

ADDIS ABABA UNIVERSITY
COLLAGE OF HEALTH SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Antimicrobial Susceptibility Profile of Bacterial Uropathogens Isolated from Pediatric Patients at Yekatit 12 Hospital Medical College

By: Yamirot Merga

Advisor: Adane Bitew (MSc, PhD)

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Approved by the Examining Board

Chair of Department or Graduate Committee

_____ Signature: _____
Date: _____

Advisor

Adane Bitew (MSc, PhD) Signature: _____ Date: _____

External Examiner

_____ Signature: _____
Date: _____
Internal Examiner

_____ Signature:_____ Date: _____

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Table of content

Acknowledgement..... i
Table of content……..…….. ii
List of tables and annexes…….. iv
Abbreviations……. v
Abstract …….. vi

1. Introduction……. 1
1.1 back ground……. 1
1.2 statement of the problem……. 2
1.3 significance of the study…….. 3
2. Literature review……... …….. 4
3. Objectives……..…… 11
3.1 General objective…….. 11
3.2 specific objectives……..... 11
3.3 Hypothesis..... 11
4. Methods and materials…… 11
4.1 Study area …… 11
4.2 Study design and period……. 12
4.3 populations……..... 12
4.3.1 Source population…….. 12
4.3.2 Study population……..... 12
4.4 **Inclusion and Exclusion Criteria** ……. 12
4.4.1 Inclusion criteria……. 12
4.4.2 Exclusion criteria……..... 12
4.5 study variables……... ……... 13
4.5.1 Dependent variables……..... 13
4.5.2 Independent variables…….. 13
4 .6 Sample size determination and sampling technique…….. 13
4.7 Data collection……..... 14
4.7.1 Demographic ……... 14
4.7.2 Specimen / urine/collection …….... 14
4.7.3Culture characterization/ identification……..... 14
4.7.4Antimicrobial susceptibility testing..... 15
4.8 Quality control…… 16
4.9 Data analysis……. 16
4.10 Ethical considerations …… .16
4.11. Dissemination of Results..... 17
5. Result……. 18
5.1. Sociodemographic characteristics..... 18
5.2. Antibiotic sensitivity testing……..…..... 20
6. Discussion..... 23
7. Limitations of the study……..……. 25
7.2 limitations……... 25
8 Conclusion……. 26
9. Recomendation…… 26
10.References……. 27
11.Annexs..... 31

List of Figures, Tables and Annexes

Table 1. Distribution of bacterial isolates in relation to male and female in each group……..
…18

Table 2. Distribution of bacterial isolates in relation to sex……..
……19

Table 3. Association of UTI in relation to circumcision…….
……..20

Table 4. Number and percent of resistant strains of gram negative uropathogens…….
…...20

Table 5. Number and percent of resistant strains of gram positive uropathogens…….
……21

Table 6.Multi-drug resistance pattern of Gram-negative bacteria isolated…….
…...22

Table 7. Multi-drug resistance pattern of Gram-positive bacteria isolated…….
……..22

Annex I. English version of participant information sheet……....31

Annex II. Amharic version of participant information sheet…….
…34

Annex III. English Versions of Consent form …….....37

Annex IV. Amharic version of consent form…….....38

Annex V. Procedure for specimen collection and processing ……...….
…40

Annex VI. Data collection form.....
.....45

Annex VII. Declaration.....
.....46

Abbreviation

AAHB	Addis Ababa Health Bureau
AAU	Addis Ababa University
AST	Antimicrobial Susceptibility Testing
BA	Blood Agar
CLED	Cystine-lactose-electrolyte-deficient
CI	Confidence Interval
CoNS	Coagulase Negative Staphylococci
CLSI	Clinical Laboratory standards Institute
DMLT	Department of Medical Laboratory Technology
ESBL	Extended-spectrum β-lactamase
KIA	Kligler Iron Agar
MSU	Mid-stream urine samples
NCCLS	National Committee for Clinical Laboratory Standards.
OPD	Outpatient Department
QC	Quality Control
SOPs	Standard Operating Procedures
Spp	Species
SPSS	Statistical Package for Social Sciences
UTI	Urinary tract infection
USA	United State of America
WHO	World Health Organization

Abstract

Background: Urinary tract infection (UTI) is considered as the most common bacterial infectious disease seen among the pediatric patients.

Objective: This study was carried out in order to determine the antimicrobial Susceptibility Profile of Bacterial Uropathogens Isolates from Pediatric Patients at Yekatit 12 Hospital Medical College

Materials and Methods Clean voided mid-stream urine specimens were obtained from patients in sterile universal bottles. Urine collected from each patient was inoculated onto CLED and blood agar plats using calibrated inoculating loop with a capacity of 0.001ml, Inoculated plates were incubated for 24- 48 hours at 37°C at inverted position aerobically. Bacterial isolates were characterized/ indented by gram stain and by using an array of standard routine biochemical test. Antimicrobial susceptibility test was carried out by using the Kirby Bauer disc diffusion.

Result: The data was analyzed by using SPSS, version 17. Descriptive statistics was computed for most of the study variables and Frequency distribution tables were used to describe the findings. In this study a total of 384 pediatric patients (199 males and 185 females) aged between 0 years to 15 years from whom urine sample were collected were enrolled. Of these patients, 61 (15.9%) had significant bacteriuria. Of the 185 females, 36 (19.5%) have positive cultures while 25 (12.6%) of the 199 males had significant bacteriuria and the largest number of study subjects were below age 3 years and the largest positive culture was obtained from this age group, accounting 35 (57.4%.) out of 61 positive culture. Bacterial species belonging to six genera were isolated and identified from 61 positive cultures and the genera were *Escherichiai, Klebsiella, Staphylococcus, Proteus, Acnitobacter* and *Entrococcus. and E. coli* was isolated in 28 cases (28/61, 49.5 %), followed by *Klebsilla* spp. in 17 cases (17/61, 27.9%), *Staphylococcus spp.* in 5 patients (5/61, 8.2%.) (*S. aureus* in one and coagulase negative staphylococci in 4 case), *Entrococcus* in 7 case (7/61, 11 5%), *Proteus spp.* in 3 cases (3/61, 4.9%)) and *Acenitobacte* in one case (1/61,1.6%). Of bacterial isolates *E. coli* was found out to be the most common pathogen followed by *Klebsiella* spp.. Furthermore *E. coli* and *klebsiella spp.* were the most common pathogen in female patients accounting 71. 4% and 64.7% respectively. percentage resistance of *Klebsilla* spp was much higher when compared to *E.coli.* Eighty eight percent of *Klebsiell spp*. were resistant to

cefotaxim, ceftazidim, trimetroprimsulfamethoxazole and cefuroxime. *Acentobacter spp*. was 100% resistances to gentamicin, trimetroprimsulfamethoxazole , agumentin, and nalidixic acid . But they were 100% susceptible to ciprofloxacin, cefuroxime , norfloxacine, cefotaxim,chloramphenicol and ceftazidim . On the other hand, *proteus spp.* were 100% sensitive to all drugs except nitrofurantion. Species of *Entrocuccus* were resistance of 71.4% to chloramphenicol and 85.7% to both trimetroprimsulfamethoxazole and Erythromycin. *S. aurues* was 100% susceptible to ciprofloxacin, cefuroxime, gentamicin, trimetroprimsulfamethoxazole, chloramphenicol, Clindamycine and Ceftriaxone while coagulase negative *staphylococci* were resistance to all of the above drugs except Clindamycine and gentamicin . Multidrug resistance to two or more drugs was observed in 73.7% of bacterial isolates.

