urine collection technique, and patient selection all have an impact on the reported prevalence of UTI. If urine samples are taken by applying a bag to the periurethral skin, a technique known to have a significant rate of bacterial contamination, falsely high infection rates are reported (Podsiadły et al., 2023).

The prevalence of UTIs in children varies according to age, gender, and other characteristics. Up to 5% of girls and 1% to 2% of boys get UTIs(D. Lin et al., 2015). All children experience UTIs most frequently in their first year of life (1%), and the incidence varies in infants from 0.1% to 1.0% in newborns to up to 10% in low-birth-weight infants. Boys are more likely than girls to get a urinary tract infection before the age of one. Girls are more likely to get UTIs and bacteriuria after the age of one. The incidence of UTI in infant boys has been estimated differently in different populations, probably because of things like circumcision, which has been linked to a lower risk of UTI(Eisenberg et al., 2018). The rising awareness of UTI as a cause of febrile illness in young children is another factor influencing incidence estimates. According to screening studies conducted in emergency rooms, up to 5% of children under the age of two who present with fever have UTIs; if the urine had not been screened as part of the study, more than half of these children would have received an alternative diagnosis, such as otitis media. According to Swedish studies, by the time they are 11 years old, at least 1% of boys and 3% of girls have a symptomatic UTI(Bacteriology, 1974). However, additional

information indicates that 8% of girls experience a symptomatic UTI during childhood, and boys older than 2 years old are likely less likely to experience a UTI for the first time than 0.5% of the time (Larkins & Hewitt, 2018).

One of the most prevalent bacterial illnesses among children in developed nations is urinary tract infections. According to estimates, 1% of boys and 3-8% of girls receive a diagnosis of UTI. In the first year of life, guys are more likely to get a UTI (2.7% against 0.7% for girls). The first three months of life are when most infections in boys happen, but by the time they are in school, girls are more likely to get infections than boys. Uncircumcised males had a 10–12-fold higher risk of UTI, according to studies(World Health Organization, 2005).

Children's urinary tract infections in poor nations Numerous studies have been conducted, mostly in hospital settings and with malnourished children, to ascertain the prevalence of UTIs in poor nations. Numerous of this research have used a variety of urine specimens and minimal patient numbers. The frequency of UTIs varies widely, according to more recent research that collected SPA and mid-stream urine specimens. According to Nigerian hospital research, 9% of febrile children (temperature > 38°C) between the ages of one and sixty months had a UTI; the frequency was noticeably greater in girls. According to prospective studies conducted in South Africa, 26% of children aged 0–12 admitted to a teaching hospital in Durban and 17% of children aged 1 week to 8 attending a primary health care setting had bacteriuria on aseptic catheter specimens(World Health Organization, 2005)

2.4 Host defense

Urine is a great medium for the growth of bacteria; as soon as these microbes enter the bladder, they quickly proliferate and start to cause inflammation. The degree of a disease is determined by the integrity of the host defense, which includes cellular and humoral immunity, in addition to other elements on the uroepithelial surface(Ambite et al., 2015). However, there are two main ways that the bladder can partially withstand bacterial invasion: (i) mechanically, by regularly emptying the bladder, which lowers the likelihood of colonization; and (ii) through the protective action of antibacterial factors secreted by the lining epithelial cells. Moreover, there are immunological and biological defenses, which include the presence of secretory IgA from the bladder lining and the phagocytic activity of macrophages(Access, 2020). Biological defenses include variable urine osmolality and the mucoid layer of the Tamm-Horsfall protein.

Patients who experience immune system failure such as from long-term steroid therapy, immunocompromised tissues, or HIV infection are more vulnerable to recurring infection(Cvitkovi et al., 2022).

2.5 Risk factor

The incidence of UTI is influenced by factors such as gender, age, status of circumcision, general health, and immune status(Storme et al., 2019). Age, gender, and colonization appear to be host factors linked to increased bacterial adherence. Increased periurethral colonization appears to increase the risk of UTI, regardless of the age (e.g., infancy) or individual factors (e.g., increased colonization linked to recurrent UTI or uncircumcised infants)(Kaur & Kaur, 2021). Numerous risk factors that contribute to UTIs have been identified by extensive clinical evidence. Determining which children have UTIs in conjunction with the progressive decline in renal function is therefore crucial(Biondo, 2023).

To preserve renal function and stop further damage to the kidneys, it is critical to detect anomalies such as renal tract obstruction and hypoplastic or dysplastic kidneys. From birth, children with these malformations may have impaired kidney function. Moreover, other factors may not be linked to the urinary tract but still increase the risk of UTIs. These factors include poor personal hygiene, using bubble baths, wearing tight clothing, and others(Larkins & Hewitt, 2018).

Because broad-spectrum antibiotics (e.g., amoxicillin, cephalexin) disrupt the urinary tract's natural defense against colonization by pathogenic bacteria, they increase the risk of UTI in children receiving these medications, which are likely to alter GI and periurethral flora(Komagamine et al., 2022). An essential bladder defense against infection is compromised when bacteria in urine remain in the bladder for an extended period because of incomplete bladder emptying or infrequent urination. An estimated 0.2-0.4% of circumcised boys get UTIs, compared to 5–20 times higher rates in uncircumcised boys (Larkins & Hewitt, 2018).

Premature infants discharged from neonatal intensive care units, children with systemic or immunologic diseases, urinary tract abnormalities or renal calculi, neurogenic bladder, voiding dysfunction, or constipation, a family history of UTI or anomalies like reflux, and girls younger than five years old with a history of UTI are the children who are more likely to have bacteriuria or symptomatic UTI with subsequent renal damage(Studies, 2022).

2.6 Virulence factor

One of the most important steps in starting a tissue attack is bacterial adhesion to urinary tract cells. The uroepithelial lining is linked to the bacteria through bacterial surface fimbriae, which bind to specific host cell receptors. This adherent state makes it easier for toxins and invasion factors to act on the bacteria. The process of attachment may involve multiple types of fimbriae, which are identified by their structural characteristics and the oligosaccharide sequences in the host glycolipids or glycoproteins that they recognize. Type L fimbriae attach to mannosylated epitopes found in integrin molecules, secretory IgA, bladder cell uroplakins, and the Tamm–Horsfall glycoprotein(W. Lin et al., 2021). Renal sialylated proteins and lipids contain sialic acid epitopes that are bound by S-fimbriae. P-fimbriae identify Galα1–4Galβ-epitopes within the glycolipid globoseries. P-fimbriae expression is highly correlated with virulence; it is expressed in approximately 80% of strains that cause simple acute pyelonephritis but less than 20% of children with ABU. Patients with compromised resistance, such as those with urodynamic abnormalities, pregnancy, or genetic abnormalities, have lower frequencies of P-fimbriated strains(Kim et al., 2022).

Gram-negative bacteria produce LPS, an endotoxin. The toxic effects of LPS are caused by a lipid that is anchored in the outer membrane. Many of the symptoms of acute pyelonephritis, Gram-negative septicemia, and other serious Gram-negative infections, such as fever and acute phase response activation, are mimicked by lipid injection. Furthermore, LPS contains a repeating oligosaccharide that identifies the O antigen and an invariable oligosaccharide core. When LPS and MD2 bind to soluble or cell surface-associated CD14, TLR4 signaling is triggered. This mechanism is less significant at mucosal surfaces because the cell-bound CD14 receptor is frequently absent there, but it is crucial for innate immune activation during systemic infection (Kim et al., 2022).

Hemolysins are pore-forming, cytotoxic proteins that seep through cell membranes. Their ability to lyse erythrocytes serves as a common means of identification. Hemolysin production in *E. coli* causing acute pyelonephritis was first detected in the 1940s, and hemolysin has been found to occur more frequently in *E. coli* causing acute pyelonephritis than in fecal isolates, though not as frequently as in P-fimbriae(Kim et al., 2022).

2.7 Pathogenesis

The human urinary tract is a distinct area covered in transitional cells called mucosa. In contrast to the GI tract, its lining is usually impermeable and sterile(Cano et al., 2021). Because most normal voiding effectively washes out contaminating bacteria, successful pathogenic colonization of the bladder is unlikely unless there is a malfunction in the bladder's defense mechanisms. Consequently, a person is more susceptible to a UTI when the normal periurethral flora is disturbed. Bacterial entry into the bladder can occur from catheterization, voiding dysfunction, or turbulent flow during normal voiding. Rarely, during systemic bacteremia (sepsis), the urinary tract may become colonized; this typically occurs in infancy(Larkins & Hewitt, 2018). Furthermore, abnormal voiding that leaves behind urine or bacteria may not be adequately cleared mechanically by voiding, which can lead to a UTI(Pigrau & Escolà-vergé, 2020).

The degree of urethral colonization and bacterial growth in the urine is determined by intricate host-parasite interactions, which are central to the pathophysiology of urinary tract infections. Certain bacteria in the urinary tract can stick to the mucosal surface and resist the mechanical effects of urination, which can lead to infection. The virulence of the microorganism and the strength of the host defense system determine how serious the infection is. Pathogenicity in bacteria is determined by several specific, known virulence factors, such as the presence of fimbriae, K antigens, capsular polysaccharides, and glycoprotein mediators, as well as the LPS content of their outer cell membranes. The uropathogenic *E. coli* is one example of a virulent organism in this category; it can promote uroepithelial colonization, cause an inflammatory response, and affect the epithelial cytokine response. There will be a spike in white cell proliferation after the first bacteria-bladder mucosa interaction, which will result in more neutrophils and interleukins in the urine(Flores-mireles et al., 2015) .

Bacterial cells project stiff hair-like projections called fimbriae, which can bind to red blood cells and identify receptor sites. Particularly in the case of upper UTI, the presence of P-fimbriae in *E. coli* is a significant determinant of virulence. The mediators secreted by the capsule consist of polysaccharides and glycoproteins that improve adhesion to epithelial cells, decrease phagocytic activity, and undermine the effectiveness of antibiotics. The outer cell membrane's LPS can block complement activation and limit adhesion to macrophages. About 70% of pediatric cases of pyelonephritis are caused by the capsular K antigens, of which there

are five known types. Adherence to uroepithelial cells, the synthesis of colicin and hemolysin, the ability to absorb iron, and resistance to serum bactericidal activity are additional virulence factors. When a child has a UTI, their body responds by secreting more IL-6 and IL-8 intracellularly(Flores-mireles et al., 2015).

2.8 Clinical presentation

A UTI can present clinically in a variety of ways. When a child has so-called "asymptomatic" bacteriuria, they may only show mild symptoms like squatting or enuresis. On the other hand, a newborn who is systemically ill may show hypotension and lethargy. Even though clinical symptoms and signs alone are frequently used to guide treatment decisions for children, they may not always accurately indicate which patients will develop scarring and pyelonephritis. However, radiologic tests to confirm reflux or pyelonephritis can be costly, time-consuming, invasive, and unpopular with doctors and parents(Larkins & Hewitt, 2018).

Signs and symptoms of UTI in children include dysuria, frequency, dribbling/hesitancy, enuresis after successful toilet training, malodorous urine hematuria, squatting abdominal/suprapubic pain, fever, vomiting/diarrhea, and flank/back pain(Larkins & Hewitt, 2018).