Conclusion: This study determines the antimicrobial Susceptibility Profile of Bacterial Uropathogen Isolates from Pediatric Patients and highlighted the major bacterial uro-pathogens involved in UTI up to my knowledge for the first time in the country. Furthermore, species bacterial pathogens and their frequency was consistent with the usually reported pattern, with *E. coli* being the most common organism isolated followed by *Klebsiella. Spp.* In this study Majority of gram negative species were susceptible to ciprofloxacin, norfloxacine and nitrofurantion (except *proteus spp)* and gram positive species were susceptible *to* ciprofloxacin Clindamycine and gentamicin so this drugs can be used to treat UTI in children however Most of bacterial isolates were multidrug resistant and **i**t is therefore suggested that appropriate antimicrobials should be administered to reduce the risk of multi drug resistant organisms developing and avert ineffectiveness of antibiotics.

1. Introduction

1.1 Back ground:

Bacterial Urinary Tract Infection (UTI) is defined as presence of significant bacteria in urine irrespective of the site of infection in the urinary tract (1). It is a serious health problem and it has been estimated that about six million patients visit outpatient departments and about 300, 000 are treated in the wards every year for UTI worldwide (2).

UTI is also a common and important clinical problem in pediatrics, as recurrent UTIs may lead to renal scarring, hypertension and end stage renal dysfunction later in life (3). UTI occurs in 3 to 5% of girls and 1% of boys during childhood, and it is common in boys during the first year of life, especially among those who are uncircumcised (4). According to Owa

the male to female ratio in UTI varies with age, observed as 2.8-5.4:1.0 in the first year of life and changing to 1:10 after the second year of life (4).

UTI is caused mainly by bacteria, although viruses, fungi and parasites can also cause urinary tract infections. Among bacteria gram negative organisms are the most commonly isolated from urine samples of children with *Escherichia coli (E. coli*) accounting for 70 to 90% of infections (5). Other include *Klebsiella* species, *Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter,* and *Serratia* are other gram negative bacteria isolated from pediatric patients However, only 10% of the cases are caused by gram positive bacteria and include *Enterococcus, Staphylococcus, and Streptococcus agalactiae* (6).

In recent years, widespread use of antibiotics has been resulted in an increasing incidence of antibiotic resistance among the urinary tract pathogens all over the world. Worldwide, emerging of antibiotic resistance is increasing among the urinary pathogens (7). More than 80% of bacterial strains causing urinary tract infections in developing countries are now resistant to trimethoprim or trimethoprim-sulphamethoxazole (8).

Urinary tract infection (UTI) is one of the most common bacterial infections encountered by clinicians in developing countries and the cause of significant morbidity and mortality (9). Several studies from the African continent have investigated the profile of common uropathogens and the pattern of their susceptibility to commonly used antimicrobial agents in order to guide choice of empiric therapy. These studies reported the emergence of antibiotic-resistant gram-negative bacilli with special emphasis on ESBL-producing isolates (10,11,12). However, to the best of our knowledge, no such studies have been published describing the spectrum of uropathogens in pediatric patients and their antimicrobial susceptibility patterns in Ethiopia. Therefore this study was conducted with the aim of determining patterns of

antibiotic resistance of uropathogens among pediatric patients from Yekatit 12 Teaching Hospital.

1.2 Statement of the problem:

UTI in children is one of the most common bacterial infections encountered by clinicians in developing countries and the cause of significant morbidity and mortality. Several studies (13-15) from the African continent have investigated the profile of common uropathogens in infants and the pattern of their susceptibility to commonly used antimicrobial agents in order to guide choice of empiric therapy. These studies reported the emergence of antibiotic-resistant. Though many studies (16, 17) in Ethiopia have been published describing the prevalence of uropathogen in adults and their drug susceptibility pattern, no such a single study has been published describing the prevalence of uropathogen and their drug susceptibility profile in pediatrics. To this end determining uropathogens isolates and their drug susceptibility pattern among pediatric patients is of a highest priority.

1.3. Significance of the study:

- The results obtained in this study may be used as base line data for future studies in other health institution and/or the country at large
- Knowledge of the isolates and drug susceptibility profiles of uropathogens in pediatrics, helps to selection of appropriate antibiotics for empiric treatment
- Bacteria isolated and maintained in this study will be used for further studies such as determining mechanisms of development of drug resistance

2. Literature Reviews

The prevalence of UTI in pediatric patients, its epidemiology, clinical features, risk factors and spectrum of bacteria causing UTI in pediatric patients and their antibiotic susceptibility profiles have been studied by various researchers across the globe in different times. This chapter reviews these studies in their chorological order.

Prevalence of UTI was studied on 945 febrile infants. Of the 945 febrile infants from whom culture specimens had been obtained the prevalence of UTI was 5.3%. The prevalence of UTI in relation to the five variables – age, sex, race, temperature and the presence or absence of an apparent source of fever showed that the prevalence of UTI was similar in infants aged less than 2 months and in infants of age greater than 2 months; higher among female than male infants; and higher among white than black infants. However, no association was apparent between the prevalence of UTI and infant temperature. If sex, race and temperature were combined, UTI was found in 16.9% of white female infants whose highest temperature had been greater than or equal to 39^0 C (18) .

A study was conducted to assess the prevalence of urinary tract infections in febrile young children in the Emergency Department of Pediatrics and Nursing, Children's Hospital of Philadelphia over a period of February 2, 1995 to February14, 1996 on 2411 study subjects. The objective of the study was to establish prevalence rates of urinary tract infection (UTI) in febrile infants and young girls in an Emergency Department by demographics and clinical parameters. Their study demonstrated 3.3% an overall prevalence of UTI, occurrence of a higher prevalence in whites,(10.7%), girls (4.3%), uncircumcised boys (8.0%) and those who did not have another potential source for their fever (5.9%) , had a history of UTI (9.3%), malodorous urine or hematuria (8.6%), appeared "ill" (5.7%) , had abdominal or suprapubic tenderness on examination (13.2%) , or had fever >39°C (3.9%). The prevalence of UTI in white girls was 6.1%. They concluded that UTI is prevalent in young children, particularly white girls, without a definite source of fever. Specific clinical signs and symptoms of UTI are uncommon, and the presence of another potential source of fever such as upper respiratory infection or otitis media is not reliable in excluding UTI (19).

The study in Taiwan carried out a retrospective investigation of 30 neonates in whom urinary tract infections were diagnosed by urine culture in Veterans General hospital, Kaohsiung. In this study, UTI was a little more common in boys (56%) and *E. coli* was the most common causative pathogen, 85% of which were sensitive to gentamycin (20).

A study was conducted in California to assess the effect of newborn circumcision on the incidence and medical costs of urinary tract infection (UTI) during the first year of life in California pediatrics and the results of the study demonstrated that new born circumcision resulted in a 9.1 fold decrease in the incidence of UTI in the first life of pediatrics thereby reducing UTI related medical costs and hospital admission (21).

There was a study conducted in IIorin to screen a total of 154 febrile children for UTI in the university of IIorin teaching hospital. The results of this study indicated that the commonest isolate was *E. coli* and *Klebsiella* species accounting 36.4% each. This was followed by *P. aeruginosa* and *S. facalis*. This study further revealed that the sensitivity of *E. coli* to ceftazidime, ciproflaxin gentamycin and ofloxacin was 88.9%, 85.7%, 66.7% and 70% respectively. The sensitivity of *Klebsiella* species was 90 % to ciprofloxacin, 71.4% to ceftazidime and 62.5 % to gentamycin (13).

Prevalence of urinary tract infection in childhood was studied in USA in University of Pittsburgh, division of General Academic Pediatrics . The results of this study revealed that among infants presenting with fever, the overall prevalence of UTI was 7.0%. With regard to gender, the pooled prevalence rates of febrile UTIs in females aged 0-3 months, 3-6 months, 6-12 months, and greater than 12 months was 7.5%, 5,7%, 8.3%, 2.1% respectively. This study further showed that among febrile male infants less than 3 months of age, the prevalence of UTI was less in of circumcised males (2.4 %) than of uncircumcised males (20.1 %) (6).

Urinary Tract Infection in neonates with serious bacterial infections admitted to the University Hospital of the West Indies was studied. Fifty-one (38%) of 135 babies with positive bacterial isolates had confirmed urinary tract infection. Male: female ratio was 6:1. *E coli* and *Klebsiella species* were most frequently identified (22).

The prevalence and antimicrobial susceptibility of bacterial uropathogens in pediatric patients was studied in Iran. In this study a total of 14199 urine specimens were collected from

pediatric patients. Of these, 2302 (16.2%) had positive results for bacterial cultures. Of these, 40% of the strains belonged to *E. coli.* The isolation percentage of other bacterial species was: *Klebsiella* spp. (17.9%); coagolase negative *Staphylococcocci* (12.3%); *Enterococcus* spp. (8.7%); *Pseudomonas* spp. (6.7%); *S. aureus* (3.6%) and remainder (10.8%) was belonged to other bacterial strains. *E. coli i*solates showed high resistance to carbenicillin, ampicillin, trimethoprimsulfomethoxazol and kanamycin. Also high degree of resistance were observed in *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp. that were resistance more than 30% to most of the commonly used antibiotics. All *Acintobacter* spp. strains were resistant to ceftazidime, cefixime and ceftizoxime. Similar trend was also seen among *Pseudomonas* spp. and *Enterobacter* spp. isolates for resistance to ampicillin. More than 50% of coagolase negative *Staphylococci, S. aureus* and *Enterococcus* spp. strains were resistant to the most of commonly used antibiotics (23).

Pathogen causing UTI in pediatric patients and their antibiotic susceptibility profile was investigated in Chong Hua Hospital. The study consisted of 140 outpatients and in patient of age 18 and below. The prevalence of UTI was more in males with ages between 28 months and one year while the prevalence of UTI in females was more with ages between 1 to 5 years. *E. coli* was the predominant isolate accounting 75% followed by *P. mirabilis* (6%), *P. aeruginosa* (6%), *Enterobacter sp.* (5%), and *K. pneumoniae*(4%). Antibiotic susceptibility profile of the isolates depicted that 76% of the isolates were resistant to ampicillin and 64.15% to cotrimoxazole (64.15%). Resistnce to ceftriaxone, imipenem, gentamicin, ciprofloxacin, netilmicin, amikacin and cefepime was less than 10% (24).

Prevalence and clinical profile of UTI in febrile children aged 3-6 years attending pediatric out patient department was conducted in Sri Siddlehartha Medical College, Tumkur, Krnataka, India. The study involved a total of 500 children of which 280 were male and 220

were female children. The prevalence of UTI was 2.9% in males and 5.5% in females with over all prevalence of 4% with male to femal ratio of 1:1.5 (25).

There was a study conducted in Nigeria to assess the prevalence of UTI, most probable etiologic agents and antimicrobial susceptibility pattern of the isolate by taking clean voided mid-stream urine samples from 301 children and adolescents between the ages of 5 and 18 years at the Obafemi Awolowo University Teaching Hospital complex (OAUTHC) from December 2005 – July 2006. The result of this study showed that 36 (11.96%) of the 301 patients studied had UTI. Of the 124 females examined, 28 (22.4%) had positive urine culture while 8 (4.56%) of the 177 males had significant bacteriuria. Of a total of 36 bacterial isolates *E. coli was* the predominant organism and was responsible for (52.77%) of the cases of UTI. This was followed by *Klebsiella* sp. (25%), *P. mirabilis* (13.89%), *S. faecalis* (5.56%) and *P. aeruginosa* (2.78%). The antibiotics sensitivity test revealed a high level of resistant to cotrimoxazole, amoxicillin and colistin as more than 60% of the isolates were resistance to these (14).

Organisms causing UTI in pediatrics patients at Murtala Muhammad Specialist Hospital was studied in Nigeria. Fifty patients of either sex ranging from neonatal period of twelve years of age were studied at the pediatric units of the hospital. Urinary tract infection was common among females except in the neonatal period. *E. coli* was the most common organism isolated (70.9%). Followed by *Klebsiella* (14%). *Proteus* (10%). *Staphylococcus* (4%) and *Pseudomonas* (2%) (15).

A study was conducted at the University of Nigeria teaching hospital, Enugu. This study demonstrated that asymptomatic bacteriuria in 6% of children with sickle cell animia and occurred more in females than males (F: M = 5:1) when compared to 2% children with normal Haemoglobin . In this study *E. coli* was the commenst bacteria isolated accounting

33.3% and all isolates were resistant to co-trimozole and ampicllin while most were sensitive to gentamicin, ceftriaxone and the quinolons (26).

Prevalence and predictors of UTI and severe malaria among febrile children attending Makongoro health centre in Mwanza city, North-Western Tanzania was carried out. A total of 231 febrile under-fives were enrolled in the study. Of all the children, 20.3% (47/231) 9.5% (22/231) and 7.4% (17/231) had urinary tract infections, Plasmodium. falciparum malaria and bacteremia respectively. Predictors of UTI were dysuria and body temperature (40-41 C). *E. coli* were the common gram negative isolates from urine (72.3%)and *E. coli* from urine were 100% resistant to ampicillin, 97% resistant to co-trimoxazole, 85% resistant to augmentin and 32.4% resistant to gentamicin; and they were 100%, 91.2% and 73.5% sensitive to meropenem, ciprofloxacin and ceftriaxone respectively (27).

The epidemiology of UTI in newborn infants admitted to neonatal intensive care unit (NICU) of Zagazig University, Egypt was studied over a period of six months. The incidence of UTI in NICU was 15.05% (33/206), prevalence among suspected cases of UTI in NICU was 41.3% (33/75), and of the 39 neonates with sepsis 33 had UTI (79.5%). Of the bacterial isolates *E.coli*, and *Klebsiella* species were the most commonly isolated bacteria accounting 58.1% and 41.9% respectively (28).

Childhood UTI, etiological organisms and antibiotic sensitivity pattern of the isolates was studied in Nigeria at University Teaching Hospital, Abakaliki between January 1, 2007 and December 31, 2009. One hundred ten subjects of the 3625 children seen in the center during the study period had UTI giving a case prevalence rate of 3.0%. Majority of the patients (59, 53.6%) were less than 2 years of age with a male: female ratio of 1:1.3. Fever was the commonest presenting symptom and the commonest organisms isolated in urine were

Klebsiella (27, 24.5%), and *S. aureus* (24, 21.8%). The drugs that were most sensitive to these organisms were Gentamicin (50, 45.5%), Ceftriaxone (49, 44.5%), and Ciprofloxacin (36, 32.7%) (29).

A prospective surveillance study was conducted to investigate the epidemiology and patterns of antibiotic resistance among uropathogens from hospitalized children in Beira, Mozambique. Additionally, information regarding determinants of UTI was obtained. Analysis of 170 urine samples from 148 children were analyzed for bacterial pathens and 34 bacterial isolates obtained of which *E. coli* and *Klebsiella* spp. Were predominant, causative agents of UTI in 29 children; 30/34 isolates (88.2%) from 26/29 children (89.7%) were considered highly resistant micro-organisms (30).