A child who may have a urinary tract infection should not have hypertension, an abdominal or flank mass, a palpable bladder, neurologic deficits, abnormal genitalia, or an abnormal urinary stream found during their physical examination. This will aid the physician in identifying related illnesses. In the absence of bacteria, the presence of irritative urinary symptoms points to a non-UTI cause, such as vaginitis, urethritis, pinworms, or bubble bath use(Larkins & Hewitt, 2018).

The younger the child, the less specific the UTI symptoms and signs that present. Numerous prospective studies have proved that fever can be the only symptom of a urinary tract infection in newborns and early children(Boon et al., 2021). Moreover, the possibility of a UTI is not excluded in the presence of another cause of fever (upper respiratory tract infection, acute otitis media, acute gastroenteritis, etc.). According to one study, the prevalence of UTI in infants and young girls who had fever was 6% in the absence of any other cause of fever but only 3% in the presence of another cause. This result emphasizes how crucial it is to take urine cultures into account for any infant who is feverish but does not have a clear cause(Kaur & Kaur, 2021).

In addition, failure to thrive and conjugated hyperbilirubinemia are fewer common signs of UTI in infants. Fever, urinary symptoms (dysuria, urgency, frequency, incontinence, macroscopic hematuria), and abdominal pain are all signs of UTI in older children. During examination, there may be costovertebral angle tenderness as well as suprapubic tenderness. Occasionally, undiagnosed urinary tract infections in older children can cause symptoms such as hypertension, nephropathy, or failure to thrive(Cano et al., 2021).

2.9 Diagnosis

The clinician's primary goal for a young child with a UTI is early diagnosis, which allows for the identification of urinary tract abnormalities and the preservation of the renal system in the growing kidney. To document a UTI in a young child, a urine specimen for culture is required. In the non-toilet-trained child, urine specimens can be obtained through suprapubic bladder aspiration, urethral catheterization, or the bag technique. The use of a urethral catheter is a dependable method for culture. The urethral technique is simple, but in boys, the catheter must be carefully inserted past the curved portion of the posterior urethra to avoid mucosal trauma in that area. The low contamination rates of urine collected via suprapubic aspiration or catheterization prevent culture results from being misinterpreted. The cumulative experience with the suprapubic aspiration technique suggests that it is an effective and easy-to-use technique(Doern & Richardson, 2016).

There is little morbidity related to this procedure. Of 654 infants, 0.6% have been reported to have transient gross hematuria. Because these complications are uncommon, case reports on bowel perforation, anterior bladder wall hematoma, peritonitis, and anaerobic infection have been published. Due to contamination by periurethral flora, this technique has a high rate of false positive cultures even though a culture-negative urine bag sample is dependable. To differentiate contamination from UTI before starting antimicrobials, urethral catheterization or suprapubic aspiration is advised in response to a positive urine bag specimen culture or urinalysis. The best method for a toilet-trained child is to use a voided midstream urine specimen. The rate of contamination of urine specimens does not seem to be significantly affected by cleaning the urethral meatus before collecting the voided specimen(Doern & Richardson, 2016).

The urine specimen should ideally be processed right away after it is collected. If that is not possible, the specimen needs to be processed in a day and kept in a refrigerator at 4°C. The

most trustworthy way to diagnose a urinary tract infection is through a quantitative aerobic culture of a correctly collected urine specimen. Urine should be streaked onto agar plates using a calibrated loop that delivers 10 or 1µl to measure the number of bacteria present in infected urine. Once the plates have been incubated for 24 hours at 37°C, count the CFU. The CFU count is multiplied by the appropriate factor to estimate the number of organisms in 1 milliliter of the urine specimen(Harris & Fasolino, 2022).

A urinalysis could be done before a culture to look for results that would point to a UTI. Urine microscopy, using a counting chamber to count white blood cells and a Gram stain to categorize bacteria, is a sensitive and specific method of detecting urinary tract infections. Unfortunately, urine microscopy is not a technique that is available in clinical practice. Rather, urine sediment is inspected for bacteria and white blood cells using a light microscope as part of a "standard" microscopy procedure. When it comes to ruling out UTI in a young child, standard microscopy is not a dependable method (sensitivity of 60 to 70% for UTI when compared with culture). An 80% sensitive dipstick test for nitrite and leucocyte esterase can be done at the patient's bedside. According to a recent study, standard microscopy contributed little to the dipstick analysis for the preliminary diagnosis of UTI and was not reliable in predicting the results of cultures (Kaufman et al., 2019).

2.10 Prevention

A common-sense approach to prevention is advised by most authors. Good hygiene (including ‘front-to-back’ wiping after urination in girls), avoidance of constipation, circumcision, and avoidance of bubble baths, chemical irritants, and tight clothing might be recommended. UTI is a common pediatric problem with the potential to produce long-term morbidity(Williams & Craig, 2019). Young children presenting with fever without localizing signs should always be evaluated for UTI(Larkins & Hewitt, 2018).

Prevention of recurrent UTI focuses on detection, and correction, if possible, of urinary tract abnormalities such as posterior urethral valves or ureterovesical obstruction. Interventions that have been associated with a decrease in symptomatic UTI in children with a history of recurrent UTI include relief of constipation and voiding dysfunction(Pigrau & Escolà-vergé, 2020)(Schlager, 2001) . Voiding dysfunction related to incomplete bladder emptying, leading to increased bladder capacity and the presence of residual bladder urine, has been described in a group of girls with recurrent UTIs. Residual urine enhances the growth of colonizing bacteria

which may lead to a symptomatic infection. Establishing regular periods where the patient can empty her bladder (patient urinates, waits a few minutes, then urinates again) has been associated with a decrease in the number of symptomatic infections(Biondo, 2023).

2.11 Treatment

Most cases of uncomplicated UTI respond readily to outpatient antibiotic treatment without further sequelae. Hospitalization is suggested for symptomatic young infants (less than two months of age) and all children with clinical evidence of acute severe pyelonephritis (high fever, toxic appearance, severe flank pain). Initial antibiotic therapy should be based on age, clinical severity, location of infection, presence of structural abnormalities, and allergy to certain antibiotics. Treatment begins with a broad-spectrum antibiotic, but it may need to be changed based on the results of urine culture and sensitivity testing. Parenteral antibiotics may be used with daily follow-up until the patient is afebrile for 24 hours and completes 10-14 days of therapy with an oral antibiotic that is active against the infecting bacteria(Larkins & Hewitt, 2018). Antibiotics commonly used to treat UTIs consist of ampicillin, nitrofurantoin, co-trimoxazole (trimethoprim/sulfamethoxazole), and ciprofloxacin. Other choices include amoxicillin/clavulanate (Augmentin) or cephalosporins, such as cefixime (Suprax), cefpodoxime, cefprozil (Cefzil), or cephalexin (Keflex)(Shaaban et al., 2021). However, increasing rates of antibiotic resistance and high recurrence rates threaten to greatly enhance the burden that these common infections place on society(Nagajothi et al., 2015).

2.12 Antibiotics resistance

Appropriate treatment of UTIs has become challenging due to high resistance to commonly prescribed antibiotics (e.g., aminopenicillins) and the globally increasing prevalence of multidrug resistance organisms causing UTIs. Evidence has shown a high prevalence of multidrug resistance in gram-negative organisms the most common cause of UTI(Vazouras et al., 2020). Of particular concern are members of the family Enterobacteriaceae, including *E. coli* and *K. pneumoniae*, which have both acquired plasmids encoding extended-spectrum β-lactamases. These plasmids rapidly spread resistance to third-generation cephalosporins as well as other antibiotics. Other Enterobacteriaceae family members produce the class C β-lactamases (AmpC enzymes) that are active against cephamycin in addition to third-generation cephalosporins and are also resistant to β-lactamase inhibitors. The expression of AmpC

enzymes is also associated with carbapenem resistance in *K. pneumoniae* strains lacking a 42 kDa outer-membrane protein (Nagajothi et al., 2015).

Multidrug resistance is also common among Enterococci, as they are naturally resistant to trimethoprim, clindamycin, cephalosporins, and penicillin. Recently, *Enterococcus* spp. has developed high-level resistance to glycopeptides, including vancomycin, which is one of the last lines of defense against multidrug-resistant organisms (Nagajothi et al., 2015). Specifically, Enterococci evolved resistance to glycopeptides through the expression of vancomycin and teicoplanin A-type resistance (van) genes that encode the penicillin-binding proteins VanA, VanB, VanD, VanE, VanG and VanL. The mechanism of resistance for VanA, the most common penicillin-binding proteins expressed by enterococci, is to replace the cell wall precursor d-alanine–d-alanine with d-alanine–d-lactose, effectively reducing the binding affinity of vancomycin1. The troubling trend toward a high prevalence of multi-drug-resistant uropathogens has spurred the development of alternative control measures and treatment options (Nagajothi et al., 2015).

CHAPTER THREE:MATERIALS AND METHODS

3.1 Study area

The study was conducted in, Adama Public Health Research and Referral Laboratory Center, Adama City, East Shoa, Oromia. Adama City is located at longitude and latitude 8.54 ^{0}N 39.27^{0}E at an elevation of 1712 meters,99 km far southeast of Addis Ababa the capital City of Ethiopia. Adama City is found between the base of an escarpment to the west and the Great Rift Valley to the east. The average annual temperature of the city is 20.7 ^{0}C or 69.3 ^{0}F and about 371 mm or 14.6 inches of precipitation falls annually. The population of Adama City is 240,779 of which 121,254 are males and 119,525 are females (CSA, 2023).

3.2 Sample size determination

The sample size (n) was calculated according to the single population proportion formula n = z^2 * p * (1 - p) / e^2(Sangeda et al., 2021)

where:

z = 1.96 for a confidence level (α) of 95%,

p = prevalence and

e = margin of error

According to a study conducted in Ethiopia, the prevalence of UTI in children was 16.7(Fenta et al., 2020), thus making p = 0.167 and taking e = 0.05.

$$n=\frac{Z^2 p(1-p)}{e^2}$$

$n=1.96^2\times0.167(1-0.167)/ (0.05)^2$

$n=3.8416\times0.139111/0.0025$

$n=213.76\approx 214$

Accordingly, the sample size was obtained to 213.76 and when it rounded, it was 214.

3.3 Socio-demographic characteristics

3.3.1 Inclusion criteria

Children under the age of 15 who reported symptoms and signs suggestive of a urinary tract infection (symptomatic) and subjects who did not suggest such complaints (asymptomatic) were the inclusion criteria. Individuals who showed two or more symptoms, such as dysuria, urgency, frequency, incontinence, and fever (38 ^{0}C) were considered symptomatic(Smelov et al., 2016).

3.3.2 Exclusion criteria

Subjects who had taken antibiotics within the last 48 hours and children older than 15 years were excluded.

3.4 Samples and sampling techniques

The study was conducted during the period from March to June 2024 on children under 15 years attending Adama Hospital Medical College and Geda Health Center. Ten urine samples were collected from Geda Health Center and the rest 204 samples were collected from AHMC locally known as Hailemariam Hospital. The attending physician and laboratory expert informed each child and the family how to collect a "clean-catch" mid-stream urine specimen.

They were further informed to void the first stream of urine but to withhold the mid-stream urine and then void it in the sterile plastic cup container. Accordingly, about 10 to 20 ml urine specimens was collected in a sterile screw-capped, wide-necked plastic cup from each child. The bottle was labeled with a unique sample code, date, and time of collection. Immediately, it was delivered to the bacteriology department of Adama Public Health Research and Referral Laboratory by using an ice box. The specimens were processed within 2 hours of collection. But in case of delays in processing the specimen, the urine specimens were refrigerated at 4°C till it was processed.