A prospective study was conducted to determine the causative agents of UTI in asymptomatic and symptomatic diabetic patients, associated risk factors and drug resistance pattern of the isolates in Ethiopia at Gondar University Hospital in 2010. a total of 422 diabetic patients with asymptomatic UTI (n=387) and symptomatic UTI (n=35) were investigated for urinary tract infection. The age range of study participants was 20 to 84 years (mean age 42.3 years). Significant bacteriuria was detected in 14.7% and 51.4% of asymptomatic and symptomatic diabetic patients, respectively. The overall prevalence of significant bacteriuria in both groups was 17.8%. A total of 82 different bacterial uropathogens were isolated. Out of the 82 bacterial isolates*, E. coli (31.7%), coagulase negative staphylococci (CONs) (22%), Klebsiella spp. (14.6%), Enterococcus spp. (11%) and S. aureus (8.5%)* were the commonest bacterial uropathogens in both groups. The gram positive and negative bacteria accounted for 42.7% and 57.3% of the bacteria isolates, respectively. Significant bacteriuria was significantly associated with history of previous UTI, antibiotic treatment, type of diabetes and blood glucose level. Both gram positive and negative bacteria showed significant level of resistance to most antimicrobial agents tested**.** Multidrug resistance to two or more drugs was observed in 59.8% of bacterial isolates (9).

3. Objectives

3.1 General objectives:

To assess the spectrum of bacterial uropathogen isolated in pediatric patients and determine their antimicrobial susceptibility profile in the study area.

3.2 Specific objectives:

- To determine the isolation rate of bacterial uropathogens with respect to sex, age and circumcision (among male pediatric patients)
- To compare and outline bacterial isolate and their drug susceptibility profile between different age group.

3. 3. Hypothesis:

The rate of isolation of bacterial uropathgens in pediatric patients in Yekatit 12 teaching Hospital is very high and most of the isolates are multidrug resistance.

4. Materials and Methods

4.1 Study area:

The study was conducted at Yekatit 12 Hospital Medical College which is located in the center of Addis Ababa. Yekatit 12 teaching hospital is one of the hospitals under Addis Ababa City Administration Health Bureau that has been giving routine health services for the city community and other referral cases from different regional states of Ethiopia. It is a tertiary level referral and teaching hospital in Addis Ababa that serves for a large number of people from the surrounding zones and nearby regions both for inpatient and outpatient treatment. This teaching hospital consists of an operating room, intensive care unit (ICU) and pediatric department with 12 beds, 13 wards with 327 beds, and outpatient departments.

4.2 Study design and period:

It was a cross-sectional study with the objective of isolating, characterizing uropathogens in pediatric patients and determining the antimicrobial susceptibility pattern of the isolates. The study was conducted prospectively from January to April 2014.

4.3 populations:

4.3.1 Source population

All pediatric patients not greater than 15 years of age attending Yekatit 12 Hospital medical college.

4.3.2 Study population

All pediatric patients from 0-15 age who were send to laboratory for urinalysis.

4.4 Inclusion and Exclusion Criteria:

4.4.1 Inclusion criteria

Pediatric patients from 0-15 age with urinalysis.

4.4.2 Exclusion criteria

Pediatric patients

a) Which were not referred to urinalysis.

b) Children taking antibiotics within the last two weeks were excluded.

4.5. Study variables:

4.5.1 Dependent variables

Bacterial isolation rate of pediatric UTI patients, susceptibility pattern of bacterial isolates from pediatrics UTI.

4.5.2 Independent variables

Age, sex and circumcisions status (among male) of pediatric UTI patients

4 .6 sample size determination and sampling technique:

Since data was not available on pediatric bacterial UTI in Ethiopia, 50% of population proportion was used to determine sample size based on single population proportion and the level of precision (d) was (0.05).

The samples size was calculated by the formula:- (31)

$$N = \frac{Z^2(P(1-P))}{D^2}$$

$$N = 1.96^2(0.5(1-0.5))/0.05^2$$

$$N = 384$$

Where: N= number of the study subjects

Z= is standardized normal distribution curve /value for the 95% confidence interval (1.96)

p =proportion of population (50%).

d = the margin of error taken (0.05 taken)

The total sample size for this study was 384 of pediatric patients from 0-15 years with urine culture.

4.7 Data collection

4.7.1. Demographic:

Age, sex, circumcision status and antibiotic history of study subject were recorded by a senior nurse by employing data collection form developed for this purpose.

4.7.2 Specimen / urine/collection:

Clean voided mid-stream urine specimens were obtained from pediatric patients in sterile universal bottles by patient's parent with the assistant of nurse and investigator and transported to the laboratory as soon as possible. When the patient is unable to provide urine catheters were used, this is done only when the child is critically ill and the sample is needed to treat the situation.

4.7.3 Culture characterization/ identification:

Urine collected from each patient was inoculated within two hours of collection onto CLED, cystine-lactose-electrolytedeficient agar (Oxoid, Basingstoke, Hampshire, England) and blood agar base (Oxoid, Basingstoke, Hampshire, England) plats to which 10% sheep blood was added and prepared following the manufacture's instruction using calibrated inoculating loop with a capacity of 0.001ml. Inoculated plates were incubated for 24- 48 hours at 37°C at inverted position aerobically. Plates with colony count of ≥10, 0000 cfu/ml was considered significant or positive also for catheterized pediatric patients 100 cfu/ml was considered significant and subjected for further study. Bacterial isolates were characterized/identified by gram stain and by using an array of standard routine biochemical test.

4.7. 4 Antimicrobial susceptibility testing:

Antimicrobial susceptibility test was carried out by the Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI - formerly NCCLS) guidelines on Muller Hinton agar (Oxoid, Basingstoke, Hampshire, England [32). Briefly, four to five bacterial colonies were inoculated into 5ml of soyabean casein digest broth (Oxoid, Basingstoke, Hampshire, England) and incubated at 37^0C for 12 hours. After an overnight incubation the growth suspension was prepared in 0.5 ml of the same broth medium and the turbidity was adjusted to match that of 0.5 McFarland standards to obtain approximately the organism number of 1x 10^6 colony forming units (CFU) per ml. Then a sterile swab was dipped into this suspension and the excess of inoculum was removed by pressing it against the sides of the tube. Then the swab was applied to the center of a 150mm size of Muller Hinton agar plate (Oxoid, Basingstoke, Hampshire, England) and evenly spread on the medium. Antibiotic discs were placed after 15 minute of inoculation to Muller Hinton agar seeded with each isolate and were incubated for 24 hours at 37°C. The diameter of the zone of inhibition around the disc was measured using sliding metal caliper. Interpretation criteria were those of Clinical Laboratory Standards Institute (CLSI) guidelines as Sensitive, Intermediate and Resistance. The following drugs and concentrations were used to determine the antibiogram of the strains: pencillin [10U], augmentin [30㎍], trimethoprim-sulfamethoxazole [1.25/23.75㎍], clindamycin [30㎍], gentamicin [30㎍], ciprofloxacin [5㎍], erythromycin [15㎍], chloramphenicol [30㎍], cefuroxime [30㎍], cephalothin [10㎍] ,Ceftazidine [30㎍], Cefotaxim [30㎍], Ceftriaxone [10㎍], Oxacillin [1㎍],

Nitrofurantion [30㎍], Nalidixicacid [30㎍] and Norfloxacine [30㎍]. All antibiotic disks were purchase from Oxoid Limited Company, United Kingdom and reference strains of *S. aureus* ATCC 25923, *E.coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 was used as control strain for susceptibility tests.

4.8 Quality control:

The reliability of the study findings was guaranteed by implementing quality control measures throughout the whole process of the laboratory work. Staining reagents, culture media and antibiotic discs were checked for their normal shelf life before use. All culture plates and antibiotic discs were stored at recommended refrigeration temperature (2-8^{0}C) after preparation. Reference strains of *S. aureus* ATCC 25923, *E.coli* ATCC 25922, and *P. aeruginosa* ATCC 27853 were used as controls. In general, all laboratory procedures were done based on recommended standard laboratory procedures by strictly following pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in standard opertional procedures (SOPs) of microbiology laboratory of Yekatit 12 hospital medical college.