3.5 Isolation of microorganisms

The procedure of urine culture and subculture were performed following the standard operation procedure (SOP) according to (Karah et al., 2020).The urine was collected from patients

suspected of UTIs, then samples were sent to clinical microbiology laboratory within 2 h. Ten microliters of urine with calibrated loop was streaked into Blood agar enriched medium (OXOID) and transferred to MacConkey agar medium. They were then incubated at 37°C for 24-48 hrs. The number of colonies was counted and calculated to concentration with unit of colony-forming units per milliliter(Mitiku et al., 2018).

The following day, a total colony count was carried out on blood agar along with an examination of the bacterial growth on the corresponding media hemolytic activity, pigment, transparency, surface, consistency, swarming, density, and color(Mitiku et al., 2018). A single organism's colony growth of greater than 10^5/ml was considered significant. Multiple growth samples were regarded as contaminated, while samples with a colony count of fewer than 10^5/ml were regarded as being free of infection(Smelov et al., 2016). Culture-positive samples do not include samples with insignificant growth or mixed growth of two or more pathogens(Sohail et al., 2023).

3.6 Identification of microorganisms

Pure isolates of the bacterial pathogen were preliminarily characterized by colony characteristics and Gram-stain. Gram-positive bacteria were further, characterized by using catalase, bile esculin test (for enterococcus identification), and mannitol salt agar for identification of *S. aureus*. Gram-negative bacteria were identified by biochemical tests like the urease test, SIM (sulfide indole motility) test, citrate utilization test, triple sugar iron test, lysine iron agar test, and oxidase test(Bitew et al., 2022). For further identification of bacterial pathogens, MALDI-TOF MS was also used.

3.6.1 Gram–staining and cell morphology

A smear of pure isolate bacteriuria was prepared on a clean slide, allowed to air dry, and then heated to perform the gram staining procedure. After soaking in crystal violet for a minute, the heat-fixed smear was rinsed with distilled water for three seconds. After rinsing the slide with distilled water for three seconds, the slide was submerged in iodine solution for a minute. Following the rinse, the slide was gently cleaned for three seconds with distilled water and the smear was decolorized for twenty seconds with 96% ethanol alcohol. After blotting the smear to air dry, safranin was used as a counterstain. The oil immersion objective lens was used to view the air-dried smear. Gram-positive bacteriuria stained blue/purple and gram-negative

bacteriuria stained red/pink after the gram-staining process was finished(Stanley & Okpara, 2015).

3.6.2 Biochemical tests

3.6.2.1 Triple sugar iron test

An isolated colony was inoculated into triple sugar iron slant by first withdrawing the needle, streaking the slant's surface, and then stabbing the butt down. After 18 to 24 hours of incubation at 37°C, the result was read. Lactose and sucrose have not been fermented, but glucose (yellow butt, red slant) and acid butt, alkaline slant have. Yellow butt, yellow slant, or acid butt, acid slant Sucrose, and/or lactose have undergone fermentation. Alkaline butt, alkaline slant (or red butt, red slant): no fermentation has occurred with glucose, lactose, or sucrose (Gopireddy, 2011).

3.6.2.2 Bile esculin test

The test organism was inoculated using a sterile, straight inoculating needle. It was then incubated at 37˚C for 18 to 24 hours, during which time its color was monitored for changes. The agar turns black, from deep brown signifying a positive test for *Enterococcus* spp., when it combines with ferric citrate in the medium to form a phenolic iron complex, which was produced by bacteria that can hydrolyze esculin in the presence of bile. If the test results are negative, the color won't change (Al-Joda & Jasim, 2021).

3.6.2.3 Catalase test

The purpose of this test is to determine which organisms make the catalase enzyme. H_2O_2 was detoxified by this enzyme, which separates it into oxygen gas and water. This test shows that catalase is present. Catalase is an enzyme that releases oxygen from H_2O_2. The sterile slide with a loopful of the organism was given a drop of 3% H_2O_2 solution. A successful outcome was indicated by foaming or bubbles (Stephan & Faloutsos, 2013).

3.6.2.4 Oxidase test

A small amount of oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) was soaked onto a piece of filter paper. Next, a swab was used to smear a colony of the test organism onto the soaked filter paper. The phenylenediamine in the reagent was oxidized to a deep purple

color if the organism can produce oxidase. In ten seconds, the color changes, indicating a successful outcome (Al-Joda & Jasim, 2021).

3.6.2.5 Lysine iron agar test

Using a straight inoculating needle, a well-isolated colony was removed from a pure culture plate and picked. To inoculate the Lysine Iron Agar Slant, stab the middle of the tube down to the bottom, then streak the slant. The tube was incubated for 18 to 24 hours at 37°C before being examined. The findings were interpreted. Decarboxylation of lysine: Positive (+): H_2S blackening or not, purple slant or butt Negative (-): yellow butt / purple slant Deamination of lysine: Positive (+): yellow butt / red slant No red slant in the negative (-)(Fenta et al., 2020).

3.6.2.6 SIM test

Different Gram-negative enteric bacteria can be identified from one another using Sulfide-Indole-Motility (SIM) media. These media were used to measure the bacteria's capacity to create hydrogen gas (H_2S), convert tryptophan into indole, and enter the medium through flagella. The test organism was cultured at 37°C for 18 to 24 hours after being stabbed two-thirds of the way into the medium. Tubes were checked for motility and H_2S production after incubation. Three to four drops of Kovac's Reagent were put into each tube, and H_2S and motility were recorded. Non-motile organisms were grown only along the line of inoculation, whereas diffuse growth/turbidity extending from the inoculating stab line indicated a positive motility test. Blackening of the medium in the places where microbial growth has taken place indicated the generation of H_2S. If there is no color change following the addition of Kovac's Reagent, the test was considered negative. Indole production was observed as the formation of a pink or red ring color (Principle et al., 2022).

3.6.2.7 Urease test

This was used to determine whether organism’s bacteria that make urease can hydrolyze urea to produce carbon dioxide and ammonia. Its main purpose is to differentiate Enterobacteriaceae other than urease-positive protease. The slant turns pink instead of bright orange when the bacteria releases urease. The agar slant and butt remain bright orange (the medium maintains its original color) if the bacterium does not make urease (Dimri et al., 2020).

3.6.2.8 Citrate test

To distinguish between species that can use citrate as a carbon source, this test was frequently performed. A bijou bottle containing Simmon's citrate agar medium was produced and left to set slanting. The test organism was inoculated into the slant medium using a sterile, straight inoculating needle. It was then incubated at 37˚C for 18 to 24 hours, during which time its color was monitored for changes. The medium's bright blue color indicated a successful citrate test (Dimri et al., 2020).

3.6.3 Identification of *S. aures*

For identification of *staphylococcus* spp. mannitol salt agar was used. Streak a plate of mannitol salt agar with appropriate culture using the quadrant streak plate method place a pure colony on nutrient agar and incubated at 370c for 24h. The result was interpreted: yellow color colonies on mannitol salt agar were *S. aureus*.

3.6.4 Identification of isolates using MALDI TOF

The isolated bacteria were investigated using the MALDI-TOF MS which analyzes the protein or peptide constituents of a sample microorganism and enables identification of the microorganisms up to genus and species level(L'Ollivier et al., 2013). MALDI TOF-MS identification of the bacterial isolates was conducted at the National Animal Health Diagnostic and Investigation Center found in Sebeta.

3.6.4.1 Preparation of pure culture

Pure culture preparation was conducted at Adama Public Health Research and Referral Laboratory Center. Each selected bacteria colony after the biochemical test was carefully picked and purified in nutrient agar and incubated at 37 ℃ for 24. The overnight-grown bacteria were carefully covered with Aluminum foil and transported to Sebeta using an ice-free glass box.

3.6.4.2 Characterization process of the isolates

The purpose of the characterization process of isolates using MALDI-TOF MS is to identify and classify microorganisms based on their protein composition. This process is crucial in

microbiology and clinical diagnostics for accurate and rapid identification of bacteria, and other microorganisms.

The extended direct transfer (eDT) procedure as per manufacturer's instruction was used to analyze the bacteria(Daltonics, 2012). The extended direct transfer method uses 70% formic acid to lyse the cell membrane of the bacteria for simple extraction of the microbial proteins or peptides and makes for easier characterization of the samples. The target plate which contains 94 wells was cleaned thoroughly and well dried before starting the procedure.

Then a distinct colony from the overnight grown bacteria was smeared directly on the wells as a thin film and left to dry. Then the spot was overlaid with 1µl of 70% (v/v) formic acid and allowed to dry at room temperature. Then immediately the spot was overlaid with 1µl of α-cyano-4 hydroxycinnamic acid solution and allowed to dry at room temperature. Then after the samples were dried the target plate was placed inside the MALDI biotyper and analyzed. The samples are then to be adequately identified at the species level when the logarithmic score value 3.00- 2.00 was with high confidence identification and 1.99-1.70 with low confidence identification. However, when the logarithmic score value was 1.69-0.00 there was no microorganism identified. (Davies et al., 2018).

3.7 Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to conduct the antimicrobial susceptibility test on Muller Hinton agar following CLSI standards.(Fenta et al., 2020). After obtaining a pure culture, a loopful of bacteria was collected from a colony and put into a tube with 5 ml of sterile normal saline. The bacteria were then gently stirred until a uniform solution was created. To standardize the inoculum size, the turbidity of the suspension was then adjusted to the optical density of a McFarland 0.5 tube (0.14-0.15 nm) measured at 500 nm absorbance. After being dipped into the suspension, a sterile cotton swab was gently rotated against the tube's surface to remove any excess. Next, the Mueller-Hinton agar (Oxoid) surface was evenly coated with the bacteria suspension using the swab. For three to five minutes, the infected plates were allowed to dry at room temperature(Mitiku et al., 2018).

On the inoculation plates, the appropriate antimicrobial discs were equally dispersed using a sterile needle and incubation was carried out at 37^{o}C for 18-24 hours. A metal caliper was used to measure the diameter of the zone of inhibition surrounding the disc to the closest millimeter.

Based on this measurement, the isolate was categorized as sensitive (S), intermediate (I), and resistant (R) following the CLSI guidelines. To repeatedly assess the effectiveness of the antibiotics, *E. Coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used control strains. Different antibiotics are used to treat UTI caused by gram-positive and gram-negative bacteria because these bacteria have different cell wall structures and characteristics that make them susceptible to specific antibiotics

For gram-negative isolates, the antibiotic discs were: amoxicillin/clavulanic acid (30µg); ciprofloxacin (5µg); nitro-furantoin (300µg); ampicillin sulbactam(10µg); amikacin (30µg); meropenem (10µg); piperacillin-tazobactam (100/10µg); cefazolin (30µg); trimethoprim-sulfamethoxazole (1.25/23.75µg), cefotaxime (30µg); ceftazidime(30µg), cephalosporin(30µg), nalidixic acid(30 µg), tetracycline(30 µg) gentamicin(10µg), cefoxitin(30 µg) and oxacillin(30 µg).