4.9 Data analysis:

Data was cleaned; entered and analyzed using SPSS, version 17. Descriptive statistics was computed for most of the study variables and Frequency distribution tables were used to describe the findings. Logistical regression was also used to estimate crude odds ratio (CORs) with 95% confidence interval (CI) of positive responses to the different variables and P values less than 0.05 was taken as statistically significant when looking for associations between dependent and independent variables.

4.10 Ethical considerations:

The study was conducted after it is ethically reviewed and approved by the Department of Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Ethical clearance was also obtained from Addis Ababa Health Bureau. Then a letter informing the hospital was written from the health bureau and permission was obtained from Yekatit 12 Hospital. Informed written consents were obtained from mothers/guardians of pediatrics before data collection. The respondent was given the right to refuse to take part in the study. All the information obtained from the study subjects was coded to maintain confidentially. When the participants are found to be positive for urine culture they were informed by the hospital clinician and receive proper treatment.

4.11. Dissemination of Results

After conducting the research, the results of the study will be submitted to Department of Medical Laboratory Sciences (DMLT) Addis Ababa University (AAU). Oral presentation of the thesis will be made. Reports will also be submitted to Yekatit 12 Hospital Medical College, Addis Ababa Health Bureau, annual conferences of professional societies and other concerned bodies. Since it is said that scientific work is incomplete until published, the manuscript will be submitted to peer reviewed journals for publication.

5. Results

5.1 Socio-demographic characteristics of patients

There were 384 patients (199 males and 185 females) aged between 0 years to 15 years from whom urine sample were collected. Of these patients, 61 (15.9%) had significant bacteriuria. Table 1 show the sex and age of patients with significant bacteriuria. Of the 185 females, 36 (19.5%) came up with positive cultures while 25 (12.6%) of the 199 males had significant bacteriuria indicating that female patients were more affected than male patients. As can be seen from table 1, the largest number of study subjects were below age 3 years and the largest positive culture was obtained from this age group accounting 35 (57.4%.) positive culture out of 61 positive culture.

Table 1. Distribution of bacterial isolates in relation to male and female in each group at Yekatit 12 Hospital Medical College, 2014.

Age in years	Males with positive culture	Females with positive culture	Total	Percent (%) of Total
< 3	16 (n= 110)	19 (n=83)	35	35/61(57.4%)
3-6	1 (n=13)	3 (n=20)	4	4/61 (6.6%)
6-9	1 (n=26)	8 (n= 30)	9	9/61(14.8%)
9-12	2 (n=24)	4 (n=29)	6	6/61 (9.8%)
12-15	5 (n= 22)	2 (n=33)	7	7/61 (11.5%)
Total	25 (40.9%)	36 (59.01 %)	N= 61	100%

Where: **n** stands for the number of patients in each age group

N stands for the total number of male female positive patients in each age group

Bacterial species belonging to six genera were isolated and identified from 61 positive cultures and the genera were *Escherichiai, Klebsiella, Staphylococcus, Proteus, Acnitobacter* and *Entrococcus* (table 2*).* As shown in Table 2*, E. coli* was isolated in 28 cases (28/61, 49.5 %), followed by *Klebsilla* spp. in 17 cases (17/61, 27.9%), *Staphylococcus spp.* in 5 patients (5/61, 8.2%.) (*S. aureus* in one and coagulase negative *staphylococci* in 4 case), *Entrococcus* in 7 case (7/61, 11 5%), *Proteus spp.* in 3 cases (3/61, 4.9%)) and *Acenitobacte* in one case (1/61,1.6%). Of bacterial isolates *E. coli* was found out be the most common pathogen followed by *Klebsiella*. Furthermore *E. coli* and *klebsiella* were the most common pathogen in female patients accounting 71. 4% and 64.7% respectively.

Table2. Distribution of bacterial isolates in relation to sex at Yekatit 12 Hospital Medical College, 2014.

Bacterial isolates	Female	Male	Total
E. coli	20/28 (71.4%)	8/28(28.6)	28/61(45.9%)
Klebsiella spp	11/17 (64.7%)	6/17 (35.3)	17/61(27.9%)
Proteus spp.	-	3/3 (100%)	3/61(4.9%)
Acenitobacter spp	-	1/1(100%)	1/61 (1.6%)
Entrococcus spp.	4/7(57.1%)	3/7 (42.9%)	7/61 (11.5)
S.aureus	1/1 (100%)	-	1/61 (1.6%)
CONS	-	4/4 (100%)(	4/61(6.6%)
Total	36 (59.0%)	25 (40.9%)	61(100%)

CONS= Coagulase negative Staphylococci

Isolation rate of UTI with regards to circumcision revealed that out of 178 circumcised male study subject 10.1% (18/178) were culture positive but from a total of 21 uncircumcised study subjects 33.3%(7/21)were culture positive. As can be seen in Table 3,circumcision was significantly associated [COR, 95% CI: 0.225(0.080-0.630), P= 0.005] with urinary tract infection.

Table 3. Association of UTI in relation to circumcision at Yekatit 12 Hospital Medical College, 2014.

		UTI			
		No	Yes	COR 95% CI	P value
circumcision	No	14(66.6%)	7(33.3%)	1	
	Yes	160(89.9%)	18(10.1%)	0.225(0.080-0.630)*	0.005

* Significant at P value < 0.05, COR: crude odds ratio,

5.2 Antibiotic sensitivity testing:

Drug susceptibility profile of bacterial isolates is given in table 4 and 5. *E. coli* and *Klebsiella spp*. were not 100% susceptible to any of 11 antibiotics tested. On the contrary *Acentobacter* spp. was 100% resistances to three antibiotics, gentamycin , trimethoprim-sulfamethoxazole, and agumentin . But they were 100% susceptible to ciprofloxacin, cefuroxim ,norfloxacline and ceftazidime . On the other hand, *proteus spp*. were 100% sensitive to all drugs except nitrofurantion (Table 4). Also high degree of resistance was observed in *Entrococcus spp.* Species of *Entrocuccus* were resistance of 71.4 to chloramphenicol and 85.7% to both trimethoprim-sulfamethoxazole and erythromycin (Table 5). As shown in table 5, *S.aurues* was 100% susceptible to almost all drugs while coagulase negative staphylococci were not as susceptible as S. aureus.

Table 4. Number and percent of resistant strains of gram negative uropathogens at Yekatit 12 Hospital Medical College, 2014.

Bacteria isolated		**Antimicrobial agents tested**										
		CIP	CXM	GM	NA	SXM	F	AMP	C	NOR	CTX	CAZ
	Total No.	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**	**No. (%)**
E. coli	28	6 (21.4)	9 (32.1)	8 (28.6)	9 (32.1)	19 (67.9)	5 (17.9)	11 (39.3)	8 (28.6)	7 (25.0)	9 (32.1)	9 (32.1)
***Klebsiella* spp.**	17	5 (29.4)	15 (88.2)	14 (82.4)	9 (52.9)	15 (88.2)	5 (29.4)	12 (70.6)	10 (58.8)	6 (35.3)	15 (88.2)	15 (88.2)

***Acinetobacter* spp.**	1	0	0	1 (100)	1 (100)	1 (100)	0	1 (100)	0	0	0	0
***Proteus* spp**	3	0	0	0	0	0	3 (100)	0	0	0	0	0
Total	49	11 (22.5)	24 (48.9)	23 (46.9)	19 (38.7)	35 (71.4)	13 (26.5)	24 (48.9)	18 (36.7)	13 (26.5)	24 (48.9)	24 (48.9)

Agumentin(AMP) [30㎍], Norfloxacine(NOR) [30㎍]. Gentamicin(GN) [30㎍], Ciprofloxacin(CIP) [5㎍], Nalidixicacid(NA) [30㎍], Nitrofurantion(F) [30㎍], Ceftazidine(CAZ) [30㎍], Cefotaxim(CTX) [30㎍], Chloramphenicol(C) [30㎍], Trimetroprimsulfamethoxazole(SXT) [1.25/23.75㎍], cefuroxime(CXM) [30㎍].