Antimicrobial disks used for gram-positive isolates were: penicillin (10 units); nitrofurantoin(300µg); vancomycin (30µg); trimethoprim-sulfamethoxazole (1.25/23.75 µg); ciprofloxacin (5µg); Ampicillin sulbactam (10µg), tetracycline(30 µg) gentamicin(10µg), cefoxitin(30 µg) and oxacillin(30 µg)(Bitew et al., 2022a).

3.8 Ethical considerations

The study was approved by the Department of Applied Biology, School of Applied Natural Sciences, Adama Science and Technology University, and the ethical clearance was obtained from Adama Science and Technology University Research and Center of Excellence Office University Ethical Review Board certificate reference number RECSoANS/BIO/20/2024. Confidentiality of any information related to the patient and clinical history was preserved. However, the attendant physicians had access to partial medical records based on the responsibility associated with the patient's treatment.

3.9 Data analysis

The data obtained from this study were entered into an MS Excel sheet and analyzed using Statistical Package for Social Science (SPSS, version 27). Descriptive data were expressed as frequency percentages and relationships between variables were analyzed by using the chi-square test wherever necessary at a 5% level of significance. A p-value < .05 was considered

as statistically significant. The results were presented through texts, graphs, tables, and pictures.

CHAPTER FOUR: RESULTS

4.1 Socio-demographic characteristics of participants

Out of the total of 214 urine specimens processed, 127/214(59.3%) and 87/214(40.7%) were females and males respectively. The samples that came from Geda Health Center 9/10 were females and 1/10 were male with a 9.5 mean age. 204 urine specimens that came from Adama Hospital Medical College 118/204(57.8%) and 86/204(42.2%) were female and males respectively with 9.78 mean age. All urine specimens from Geda Health Center were negative results because 9/10(90%) were insignificant and 1/10(1%) was a negative culture result when counted on blood the agar also.

For the Adama Hospital Medical Collage samples counted on the blood agar 112/204(54.9) were negative, 67/204(32.8) were insignificant and only 25/204(12.3%) were found significant and most bacteriuria were found at age 6-10(Table 4.1). In the overall urine specimens processed 113/214(52.8%) were found negative,76/214(35.5%) were found insignificant and 25/214(11.7%) were found positive which showed the prevalence of UTI of children in this study was 11.7%.

Table 4.1 Relation of culture results with age and gender.

age			culture result			Total
			insignificant	negative	significant	
0 - 5	sex	F	1	4	0	5
		M	4	9	1	14
	Total		5	13	1	19
6 - 11	sex	F	28	31	12	71
		M	9	34	3	46
	Total		37	65	15	117
11-15	sex	F	27	18	6	51
		M	7	17	3	27
	Total		34	35	9	78
Total	sex	F	56	53	18	127
		M	20	60	7	87
	Total		76	113	25	214

From a total of 214 urine specimens 209/214(97.7%) were asymptomatic and 5/214(2.3%) were symptomatic for UTI. Also, from this study 124/127 females were asymptomatic and 3/127 were symptomatic while 85/87 males were asymptomatic and 2/87 were symptomatic (Table 4.2).20 urine specimens collected from asymptomatic children have $\geq 10^5$ growth which means significant.

Table 4.2 Asymptomatic and symptomatic children with gender and age

sex			age			Total
			0 - 5	6-10	11-15	
F	statues of patient	asymptomatic	5	71	48	124
		symptomatic	0	0	3	3
	Total		5	71	51	127
M	statues of patient	asymptomatic	14	45	26	85
		symptomatic	0	1	1	2
	Total		14	46	27	87
Total	statues of patient	asymptomatic	19	116	74	209
		symptomatic	0	1	4	5
	Total		19	117	78	214

The overall prevalence of urinary tract infection in children in this study was 25/214 (11.7%). Age and sex did not have a statistically significant correlation with UTI($p>0.05$) which means UTI was not dependent on age and sex (Table 4.3).

Table 4.3 Prevalence of bacteriuria vs host factor (gender and age)

result		Value	df	Asymptotic Significance (2-sided)
positive	Pearson Chi-Square	10.828[a]	7	.146
	Likelihood Ratio	12.449	7	.087
	N of Valid Cases	25		
Total	Pearson Chi-Square	10.828[a]	7	.146
	Likelihood Ratio	12.449	7	.087
	N of Valid Cases	25		

A total of 25 bacteria were isolated from both asymptomatic and symptomatic males and females at different ages (Table 4.4). From the asymptomatic group, the prevalence of bacteria was 14/20(70%) isolated at ages between 6-10 years 12 from females and 2 from males. On the other hand, from the symptomatic group more prevalent bacteria 4/5 (80%) were isolated at age between11-15 years which were 3 from females and 1 from male. Totally 18/25 bacteria were isolated from females and 7/25 bacteria was isolated from male' children 20 from an asymptomatic and 5 from a symptomatic group.

Table 4.4 Prevalence of bacteria related to age, sex, and status of children

statues of patient			sex		Total
			F	M	
asymptomatic	age (Binned)	0 - 5	0	1	1
		6-10	12	2	14
		11-15	3	2	5
	Total		15	5	20
symptomatic	age (Binned)	6-10	0	1	1
		11-15	3	1	4
	Total		3	2	5
Total	age (Binned)	0 - 5	0	1	1
		6-10	12	3	15
		11-15	6	3	9
	Total		18	7	25

4.2 Isolation and identification of bacteriuria

4.2.1Gram staining

Out of 25 bacteria isolated from the urine specimens, more than half (56%) were gram-negative rods followed by gram-positive cocci 7/25(28%) and gram-positive short rods 4/25(16%) (Figure 4.1 and Table 4.5).

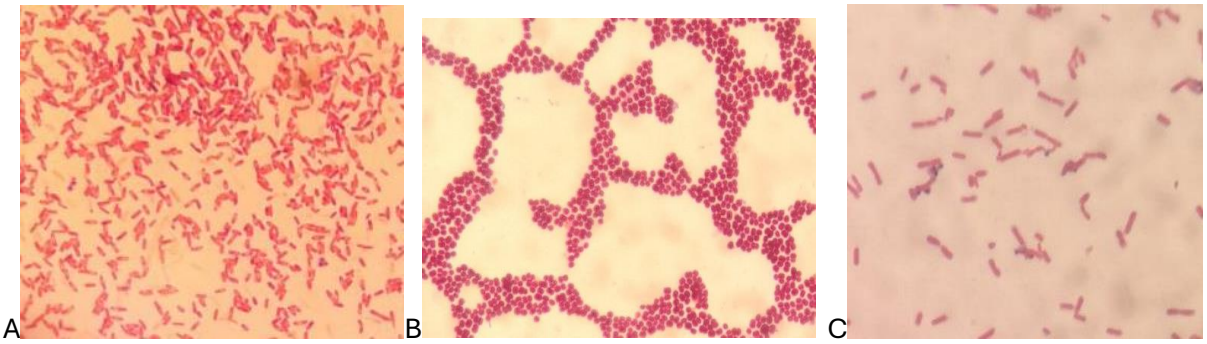


Figure 4.1 A: Gram-negative rod B: Gram-positive cocci C: Gram-positive short rod

Table 4.5 Gram staining result of the isolated bacteriuria

Sample code	color	shape	result
UBI 7	purple	cocci	GPC
UBI 13	pink	rod	GNR
UBI 17	purple	short rod	GPSR
UBI 21	purple	cocci	GPC
UBI 45	purple	cocci	GPC
UBI 48	purple	short rod	GPSR
UBI 57	purple	short rod	GPSR
UBI 60	purple	short rod	GPSR
UBI 62	purple	cocci	GPC
UBI 86	purple	cocci	GPC
UBI 94	pink	rod	GNR
UBI 102	pink	rod	GNR
UBI 111	purple	cocci	GPC
UBI 115	purple	cocci	GPC
UBI 125	pink	rod	GNR
UBI 135	pink	rod	GNR
UBI 136	pink	rod	GNR
UBI 138	pink	rod	GNR
UBI 138	pink	rod	GNR
UBI 139	pink	rod	GNR
UBI 141	pink	rod	GNR
UBI 143	pink	rod	GNR
UBI 145	pink	rod	GNR
UBI 150	pink	rod	GNR
UBI 182	pink	rod	GNR

UBI=urine bacterial isolate GNR=gram negative rod GPC=gram positive cocci GPSR=gram positive short rod

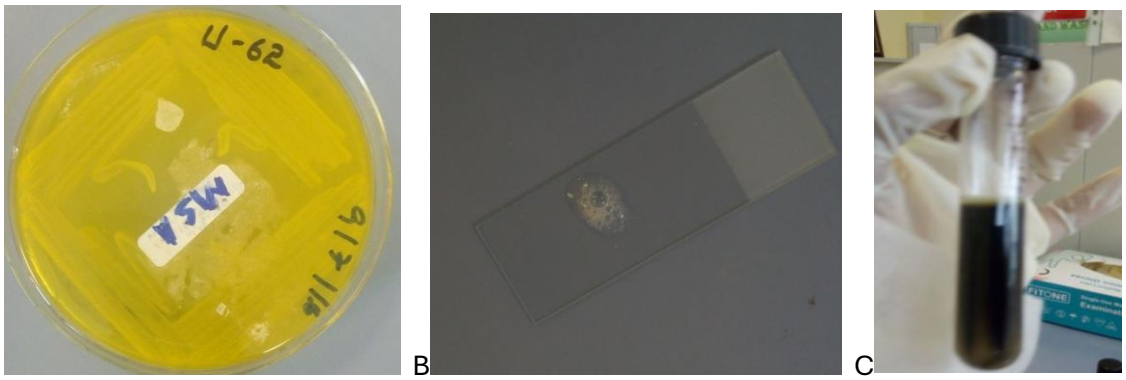
4.2.2 Biochemical test and mannitol salt agar

Following the results of the gram staining processes the gram-positive bacteria were further characterized by biochemical test using catalase, bile esculin test (for enterococcus identification), and mannitol salt agar for the identification of *S. aureus* (Figure 4.2 and Table 4.6). Likewise, gram-negative bacteria were identified by biochemical tests as follows: the urease test, SIM (sulfide indole motility) test, citrate utilization test, triple sugar iron test, lysine iron agar test, and oxidase test (Figure 4.3 and Table 4.7).