Table 5. Number and percent of resistant strains of gram positive uropathogens at Yekatit 12 Hospital Medical College, 2014.

Bacteria isolated		**Antimicrobial agents tested**												
	Total No	P **No. (%)**	CIP **No. (%)**	C **No. (%)**	E **No. (%)**	OX **No. (%)**	CXM **No. (%)**	GM **No. (%)**	KF **No. (%)**	SXM **No. (%)**	F **No. (%)**	DA **No. (%)**	AMP **No. (%)**	CRO **No. (%)**
Enterococcus spp.	7	0	1 (14.3)	5 (71.4)	6 (85.7)	_	3 (42.9)	0	3 (42.9)	6 (85.7)	1 (14.3)	0	3 (42.9)	3 (42.9)
CONS	4	0	1 (25.0)	1 (25.0)	4 (100)	_	4 (100)	0	0	4 (100)	0	0	1 (25.0)	1 (25.0)
S. aureus	1	1 (100)	0	0	1 (100)	0	0	0	1 (100)	0	0	0	1 (100)	0
Total	12	1 (8.3)	2 (16.6)	6 (50)	11 (91.6)	0	7 (58.3)	0	4 (33.3)	10 (83.3)	1 (8.3)	0	5 (41.6)	4 (33.3)

CONS= Coagulase negative Staphylococci

penicillin(P) [10U], Agumentin(AMP) [30㎍], Cepalothin(KF) [10㎍], Clindamycine(DA) [30㎍], ,Ceftriaxone(CRO) [10㎍], Gentamicin(GN) [30㎍], Erythromycin(E) [15㎍], Ciprofloxacin(CIP) [5㎍], Oxaciline(OX) [1㎍], Nitrofurantion(F) [30㎍], Chloramphenicol(C) [30㎍], Trimetroprim-sulfamethoxazole(SXT) [1.25/23.75㎍], cefuroxime(CXM) [30㎍].

Multidrug resistance (MDR) to two or more drugs was observed in 11/12 (91.7%) and 34/49 (69.4%) of grampositive and gram negative bacteria, respectively (Tables 6 and 7). The overall prevalence of MDR in both groups was 45/61 (73.7%).

Table 6; Multi-drug resistance pattern of Gram-negative bacteria isolated at Yekatit 12 Hospital Medical College, 2014.

Combination of antibacterial agent	*E. coli*	*Klebsiella* Spp	*Acinetobacter* spp.	Total
	No %	No %	No %	No %
AMP,SXT	3(16.6)			3(8.8)
C,SXT	3(16.6)			3(8.8)
CIP,NOR,SXT	1(5.6)			1(2.94)
AMP,SXT,NA,GN	1(5.6)		1(100)	2(5.9)
AMP,CIP, SXT,NOR,GN,CAZ,CTX,CXM	4(22.2)			4(11.8)
AMP,SXT,NA,GN,CAZ,CTX,CXM,C	4(22.2)	4(26.7)		8(23.5)
CIP,SXT,NA,GN,CAZ,CTX,CXM,NOR,F	1(5.6)	5((33.3)		6(17.6)
AMP,SXT,NA,GN,CAZ,CTX,CXM,NOR,F	1(5.6)	6(40)		7(20.6)
Total	18(100)	15(100)	1(100)	34(100)

Agumentin(AMP) [30㎍], Norfloxacine(NOR) [30㎍]. Gentamicin(GN) [30㎍], Ciprofloxacin(CIP) [5㎍], Nalidixicacid(NA) [30㎍], Nitrofurantion(F) [30㎍], Ceftazidine(CAZ) [30㎍], Cefotaxim(CTX) [30㎍], Chloramphenicol(C) [30㎍], Trimetroprimsulfamethoxazole(SXT) [1.25/23.75㎍], cefuroxime(CXM) [30㎍].

Table 7. Multi-drug resistance pattern of Gram-positive bacteria isolated at Yekatit 12 Hospital Medical College, 2014.

Combination of antibacterial agent	*Enterococcus* spp	*S.aureus*	*CONS*	Total
	No %	No %	No %	No %
P , AMP,KF		1 (100)		1(9.09)
P, SXT ,E,CXM			3(75)	3 (27.2)
P,, SXT , E,C	2(33.3))			2(18.2)
P, SXT , E,C,AMP	1(16.7)		1(25)	2(18.2)
CXM,E,F,AMP,SXT	1(16.7)			1(9.09)
CXM,E,F,KF,CRO,SXT,C	1(16.7)			1(9.09)
C,E,F,KF,CRO,SXT	1(16.7)			1(9.09)
Total	6(100)	1(100)	4(100)	11(100)

penicillin(P) [10U], Agumentin(AMP) [30㎍], Cepalothin(KF) [10㎍], Clindamycine(DA) [30㎍], ,Ceftriaxone(CRO) [10㎍], Gentamicin(GN) [30㎍], Erythromycin(E) [15㎍],Ciprofloxacin(CIP) [5㎍], Oxaciline(OX) [1㎍], Nitrofurantion(F) [30㎍], Chloramphenicol(C) [30㎍], Trimetroprim-sulfamethoxazole(SXT) [1.25/23.75㎍], cefuroxime(CXM) [30㎍].

6. DISCUSSION

The present study was undertaken to determine the prevalence of UTIs, to evaluate the bacterial agents involved in UTI and determine the drug susceptibility profile of bacterial uropathogens. A total of 384, study participants were enrolled in the present study of which 199 (51.8%) were males and 185 (48.2%) females. The ages of study subjects ranged from 0 to 15 years. .

Out of the 384 study subjects (185 female and 199 males) between the ages of 0 and 15 years) that participated in this study only 61(15.8%) had urine samples with significant bateriuria. The findings of this study was similar to that reported in Nigeria (14), Iran (23) and Egypt (28) where a prevalence rate of UTI documented were 11.96% 16.2% and 15.05% respectively. However, the result obtained in this study (15.8%) appeared to be higher when compared with those reported in Philadelphia (19), USA (6), India (25) and Nigeria (29). A prevalence rate reported were 3.3%, 7.0 %, 4% and 3.0% respectively. On the other hand prevalence rate of UTI 0btained in this study was much less than reported by Olowu (1996) which was 28.1%.

The present study revealed that a higher percentage of urinary tract infection in females than males. Of the 185 females, 36 (, 19.5%) came up with positive cultures while 25 (12.6%) of the 199 males had significant bacteriuria indicating that female patients were more affected than male patients. Furthermore, the largest numbers of study subjects were patients below age 3 years and out of 61 culture positive study subjects below age group three accounted 35 (57.4%). Our result in this regard was comparable to that of Nigeria (14). The higher incidence of urinary tract infections in females might be as a result of shorter urethra and the proximity of their reproductive organ to the anus.

Sixty one bacterial isolates were recovered from this study. They were *E. coli, Klebsiella spp*., *proteus spp*. *Entrococcus spp*. *Acinetobacter spp. S.aureus* and Coagulase negative *staphylococci* Of these, *E. coli* was the most common organism isolated from patients with significant bateriuria and was isolated from 28 (45.9%) followed by *Klebsiella spp.* in 17 cases (27.9%), *Entrococcus spp* from 7 case ((11.5%, *coagulase negative staphylocci* from 4 case (6.6%)) *proteus spp*. from 3 cases (4.9%) and *S. aureus* from 1 case((1.6). The pattern and the frequency of bacteria isolates obtained in this study were comparable with the

findings earlier similar studies. The findings of a study conducted in Iran (23), showed that among bacterial isolates 40% is accounted by *E. coli* and this was followed by *Klebsiella spp.* (17.9%); Coagolase negative *Staphylococcocci* (12.3%); *Enterococcus spp.* (8.7%); *Pseudomonas spp*. (6.7%); and *s.aureus* (3.6%). A study in Nigeria (14) reported a total of 36 bacterial isolates of which *E.coli* was the predominant organism and was responsible for (52.77%) of the cases of UTI and this was followed by *Klebsiella sp*. (25%), *Proteus mirabilis* (13.89%), *Streptococcus faecalis* (5.56%) and *Pseudomonas aeruginosa* (2.78%). Similarly, a study conducted in Egypt (28), indicated that among all bacteria isolated in their study *E. coli*, and *Klebsiella spp.* were the most commonly isolated bacteria accounting 58.1% and 41.9% respectively.