Table 4.6 Biochemical characterization of gram-positive bacteria (Koneman et al.,2017)

Sample code	catalase	MSA	Bile esculin	Identified bacteriuria
UBI 7	+	+		*S. aurues*
UBI 17	+			*Corynebacterium spp.*
UBI 21	+	-		*CNS*
UBI 45	-	+	+	*Enterococcus spp.*
UBI 48	+			*Corynebacterium spp.*
UBI 57	+			*Corynebacterium spp.*
UBI 62	+	+		*S. aurues*
UBI 86	+	+		*S. aurues*
UBI 111	-	+	+	*Enterococcus spp.*
UBI 115	+	-		*S. saprophytices*

UBI=urine bacterial isolate CNS=coagulase negative staphylococcus MSA=mannitol salt agar



A B C

Figuer 4.2 A:Mannitol salt agar positive B:Catalase potive C:Bile esculin postive

Table 4.7 Biochemical identification for gram-negative bacteria (Koneman et al.,2017)

Sample code	citrate	TSI	LIA	urease	H_2S	indole	Motility	oxidase	gas	Identified bacteriuria
UBI 13	-	K/A	+	-	-	+	+	-	-	*E. coli*
UBI 94	-	K/A	+	-	-	+	+	-	-	*E. coli*
UBI 102	-	A/A	+	-	-	+	+	-	-	*E. coli*
UBI 125	-	A/A	+	-	-	+	+	-	-	*E. coli*
UBI 135	-	K/A	+	-	-	+	+	-	-	*E. coli*
UBI 136	-	K/A	+	-	-	+	+	-	-	*E. coli*
UBI 138	-	K/A	+	-	-	+	+	-	-	*E. coli*
UBI 139	+	A/A	-	+	-	-	+	-	-	*E. cloace*
UBI 141	-	K/A	-	+	-	+	+	-	-	*P. vulgaris*
UBI 142	-	A/A	+	-	-	+	+	-	-	*E. coli*
UBI 143	-	A/A	+	-	-	+	+	-	+	*E. coli*
UBI 145	-	A/A	+	-	-	+	+	-	-	*E. coli*
UBI 150	-	A/A	-	-	+	+	+	-	-	*P. vulgaris*
UBI 182	+	A/A	+	+	-	+	-	-	-	*K. oxtyoca*

UBI=urine bacterial isolate +=positive -=negative A/A=sucrose or lactose fermented but not glucose K/A=glucose fermented but not sucrose or lactose

From 25 isolated bacteria categorized in 9 spp.,10/25(40%) were identified as *E. coli* and the dominant bacteria among all of them. From gram-positive bacteria *Corynebacterium* spp. were dominant 4/25(16%) (Figure 4.3). From the identified bacteria CNS 1/25 from male, *Corynebacterium* spp. 4/25 from females, *E. coli* 7 from females and 3 from males in total 10/25 were identified. *Enterococcus* spp. 2/25 from females, *P. vulgaris* 2/25 males, *S. aurues* 2 from males and 1 from females total 3/25, *E. cloace* 1/25 from female, *S. saprophytices* 1/25 from female and *K. oxytoca* 1/25 from female were identified (Table 4.8).

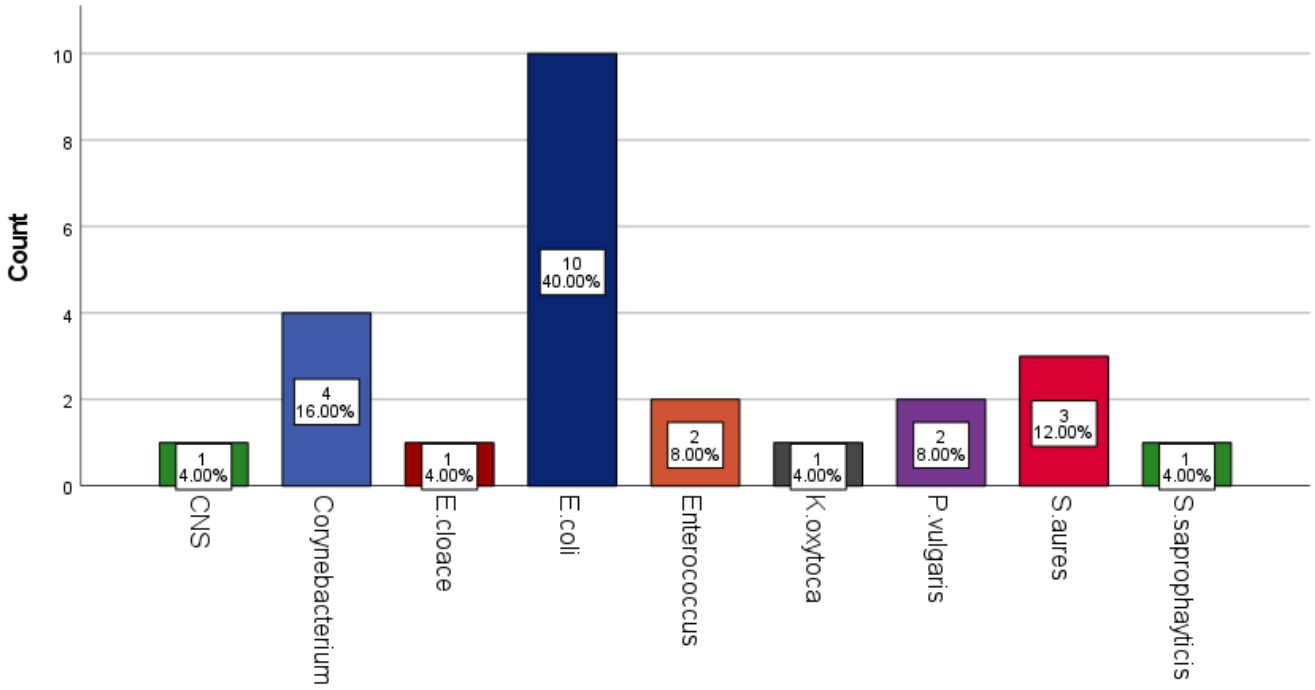


Figure 4.3: The etiology and frequency of identified bacteria

Table 4.8 The etiology and frequency of identified bacteria based on sex and age

identified bacteria			sex		Total
			F	M	
CNS	age (Binned)	11-15		1	1
	Total			1	1
Corynebacterium	age (Binned)	6-10	4		4
	Total		4		4
E. coli	age (Binned)	6-10	4	3	7
		11-15	3	0	3
	Total		7	3	10
E. cloace	age (Binned)	11-15	1		1
	Total		1		1
Enterococcus	age (Binned)	6-10	2		2
	Total		2		2
K. oxytoca	age (Binned)	6-10	1		1
	Total		1		1

P. vulgaris	age (Binned)	11-15		2	2
	Total			2	2
S. aures	age (Binned)	0 - 5	0	1	1
		6-10	1	0	1
		11-15	1	0	1
	Total		2	1	3
S. saprophayticis	age (Binned)	11-15	1		1
	Total		1		1
Total	age (Binned)	0 - 5	0	1	1
		6-10	12	3	15
		11-15	6	3	9
	Total		18	7	25

From the asymptomatic group 20 bacteria were isolated and among that 8 were *E. coil* from the symptomatic group 5 bacteria were isolated 2 E. *coli* were identified (Figure 4.4).

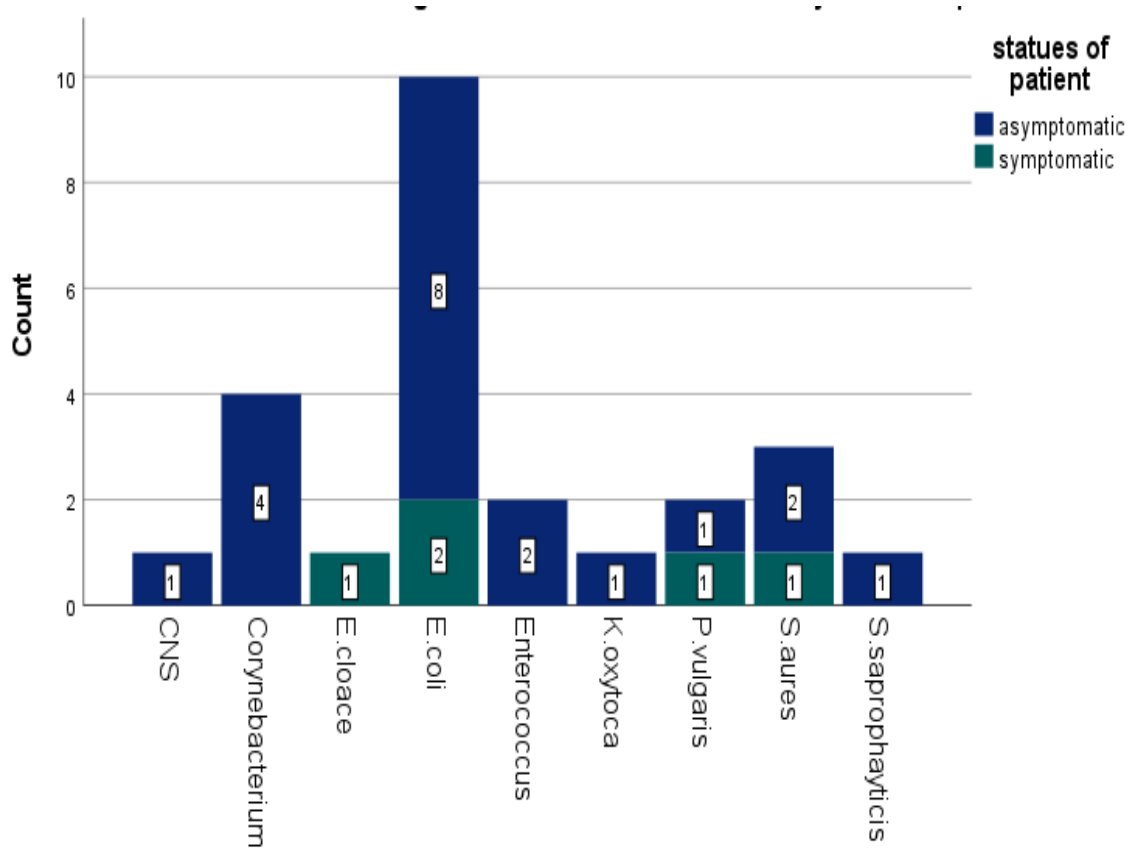


Figure 4.4: The etiology and frequency of identified bacteria based on statues of children

4.3 Identification of isolates using MALDI TOF MS

Of 8 isolates were subjected to MALDI TOF MS analysis with sample Id 16142(UBI 86),16143(UBI 187),16144(UBI 115),16145(UBI 102),16146(UBU 111),16147(UBI 45),16148(UBI 139) and 16149(UBI 125). According to the identification list suggested by the MALDI biotyper 3.0 software (Bruker), these isolates were identified as 16143(UBI 182) and 16148(UBI 139) *K. pneumoniae* with 2.46 and 2.14 score values.16145(urine UBI 102) and 16149(UBI 125) were identified as *E. coli* with 2.25 and 2.41 score values these 4 isolates' score values indicate high confidence identification. The last one that coded with 16144(UBI 115) was identified as *S. hemolytices* with a 1.97 score value indicating low confidence identification. However, 3 isolates 16142(UBI 86),16146(UBI 111) and 16147(UBI 45) has 0.00,0.00 and 1.62 score values respectively which means no organism identification is possible (Table 4.9).

Bacterial isolates with sample Id 16145 (UBI 102) and 16149 (UBI 125) were identified as *E. coli* in biochemical and MALDI TOF MS analysis. Whereas bacterial isolate with sample Id 16143(UBI 182) and 16144 (UBI 115) identified as *S. saprophytices* and *K. oxtyoca* respectively in biochemical test but when identified by MALDI TOF MS analysis it varies by spp. *S. saprophytices* was identified as S. epidermis and *K. oxtyoca* was identified as K. pneumoniae. Bacteria isolates named by sample Id 16148 (UBI 139) was identified as *E. cloace* but in MALDI TOF MS analysis it was identified as *K. pneumoniae*. Bacterial isolate with sample Id 16142 (UBI 86),16146 (UBI 111) and 16147 (UBI 45) identified bacteriuria by biochemical test but didn't identify by MALDI TOF MS analysis due to the score value was described as no organism identification possible.

Table 4.9 Rate classification as determined by Bruker MALDI TOF MS biotyper

Sample code	**Biochemical identification**	**Sample ID**	**Organism (best match)**	**Score value**	**Organism (second best match)**
UBI 86	*S. aures*	16142(standard)	No peak found	0.00	No peaks found

UBI 187	*K. oxytca*	16143(standard)	*K. pneumoniae*	2.46	*K. pneumoniae*
UBI 115	*S. saprophytices*	16144(standard)	*S. hemolytices*	1.97	*S. hemolytices*
UBI 102	*E. coli*	16145(standard)	*E. coli*	2.25	*E. coli*
UBI 111	*Enterococcus spp.*	16146(standard)	No peak found	0.00	No peak found
UBI 45	*Enterococcus spp.*	16147(standard)	No organism identification possible	1.62	No organism identification possible
UBI 139	*E. cloace*	16148(standard)	*K. pneumoniae*	2.12	*K. pneumoniae*
UBI 125	*E. coli*	16149(standard)	*E. coli*	2.41	*E. coli*

4.4 Antimicrobial Susceptibility Testing

4.4.1 Antimicrobial susceptibility testing from gram-positive bacteria

From identified gram-positive bacteria only 3 spp. were done for antimicrobial susceptibility test. Antimicrobial susceptibility test was not done for two gram-positive bacteria i.e., *Corynebacterium* spp. and CNS because it needs MIC other than the Kirby-Bauer disc diffusion method due to *Corynebacterium* spp. and CNS can have variable growth rates and may not produce clear zones of inhibition in the Kirby-Bauer test, making it difficult to interpret results accurately(N.a., 2019). Out of 6 bacteria 3 were *S. aures* and tested against cefoxitin, gentamicin, tetracycline, penicillin, sulfa/trimeth, nitrofurantoin, and ciprofloxacin. From that *S. aures* were 100% sensitive to cefoxitin, gentamicin, nitrofurantoin, and ciprofloxacin also 66.6% resistance to sulfa/trimeth. Out of 6 bacteriuria 2 were *Enterococcus* spp. and tested against vancomycin, ciprofloxacin, and ampicillin sulbactam, and *Enterococcus* spp. was 100% sensitive to vancomycin and resistant to ciprofloxacin, ampicillin sulbactam. *S. sapropahtices* bacteria was sensitive against tetracycline, ciprofloxacin, and oxacillin and resistance to penicillin and gentamicin. Overall gram-positive bacteria were more sensitive to ciprofloxacin (83.3%) and resistant to sulfa/trimeth (66.6%) followed by tetracycline (50%) (Figure 4.5 and Table 4.10).

Table 4.10Antimicrobial susceptibility pattern of Gram-positive bacteriuria

Bacteriuria species	Antimicrobial agents tested									
	Pattern	FOX	NOR	T	GM	PG	CIP	SAM	VAN	OX	TS
S. aureus (n=3)	R	0	0	2(66.6%)	0	1(33.3%)	0	ND	ND	ND	2(66.6%)
	I	0	0	0	0	0	0	ND	ND	ND	1(33.3%)
	S	3(100%)	3(100%)	1(33.3%)	3(100%)	2(66.6%)	3(100%)	ND	ND	ND	0
S. saprophytices (n=1)	R	ND	ND	0	1(100%)	1(100%)	0	ND	ND	0	ND
	I	ND	ND	0	0	0	0	ND	ND	0	ND
	S	ND	ND	1(100%)	0	0	1(100%)	ND	ND	1(100%)	ND
Enterococcus (n=2)	R	ND	ND	0	ND	ND	1(50%)	1(50%)	0	ND	ND
	I	ND	ND	1(50%)	ND	ND	0	0	0	ND	ND
	S	ND	ND	1(50%)	ND	ND	1(50%)	1(50%)	2(100%)	ND	ND
Total (n=6)	R	0	0	3(50%)	1(33.3%)	2(50%)	1(16.6%)	1(50%)	0	0	2(66.6%)
	I	0	0	1(16.6%)	0	0	0	0	0	0	1(33.3%)
	S	3(100%)	3(100%)	2(33.3%)	3(66.6%)	2(50%)	5(83.3)	1(50%)	2(100%)	1(100%)	0

CIP=Ciprofloxacin FOX=cefoxitin GM=Gentamicin PG=Penicillin TS=Sulfa/Trimeth NOR=nitrofurantoin OX=oxacillin T=Tetracycline VAN=vancomycin and SAM=ampicillin sulbactam R=resistant I=intermediate S=susceptible ND=not done n=number of strains

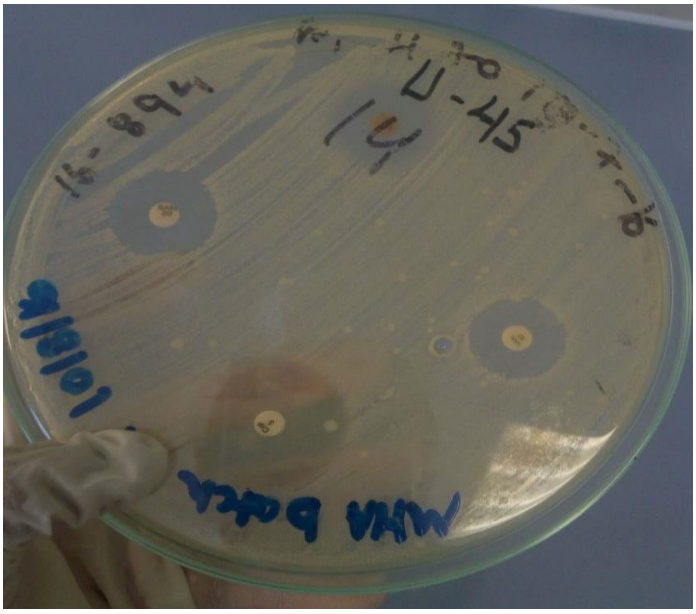


Figure 4.5 Antimicrobial susceptibility test for *Enterococcus*

4.4.2 Antimicrobial susceptibility testing from gram-negative bacteria

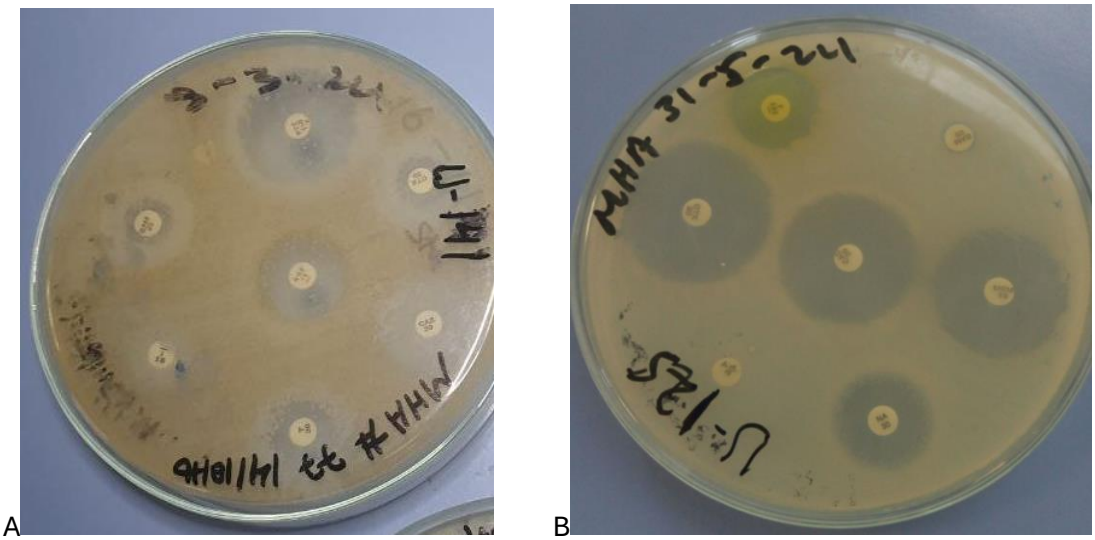
All 14 identified gram-negative bacteria done antimicrobial susceptibility test. *E. coli* was the dominant bacteria constituting 40% of all identified bacteria and 71.4% of gram-negative bacteria highly sensitive to meropenem (80%), sulfa/trimeth (87.5%), and cefazolin (77.7%). However, *E. coli* was highly resistant to ampicillin-sulbactam (100%) followed by ceftazidime (66.6%). *P. vulgaris* which constitutes 8% of all identified bacteria and 14.2% of gram-negative bacteriuria-sensitive to meropenem, piperacillin/tazobactam, sulfa/trimeth, cefazolin and nitrofurantoin. However, *P. vulgaris* was highly resistant to ceftazidime and ampicillin sulbactam. Overall gram-negative bacteria were sensitive to meropenem (71.4%), and cefazolin (83.3%) and highly resistant to ampicillin-sulbactam (100%) and ceftazidime (76.9%) (Figure 4.6 and Table 4.11).

Table 4.11 Antimicrobial susceptibility pattern of Gram-negative bacteria

Bacteria species		Antimicrobial agents tested													
	Pattern	MEM	CIP	GM	PTZ	CEP	TS	CZ	FOX	CAZ	SAM	NA	CTX	T	OX	NOR
E. coli (n=10)	R	1(10%)	2(20%)	4(44.4%)	2(22.2%)	0	1(12.5%)	2(22.2%)	1(100%)	6(66.6%)	9(100 %)	0	0	1(100%)	-	0
	I	1(10%)	3(30%)	1(100%)	2(22.2%)	0	0	0	0	1(11.1%)	0	0	0	0	-	0
	S	8(80%)	4(40%)	4(44.4%)	5(55.5%)	1(100%)	7(87.5%)	7(77.7%)	0	2(22.2%)	0	1(100%)	1(100%)	0	-	1(100 %)
P. vulgaris (n=2)	R	0	0	1(100%)	0	ND	0	0	ND	2(100%)	2(100%)	ND	1(100%)	1(100%)	1(100%)	0
	I	0	1(100%)	0	1(50%)	ND	0	0	ND	0	0	ND	0	0	0	0
	S	1(100%)	0	0	1(50%)	ND	1(100%)	1(100%)	ND	0	0	ND	0	0	0	1(100%)
K. oxytoca (n=1)	R	0	0	0	0	ND	1(100%)	0	ND	1(100%)	1(100%)	ND	ND	ND	ND	ND
	I	0	0	0	0	ND	0	0	ND	0	0	ND	ND	ND	ND	ND
	S	1(100%)	1(100%)	1(100%)	1(100%)	ND	0	1(100%)	ND	0	0	ND	ND	ND	ND	ND

E. cloace (n=1)	R	1(100 %)	1(100 %)	1(100%)	0	ND	0	0	ND	1(100 %)	1(10 0%)	ND	ND	ND	ND	ND
	I	0	0	0	1(10 0%)	ND	0	0	ND	0	0	ND	ND	ND	ND	ND
	S	0	0	0	0	ND	1(10 0%)	1(1 00 %)	ND	0	0	ND	ND	ND	ND	ND
Total (n=14)	R	2(14.28 %)	3(25%)	6(50%)	2(15 .3%)	0	2(18. 18)	2(1 6.6 %)	1(1 00 %)	10(76 .9%)	13(1 00%)	0	0	2(100 %)	1(100 %)	0
	I	2(14.28 %)	4(33.3 %)	1(8.3%)	4(30 .7%)	0	0	0	0	1(7.6 9%)	0	0	0	0	0	0
	S	10(71.4 %)	5(41.6 %)	5(41.6%)	7(58 .8)	1(100 %)	9(81. 8%)	10(83. 3%)	0	2(15. 3%)	0	1 (100 %)	1(100 %)	0	0	2(100%)

CZ=Cefazolin CTX=Cefotaxime CAZ=Ceftazidime CEP=cephalosporin CIP= Ciprofloxacin FOX=cefoxitin GM=Gentamicin MEM=Meropenem PTZ=Piperacillin/Tazobactam TS=Sulfa/Trimeth NA=Nalidixic acid NOR=nitrofurantoin OX=oxacillin T=Tetracycline and SAM=ampicillin sulbactam R=resistant I=intermediate S=susceptible ND=not done n=number of strains



A B

Figure 4.6 A: Antimicrobial susptability test for *P.vulgaris* and B:Antimicrobial suceptabilty test for *E.coli*

4.4.3 Multidrug resistance of bacteria

Most gram-negative bacteria were resistant to many antimicrobial agents when compared with gram-positive bacteria. From gram-negative bacteriuria *E. coli* was resistant to more than two ten antimicrobial agents followed by *P. vulgaris* which showed resistance to six.