A study conducted in Philadelphia (19), indicate that uncircumcision as one of the risk factors for UTI also a study in USA (6), showed that the prevalence of UTI was less in of circumcised males (2.4 %) than of uncircumcised males (20.1 %). This study has also showed that circumcised (10.1%) male patients were less susceptible to bacterial pathogen than uncircumcised (33.3%) patients.

Drug susceptibility profile of gram negative and gram positive bacteria tested in the present study was variable. For instance, *E. coli* and *Klebsiella spp*. were not 100% susceptible to any of 11 antibiotics tested. However percentage resistance of *Klebsilla* was much higher when compared to *E.coli.* Eighty eight percent of *Klebsiell spp*. were resistant to cefotaxim, ceftazidim, trimetroprimsulfamethoxazole and cefuroxime. The drug susceptibility profile of *E.coli* and *klebsiell spp*. obtained in the present study were similar with the findings in IIorin (13). However, drug susceptibility profile of *E. coli* in this study strongly contradicts with the findings reported in Tanzania (27). On the contrary *Acentobacter spp*. was 100% resistances to three antibiotics, gentamicin, trimetroprimsulfamethoxazole , agumentin, and nalidixic acid and 100% susceptible to ciprofloxacin, cefuroxime , norfloxacine, cefotaxim,chloramphenicol and ceftazidim . The present finding with regards to this isolated strongly contradicted with the findings in Iran (23). On the other hand, *proteus spp*. were 100% sensitive to all drugs except nitrofurantion. Also high degree of resistance was observed in *Entrococcus spp.* Species *Entrocuccus* were 100% senstive to penicillin but were resistantnt to71.4% to chloramphenicol and 85.7% to both trimetroprimsulfamethoxazole and Erythromycin. Simiarily, *S. aurues* was 100% susceptible to almost all drugs while coagulase negative staphylococci were not as susceptible as *S.*

aureus. This study also showed that both gram positive and gram negative bacteria's were Multidrug resistance (MDR) to two or more drugs with overall prevalence of 45/61 (73.7%) in both groups.

7. Limitations of the study

- Lack of such similar studies in Ethiopia for the purposes of comparison.
- Absence of some biochemical reagents and antibiotic discs might influence the appreciation of complete profile of isolates
- Absence of antibiotic to done minimal inhibitory concentration .
- Serotyping and genotyping of bacterial isolates especially multidrug resistant organisms was not possible due to limited laboratory facility.
- The data was not linked with chemical findings.

8. Conclusions

This study determines the antimicrobial Susceptibility Profile of Bacterial Uropathogen Isolates from Pediatric Patients and highlighted the major bacterial uro-pathogens involved in UTI up to my knowledge for the first time in the country. Furthermore, species bacterial pathogens and their frequency was consistent with the usually reported pattern, with *E. coli* being the most common organism isolated followed by *Klebsiella. Spp.* In this study Majority of gram negative species were susceptible to ciprofloxacin, norfloxacine and nitrofurantion (except *proteus spp)* and gram positive species were susceptible *to* ciprofloxacin Clindamycine and gentamicin so this drugs can be used to treat UTI in children however Most of bacterial isolates were multidrug resistant and **i**t is therefore suggested that appropriate antimicrobials should be administered to reduce the risk of multi drug resistant organisms developing and avert ineffectiveness of antibiotics.

9. Recommendations

- A widespread screening program for UTI in pediatric should be implemented to know the exact prevalence, as there are no studies on the subject.
- Another study should be implemented to measure resistance rate of uropathogens to commonly used antibiotics;
- Policies must be put on antibiotic prescribing as the resistant rate in all antibiotics in this study was high.

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11. Annexes

Annex I: English Versions of Participant Information Sheet (for mothers or guardians)

You are invited to participate in a study to be conducted by MSC student at Addis Ababa University, college of health sciences, School of allied health science, Department of medical

laboratory science, please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of this study is the Prevalence and Antimicrobial Susceptibility of Bacterial Uropathogens Isolated from Pediatric Patients at yekatit 12 hospital medical college in Addis Ababa Ethiopia. It is aimed to identify microorganisms responsible for UTI, to explore sensitivity patterns of identified microorganisms to certain antibiotics used in the treatment of UTI, and to study the relation between some demographic variables and UTI.

Participation in this study is exclusively voluntarily. If you are not interested to participate to your child or if you once decide to participate and with draw your children at any time, there will be no consequences and your child will get all the services provided in the hospital with no problems. If you decide to participate your child, you have to sign on the consent form and you may obtain a copy of this information sheet.

What is expected from me and my child as a participant of the study?

As a participant of this study you are expected to agree that 2-3 ml urine from your child which is drawn for your own diagnostics test and the left over sample will be taken for the research after the requested test is done. In addition you are expected to give answers for some questions about yours and your child health and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals out of this hospital. But the name of you or your child, address and phone number will not be disclosed and rather than identification code will be used in such conditions.

How much time will I and my child spent to participate in this study?

You will spend 20-25 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

What are the risks of participating in this study?

There are no anticipated risks to your participation. Because I took left over urine specimen after the requested test is done.

How our information is to be kept in secret?

All information that you give and the results from your child specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What are the benefits from participation?

Since this study is Msc student research, there will not be payment for participants. But your child participation is important for assessing Prevalence and Antimicrobial Susceptibility of Bacterial Uropathogens Isolated from Pediatric Patients

What are our rights as a participant of this study?

You have the right to withdraw your child from the study at any time and all the services provided in the hospital will not be discontinued. You have also welcomed if you have any question for further explanations about the study. You can get the results of the analysis.

What can I do if I have a problem or question?

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are: **Investigator:** Yamirot Merga (BSc), +251911398493

Email- ymerga@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU +251911039162

For additional information, please contact Addis Ababa University, College of Health Sciences,

Department of Medical Laboratory Sciences at: Telephone +251112755170

Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign,please understand purpose of the study, procedure,risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question. I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Agree to participate? Yes---------- No----------

Annex II: Amharic Versions of Participant Information Sheet (for mothers /guardians)

አዲስ አበባ ዩኒቨርሲቲ,የጤና ሣይንስ ኮሌጅ,የአላይድ ጤና ሣንይስ ት/ቤት,የሕክምና ላቦራቶሪ ሣንይስ ክፍል

እድሚያቸው ከ 15 ዓመት በታች የሆኑ ልጆች ላይ የሽንት ናሙና ተወስዶ ለሚሰራው ጥናት ለሚሳተፉ ልጆች ለእናቶች / አሳዳጊዎች የተዘጋጀ መረጃ

በአዲስ አበባ ዩኒቨርሲቲ፣ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል።እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን/ትን ማንኛዉም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ ዕድሚያቸው ከ 15 ዓመት በታች በሆኑ ልጆች ላይ የሽንት ፈሳሽ ተወስዶ ለሚሰራው ጥናት ነው ። አላማውም የኩላሊት ህመም አምጪ የሆነ ባክቴሪያ ለመለየትና ባክቴሪያውንም ለማጥፋት የሚረዳውን የተሻለ መድኃኒት ለመለየት ነው ። እርስዎ ና ልጅዎ በዚህ ጥናት ላይ የሚኖራችሁ ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ።በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቆረጥም።በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል።ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ።