In this study, gram-positive identified bacteria *S. aures* was resistant to tetracycline, penicillin, and sulfa/trimeth. *S. saprophatices* is also resistant to gentamicin and penicillin. *Enterococcus* was resistant to ciprofloxacin and ampicillin sulbactam.

CHAPTER FIVE: DISCUSSION

UTI remains one of the most common causes of morbidity in pediatric practice. Early diagnosis of UTI in children is central as it can be the indicator of renal abnormalities such as scarring, hypertension, and end-stage renal diseases. The main objective of this study was to determine the prevalence and antimicrobial resistance patterns of the bacterial pathogens causing urinary tract infection among outpatient children under 15 years old in, Adama, Ethiopia. The overall prevalence of UTI in children between the age group 0-15 years in this study was 11.7%. The prevalence of bacteria was 18/25 for females,7/25 for male children and higher prevalence rate was observed 6-10 age group. Among the identified bacteria 40% were *E. coli* the dominant one of all isolated bacteria.

UTI according to gender was found at the rate of females with higher UTI prevalence when compared to male children this finding similar with the study done in Ethiopia(Fenta et al., 2020)and in Iraq(Shahab et al., 2017) because they have shorter urethra when compared to male children. In this study most bacteria were isolated from 6-10 age group due to in this age the rate of exposure become high that means may attend school or daycare, increasing exposure to pathogens and infection also may they not well experienced good hygienic practices. In contrast with the study an assessment done in Ethiopia reported that most bacteria was identified among <3 year children(Bitew et al., 2022a).Also another study done in Ethiopia showed that most bacteriuria was isolated among <12 month(Mekonnen et al., 2023).An assessment done in Tanzania and Iraq reported that most bacteriuria were identified between 12 and 59 month and 4 days-2 years respectively (Sangeda et al., 2023; Shahab et al., 2017)(Shahab et al., 2017) .This is due to the study population of the study were under five-year children in the age group the UTI became high.

The overall prevalence of this study was 11.7% this finding has slight agreement with the prevalence report from Ethiopia 16.7(Fenta et al., 2020),16.4(Girma et al., 2022) and from Tanzania 16.4(Sangeda et al., 2023).However in contrast to our study the prevalence report from Ethiopia 27.5(Mitiku et al., 2015),24.1(Mekonnen et al., 2023), 28.6(Bitew et al., 2022b) from

Turkey 18.5(Yilmaz et al., 2016) and from Iraq 38.09(Shahab et al., 2017) has higher prevalence when compared to our study this is due to the geographical location has a negative impact on health, children in that area not well experienced good hygiene practice, environmental factors, the study population of the study were under five-year children in the age group the UTI became high, the fungal pathogens were identified in the study in addition to bacteria, the proximity of anal and urethral openings, and relatively wide urethra.

In this study totally 25 bacteria isolated with 9 spp. were identified 4 gram negative and 5-gram positive spp. characterized by biochemical test. Those *are E. coli, P. vulgaris, E. cloacec, K. oxytca, S. aures, S. saprophaytices, Enterococcus* spp., *Corynebacterium* spp. and CNS. In line with this study an assessment done in Ethiopia got 8 spp. out of that 5 were *E.coli, klebsiella* spp., *S. aures , Enterococcus* spp., and *Protues* spp. similar with our study(Mitiku et al., 2015). Similar study with reported in Tanzania who reported that 35 isolates from 214 children with 5 spp. out of that 4 were *Protus* spp., *Klebsilla* spp. ,*E.coli* and *Staphylococcus* spp.(Sangeda et al., 2023). In line with this study an assessment reported in Turkey got 6 spp. bacteria among that 5 were *E.coli, Proteus* spp., *Klebsiella* spp., *Enterococcus* spp., and CNS(Yilmaz et al., 2016).Another study done in Iraq slightly similar with this study they reported that 40 isolates with 6 spp. out of 6 spp. 3 were similar with this study(Shahab et al., 2017). However in contrast to this study an assessment done in Ethiopia reported that they isolated 80 bacteria with 13 spp. but similar to this study they found *E. coli, K. pnemumoniae, S. aureus ,and Enterococcus* spp.(Mekonnen et al., 2023).Also contrast with another study done in Ethiopia reported that 65 isolates which 49 were bacterial pathogens while 16 were fungal pathogens but in this study no fungal pathogens were identified.49 bacterial pathogens with 8 spp. among that 5 spp. were similar with this study those are *E.coli, K. pnemounia, Enterocoocus* spp. ,*S. aures* and CNS(Bitew et al., 2022).

In contrast with study another study done in Ethiopia reported that they found that 50 isolates with 8 spp. but among 8 spp. 3 were similar with our study those are *E.coli, K. pneumonia and P. vulgaris*(Fenta et al., 2020) .In contrast with this study another study done in Ethiopia reported that 141 isolates with 16 spp. this have higher number of isolates and spp. when compared to this study(Girma et al., 2022).The disparity between studies could result from the difference in sample size, the study population, the definition of bacteria, and the duration of the study, method of lab

diagnosis. Overall *E.coli* was the most frequent bacteria similar to the study reported by in different parts of Ethiopia (Bitew et al., 2022;Fenta et al., 2020),in Tanzania (Sangeda et al., 2023),in Iraq(Shahab et al., 2017) and in Turkey(Yilmaz et al., 2016).

According to MALDI TOF MS analysis there 3 bacteria 2 *Enterococcus* spp. and 1 *S. aures* identified bacteria by biochemical test but didn't identify by MALDI TOF MS analysis due to the score value was described as no organism identification possible as we know those were gram positive bacteria. Identifying gram-positive bacteria using MALDI-TOF MS analysis can be challenging for several reasons. Gram-positive bacteria have thick peptidoglycan layers, which can affect the extraction of proteins necessary for MALDI-TOF analysis. The robustness of their cell walls may lead to incomplete lysis, resulting in lower quality or quantity of protein. Different gram-positive species can have similar protein profiles, making it difficult to differentiate between them based solely on mass spectrometric data. The high similarity in their ribosomal proteins can lead to ambiguous results. Also, while the databases for MALDI-TOF have been expanding, there may still be gaps in the representation of gram-positive bacterial species. If the species is not well-represented in the database, identification will be more difficult. The choice of matrix used in MALDI-TOF can also influence the ionization and detection of proteins from Gram-positive bacteria.

According to the overall finding of antimicrobial susceptibility test for gram-positive bacteria were more sensitive to ciprofloxacin (83%) and resistant to sulfa/trimeth (66.6%) followed by tetracycline tetracycline (50%) and gram-negative bacteria were sensitive to meropenem (71%), and cefazolin (83%) and highly resistant to ampicillin-sulbactam (100%) and ceftazidime (76%).For instance *E. coli* was the dominant bacteriuria constituting 40% of all identified bacteria highly sensitive to meropenem(80%), sulfa/trimeth (70%), and cefazolin (70%) however, highly resistant to ampicillin-sulbactam (100%) followed by ceftazidime (66.6%) in this study.

In line with this study, an assessment done in Ethiopia showed that *E. coli* was sensitive to meropenem and gram-positive bacteria were sensitive against ciprofloxacin and showed resistance to tetracycline and trim/sulpha(Mekonnen et al., 2023). An assessment done in Ethiopia showed that meropenem was the active drug that showed higher susceptibility than other drugs for gram-

negative bacteria and gram-positive bacteria were show resistance to trime/sulpha like this study. However, they found that cefazolin was resistant to gram-positive bacteria opposite to this study because cefazolin was an active drug against gram-negative bacteria(Bitew et al., 2022b).Similar to this study showed that the highest resistance rate of *Klebsiella* spp. was to ampicillin-sulbactam and *Enterococci* was resistant to ampicillin and sensitivity to vancomycin. Although, the highest resistance rates of *E. coli* and *Proteus* spp. were to trimethoprim-sulfamethoxazole in their study(Yilmaz et al., 2016) .Another study done in Ethiopia reported that among tested Gram-negative bacterial isolates, were susceptible to meropenem. *E. coli* isolates were susceptible to cefotaxime, ciprofloxacin, meropenem, and nitrofurantoin. However, it contrast in resistance rate was observed to ampicillin and tetracycline(Fenta et al., 2020).An assessment done in Ethiopia showed that ceftazidime was the resistant antibiotic against gram-negative bacteria similar to this study but their study reported that meropenem was the highly resistant antibiotic whereas meropenem was the most active drug against the gram-negative bacteria in this study(Gebretensaie et al., 2023).

In contrast with study an assessment done in Tanzania showed that, *Proteus* spp. was resistant to ampicillin and erythromycin and sensitive to amoxicillin-clavulanate. However, in this study, the antibiotics were assessed differently from their study due to the administration differences of the antibiotics in different countries. In this study, *Proteus* spp. were sensitive to meropenem, trim/sulfs, piperacillin-tazobactam, cefazolin, and nitrofurantoin and resistant to ampicillin-sulbactam and ceftazidime(Sangeda et al., 2023).Another study contrast with this study done by Mitku et al reported that *E.coli* was resistant to ampicillin, penicillin, and oxacillin(Mitiku et al., 2015). This difference came from due to the nature of each bacteria response to drug have some variation, of the gradual increase of drug resistance/ selective pressure of bacteria to the drug /mutation, or the difference in antibiotic practices in the study area.

According to multi drug resistance an assessments done in Ethiopia reported that a higher rate of multidrug resistance was observed in gram-negative bacterial isolates compared to gram-positives like this study(Bitew et al., 2022b;Mekonnen et al., 2023). However, the highest multidrug resistance was observed in *Klebsiella* spp. ,followed by *Citrobacter* spp., and *E. coli*, but in this study, the highest multidrug resistance was observed in *E.coli, P. vulgaris,* and *S. aures* from

gram-negative bacteriuria due to the continuous use of these drugs for many years, easily availability, self- prescription, the tendency of patients using relatively cheaper antibiotics for all types of infection, and misuse(Fenta et al., 2020).

CONCLUSION

The prevalence of UTIs for children under the age of 15 in this study was 11.7%. In this study, females have higher UTI than male children and asymptomatic children have a high number of UTI cases. No association was found between age, gender, and the UTI prevalence. Nine species of bacteria were identified in the study and *E. coli* was the most frequently isolated bacteria of all isolates whereas *Corynebacterium* spp. was the most common bacteria among gram-positive bacteria. There was high antimicrobial resistance to sulfa/trimeth and tetracycline for gram-positive bacteria. Gram negative bacteria were resistant to ampicillin sulbactam and ceftazidime. Generally, gram-negative bacteria were resistant to most drugs imply that they were multidrug resistance strains.

RECOMMENDATION

Based on the findings of this study on the prevalence and antimicrobial resistance patterns of pathogens causing urinary tract infections (UTIs) in children under 15 years, the following recommendations are proposed:

1. Enhanced Surveillance:

Regular Monitoring: Implement continuous surveillance programs to monitor the prevalence and antimicrobial resistance patterns of uropathogens in pediatric populations. This will help in the early detection of emerging resistance trends and inform treatment guidelines.

2. Antibiotic Stewardship:

Guideline Development: Develop and regularly update clinical guidelines for the empirical treatment of pediatric UTIs based on local antimicrobial resistance patterns. This will ensure that the most effective antibiotics are used, reducing the risk of treatment failure.

3. Public Health Initiatives:

Awareness Campaigns: Launch public health campaigns to raise awareness about the importance of proper hygiene and preventive measures to reduce the incidence of UTIs in children. Educating parents and caregivers on recognizing early symptoms and seeking timely medical care is crucial.

5. Policy and Regulation:

Funding and Support: Allocate funding and resources to support research, surveillance, and public health initiatives aimed at combating antimicrobial resistance in pediatric UTIs.

I recommend that prospective study have to be done in rural area to figure out the prevalence and antibiotic resistance among children. By implementing these recommendations, healthcare providers and policymakers can work together to reduce the prevalence of UTIs in children and mitigate the impact of antimicrobial resistance, ensuring better health outcomes for this vulnerable population.

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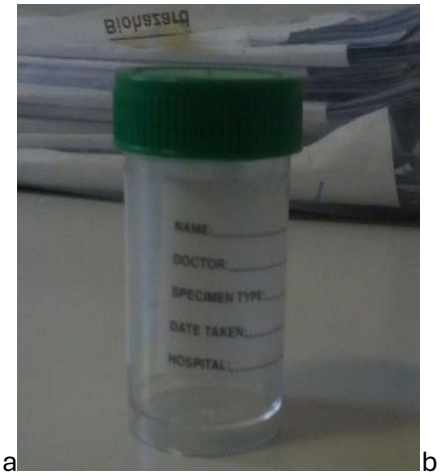
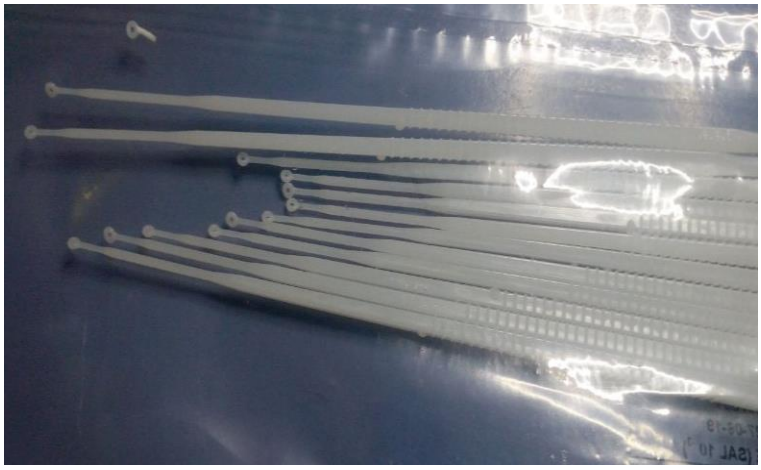
APEPENDICES

Appendix 1: Representative picture of sterile urine plastic cup and inoculating needle

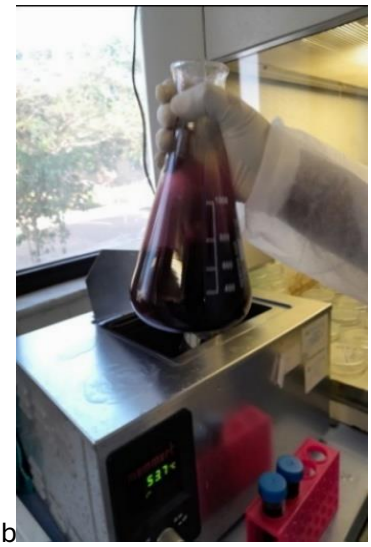
a 

b 

Figure(a)sterile urine plastic cap used for sample collection (b)sterile inoculating needle used for inoculation

Appendix 2: Representative picture of media preparation procedure


a


b


c

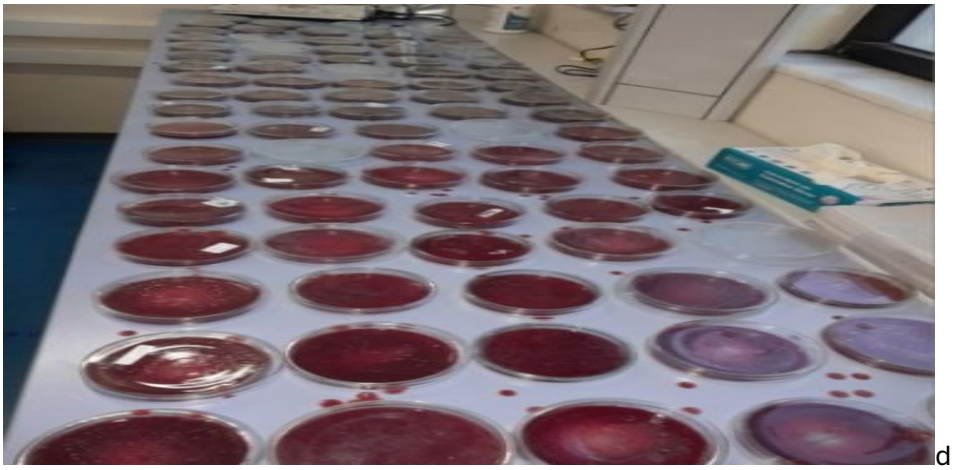

d

Figure (a): sterilized Petri dishes waiting for media dispensing (b): blood agar preparation (c): Dispensing blood agar on sterilized Petri dishes (d): prepared media that left for dry

Appendix 3: Representative picture of bacteriuria growth on blood and macconkey agar

a  b  c 

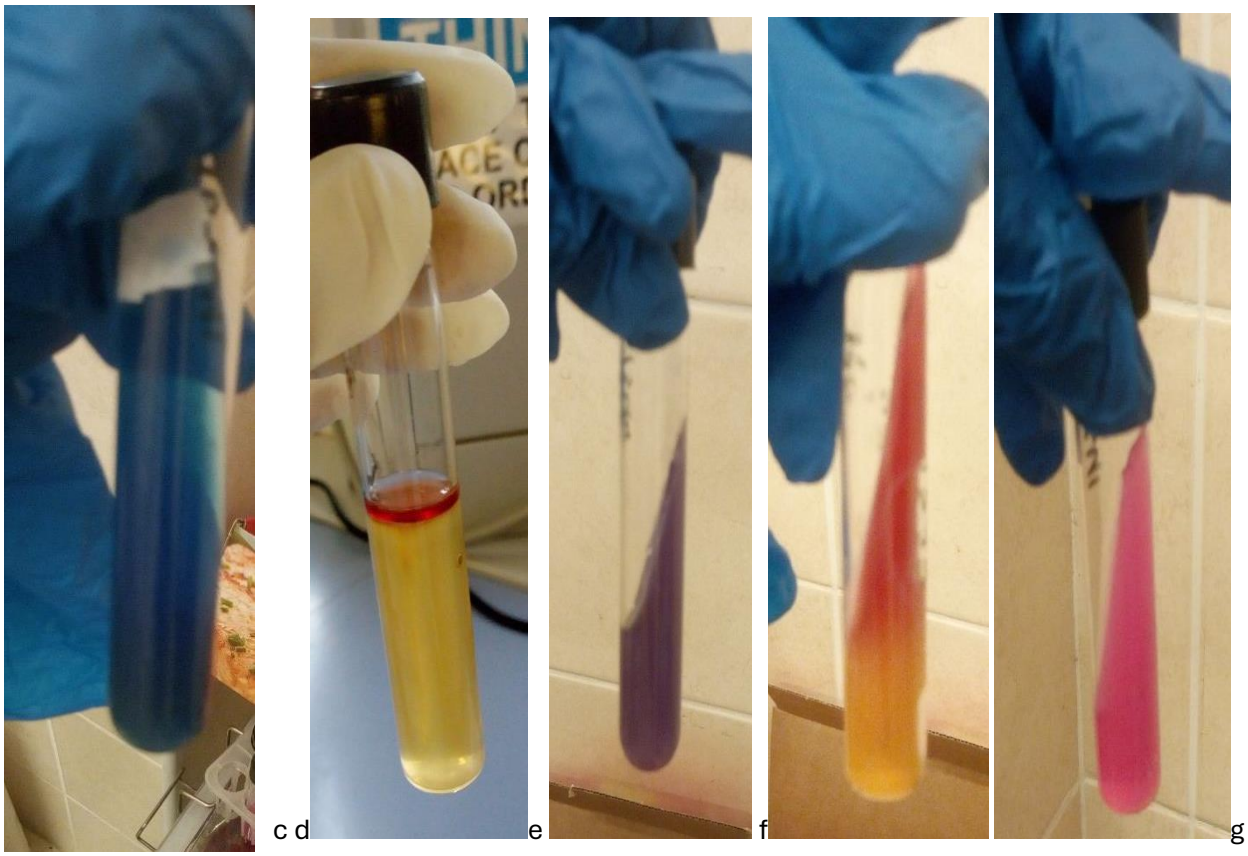
Figure (a)bacteriuria growth on macconkey agar (b) and (c)bacteriuria growth on blood agar

Appendix 4: Representative picture of Biochemical test procedure and result

a 

b 

Figure (a) Biochemical test reagents oxidase, catalase, indole (b)results of biochemical tests



c d e f g

Figure c:Citrate postive d:Indole postive,motile and sulphide negative e:Lysin iron agar test postive f:K/A triple sugar iron test g:Urea postive result

Appendix 5:represenative procedure of MALDI TO MS

a 

Figure (a)MALDI TOF MS analysis

Appendix 6: Representative picture of Antimicrobial Susceptibility test

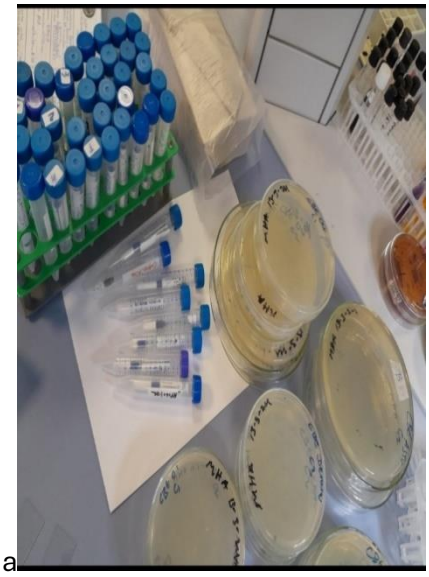
a  b 

Figure (b): Antibiotics and Muler hinton agar (b): Antibiotics that used for antimicrobial susceptibility test