ልጄ የጥናቱ ተሳታፊ በመሆኑ የሚጠበቅበት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ 3 ሚሊ ሌትር የሽንት ፈሳሽ ናሙና ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚወሰድና ለጥናቱ እንዲዉል መስማማት ይጠበቅብወታል።ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል።ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን እንዲሁም የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል ልጅዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል።በተጨማሪም ስለርስዎና ስለልጅዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል።

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፤የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ 20-25 ደቂቃ ያስፈልጋል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮቸ ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም።ናሙና የሚሰበሰበዉ ከልጅዎ ከተወሰደ የሽንት ናሙና ሃኪሙ ያዘዘለት ቤተ-ሙከራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና

የልጄ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስና ስለልጅ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው።ከዚያም በላይ ስለ እርስና ስለ ልጅዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም። ነገር ግን የእርስ ተሳትፎ ህጻናትን ለመርዳትና ህጻናቱ በባክቴሪያ ሲጠቁ በአጭር ጊዜ ለመመርመር ይረዳል።

የልጄ በዚህ ጥናት ተሳታፊ መሆኑ መብቱ ምንድን ናቸው ?

በጥናቱ ውስጥ ያላችሁን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጅን ከጥናቱ በማግለል ምክንያት የሚቀርበት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱ በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለበት። የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ያምሮት መርጋ (ቢኤስሲ) +251911398493

Email- ymerga@gmail.com

አማካሪ ፡ አዳነ ቢተው(ፒኤችዲ), DMLT, AAU +251911039162

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርስቲ የጤና ትምህርት የህክምና ላቦራቶሪ ክፍልን በ ስልክ ቁጥር +251112755170 እባክዎ ያነጋግሩን ።

Annex III: English Versions of Consent form (for mothers/guardians)

Code number----------

Name of mother/guardian for child study subject----------

I have been informed about the study which is aimed to assess Prevalence and Antimicrobial Susceptibility of Bacterial Uropathogens Isolated from Pediatric Patients. For this study urine will be collected from my child which is taken for the child's own diagnostics test and the left over sample will be taken for the research after the requested test is done. The aims of the study were well explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make my child withdraw from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the urine taken from my child for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child sample measured for free via the counselor nurse.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Participant's mother/guardian signature /finger print ----------

Name of deponent ----------signature----------

(For mothers unable to read)

Name of counselor nurse --------signature----------

Date----------

Annex IV: Amharic Versions of Consent form (for mothers/guardians)

<u>የተሳታፊወች ስምምነት ማረጋገጫ ቅጽ</u>

የሚስጥር ቁጥር ----------

የተሳታፊዉ ልጅ እናት/አሳዳጊ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ‹‹ እድሚያቸው ከአስራ አምስት አመት በታች በሆኑ ልጆች ላይ የሽንት ናሙና ተወስዶ ስለሚሰራው ጥናት በቂ ገለጻ ተደርጎልኛል። ለጥናቱም ከልጆች የተወሰደ ፈሳሽ ናሙና ከተወሰደ የታዘዘለትን የላቦራቶሪ ምርመራ ተሰርቶ ሲያልቅ የተረፈዉ ናሙና እንደሚያስፈልግ ተገልጾልኛል።የጥናቱን አላማዎችም ተረድቻለሁ።

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ።በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቱ የተጠበቀ እንደሆነ ተገልጾልኛል።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው። የሽንት ናሙና ለምርምር እንደሚውልም ተረድቻለው ።በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ።የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

የተሳታፊዉ ወላጅ ወይም አሳዳጊ ፊርማ /የጣት አሻራ ----------

የምስክር ስም 1---------- ፊርማ---------

2----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች**)**

የአማካሪ ነርስ ስም ---------- ፊርማ

ቀን----------

Annex VII: Data collection form

Date	Patient name	Ser. No	age	sex	Circumcision status(for	Antibiotic treatment	Type of specimen	Culture result	Sensitiv

					male)									
					yes	**no**	**yes**	**no**			**C**	**CIP**	**CXM**	**OX**

Annex V: Procedure for specimen collection and processing

I. Laboratory procedure for collection and culturing of urine sample

1. In infant and pre-toilet trained children urine will be obtained by catheterization method and in older children Mid-stream urine samples (MSU) will be collected in sterile disposable containers under the supervision patient family.
2. Label the sample as soon as possible with the patient code number
3. Urine samples will be inoculated on MacConkey/ CLED and blood agar plates using calibrated inoculating loop with a capacity of 0.001ml
4. Incubate the plate aerobically at 35-37 oC for 18-24 hours.
5. Examine and report the culture; look for colony characteristics gram reaction and perform biochemical
6. Determine drug susceptibility pattern of the isolated organism

II. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with the date and patient's name and number.

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or chemicals (methanol).

5. Cover the fixed smear with crystal violet stain for 30-60 seconds.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

7. Tip off all the water, and cover the smear with lugol's iodine for 30-60 seconds.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean

water.

10. Cover the smear with neutral red or safranine stain for 2 minutes.

11. Wash off the stain with clean water.

12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram-negative bacteria ----------pale to dark red

III. Laboratory procedure for Biochemical testing

Biochemical tests for gram positive bacteria: Gram-positive cocci will be identified based on their gram reaction, catalase and coagulase tests results.

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure

1. pour 2-3 ml of 3% hydrogen peroxide to a test tube

2. using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution

3. look for immediate bubbling

4. interpretation :

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Positive catalase control: *Staphylococcus* species

Negative catalase control: *Streptococcus* species

Coagulase test

This test is used to identify *S. aureus* which produces the enzyme coagulase

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

1. place a drop of physiological saline on two separate slides

2. emulsify the test organism in each of the drop to make thick suspension

3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the

organism within 10 seconds

4. Clumping within 10 secs . . . . . *S. aureus*

No clumping within 10 secs . . . . . No bound coagulase

Controls

Positive coagulase control: *Staphylococcus aureus*

Negative coagulase control: *Escherichia coli*

Biochemical test for gram negative bacteria:- Identification of gram negative bacteria will be based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colony of test organism in 5 ml nutrient broth.

2. A loop full of the bacterial suspension is inoculated in to KIA, indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, manitol, urea agar ,oxidase and motility medium.

3. Incubate at 35-37 Oc for 18-24 hours

4. Look for color change (turbidity for motility) of the medium

5. Identify the test organism by considering the result of the ten biochemical tests

IV. Laboratory procedure for Antimicrobial sensitivity testing

Procedure

1. Emulsify several colonies of similar appearance of test organism in small volume of nutrient broth.
2. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an over night broth culture.
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
4. spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a similar inoculation technique, inoculate an overnight broth culture of the Control organism evenly across the upper and lower third of the plate.
6. using a sterile forceps or needle ,place the antimicrobial disc on the inoculated plate
7. incubate the plate aerobically at 35-37oC For 18-24 hours
8. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light
9. Measure the radius of the inhibition zone. interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard
   Sensitivity (S): Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.
   Intermediate (I): Zone radius is more than 3mm smaller than the control but not less than 3mm.
   Resistant (R): No zone of inhibition or zone radius measure 2mm or less.

Annex VI: Declaration

Title of Project: The Prevalence and Antimicrobial Susceptibility Profile of Bacterial Uropathogens Isolated from Pediatric Patients at Yekatit 12 Hospital Medical College**,** Addis Ababa, Ethiopia.

I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Principal investigator:

Name: **Yamirot Merga (BSc)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Advisor:

Name: **Adane Bitew (MSc, PhD)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____