ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

SPECTRUM AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIURIA ISOLATED FROM PATIENTS ATTENDING ARSHO ADVANCED MEDICAL LABORATORY WITH URINARY TRACT INFECTIONS BY USING VITEK 2 COMPACT SYSTEM, ADDIS ABABA, ETHIOPIA

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A MSC THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF ALLIED HEALTH SCIENCE, COLLEGE OF HEALTH SCIENCE, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CLINICAL LABORATORY SCIENCE (Diagnostic and Public Health Microbiology Specialty track)

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LIST OF ABBREVIATIONS/ACRONYMS

AAU	Addis Ababa University
AES	Advanced Expert System
AAML	Arsho Advanced Medical Laboratory
AST	Antibiotics Susceptibility Test
BAP	Blood agar plate
CA-UTI	Community-acquired urinary tract infection
CFU	Colony forming unit
CLED	Cysteine lactose electrolyte deficient medium
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase negative *Staphylococci*
DST	Drug Susceptibility Test
ESBL	Extended-spectrum-ß-lactamase
EUAST	European Antimicrobial Susceptibility Testing
HA-UTI	Hospital-acquired urinary tract infection
ID	Identification
MIC	Minimum inhibitory concentration
MSU	Mid stream urine
NaCl	Sodium Chloride
PC	Personal computer
PI	Principal Investigator
SOPs	Standard operating Procedures
SPSS	Statistical Package for Social Sciences
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

Background: Urinary tract infection (UTI) is a very common infection both in the community and hospital patients. Development of drug resistance by bacteriuria is a growing problem. Accurate identification of bacteria implicated causing UTIs and determining their drug susceptibility pattern is critical for efficient management of patients with UTIs; that has significant clinical and financial benefits, via reduction of mortality rates and overall hospitalization costs. The aim of this study was to determine the spectrum of bacteria implicated in causing urinary tract infections and their drug susceptibility profile.

Methodology: The present study was a single institutional cross-sectional study carried out at Arsho Advanced Medical laboratory, Addis Ababa, Ethiopia from March to July 2016. Clean-catch midstream urine was collected from study participants and was inoculated onto primary isolation culture media with calibrated loop. All plates were incubated at 37 ^{0}C for 18-24 hours and the number of colonies was counted. Species identification and antibiotic susceptibility testing of bacteria were determined with the automated VITEK 2 compact system.

Result: Out of 712 urine samples processed, 256 (36%) yielded significant bacteriuria of which 208 (81.25%**)** were obtained from female study participants and 48 (18.75%) were from male study participants. Cases of 75 % UTIs were recorded among age group of 15-64 years. Of the 256 bacterial isolates recovered, *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus haemolyticus* and *Enterococcus faecalis* were the dominant bacterial isolates. Ampicillin (21.7%) and trimethoprim/sulfamethoxazole (33.7%) were the least effective drugs against gram negative bacteria while piperacillin/tazobactam (82.3%) was the most effective drug. Erythromycin (17.8%) was the least effective drug against gram positive bacteria and daptomycin (98.1%) was the most active drugs. The overall resistance to two and more antibacterial drugs was high (75.4% and 91.3% for gram negative and positive respectively).

Conclusion: Isolation of many unpredictable bacteria, urinary tract infection rate of 36%, and an overall higher resistance rate to two and more antibacterial (75.4% and 91.3% for gram negative and gram positive bacteria respectively) were documented. These highlight the need for nationwide study on the spectrum and drug sensitivity pattern bacterial uropathogens.

Key words: Antibiotics Resistance, Bacterial Profile, AAML, Urinary Tract Infection, VITEK 2 Compact.

1. INTRODUCTION

1.1. Back ground

Urinary tract infections (UTIs) are infections of the bladder (cystitis), the kidneys (pyelonephritis) and the urethra (urethritis). They are the second most common type of infection in the body and account for around 8.1 million visits to health care providers each year [1]. In contrast to men, women are more susceptible to UTI, and this is mainly due to anatomical differences (shorter urethra in woman, the proximity of the urethra to the anus), absence of prostatic secretion and pregnancy. Over 50% of all women will experience at least one UTI during their lifetime, with 20-30% experiencing recurrent UTI [2, 3]. UTIs are a morbid disease in terms of loss of working days and treatment cost [4]. In the United States alone, UTIs have been reported to cause >6 million outpatient visits [5] and 479 000 hospitalizations annually [6]. Furthermore, the annual treatment cost of UTIs in this part of the world has been estimated to be greater than 2.47 billion USD [2]. They are also important cause of septicemia resulting in high mortality rates [7].

Infants, pregnant women, patients with spinal cord injuries, diabetes, multiple sclerosis, acquired immunodeficiency disease syndrome or underlying urologic abnormalities are subjects that are at increased risk of UTI. In addition catheter-associated UTI is the most common nosocomial infection and in non-institutionalized elderly population [7].

Many previous studies have shown that *Escherichia coli* is the most common etiological agent of UTI in both hospital and community acquired infections while hospital acquired UTIs characteristically associated with a higher prevalence of *Enterococci* and *Coagulase-Negative Staphylococci (CNS)* [8–12]. In addition *Klebsiella pneumonia, Streptococcus agalactiae*, *Proteus mirabilis, Viridans streptococci*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Staphylococcus aureus* have been identified as etiologic agent of UTI [7].

Due to the rapidly evolving adaptive strategies of bacteria, the etiology of UTI and the antibiotic resistance profile of bacterial uropathogens have changed considerably over the past years, both in community and nosocomial infection [12]. Many studies conducted from USA and Europe

have revealed increasing antibiotic resistance among uropathogenic *E. coli* to ampicillin, trimethoprim and sulfonamides [9, 10, 11]. Apparent shift in the etiological agents of urinary tract infection and the associated problem of antibiotic resistance amongst bacterial uropathogens from time to time and from one institution to another has initiated health institution to carry out continuous evaluation of UTIs from the view point of their spectrum and drug susceptibility testing.

Accurate identification of bacteria implicated causing UTI and determining drug susceptibility pattern of the etiologic agents is critical for efficient management of patients with UTI and is associated with significant clinical and financial benefits, via the reduction of mortality rates and overall hospitalization costs [13]. In view of this, identification and antimicrobial susceptibility testing (AST) of clinical isolates by means of fully automated systems have become a common practice in many laboratories. The VITEK 2 system is a new automated system designed to provide accurate identification and susceptibility testing results for most clinical isolates of both gram positive and gram negative bacteria. Apart from accurate identification and susceptibility testing shortened turnaround times, improved specimen handling, enhanced quality control, reproducibility and the ability to track results are further advantages of the system [14].

Unfortunately, in Ethiopian health care providing institutions identification and drug susceptibility profile of bacterial uropathognes have been carried by conventional methods that appeared to be inferior to fully automated systems. This study was designed to determine the spectrum of bacterial uropathogens and their antimicrobial susceptibility profile by employing VITEK 2 compact system among patients referred to Arsho Advanced Medical Laboratory private limited company with a complain of UTIs.

1.2. Statement of the Problem

Urinary tract infections (UTIs) are the second most common community and hospital acquired infections. Nearly 10% of people will experience a UTI during their life time [15]. Urinary tract infection (UTI) is the most commonly encountered nosocomial infection, and the major risk factor is urinary catheterization [16].

Despite of wide spread availability of antibiotics, urinary tract infection remains a worldwide therapeutic problem; not only as a nosocomial infection but also as a community acquired infection [17]. Resistance to commonly prescribed antibiotics for UTI is an expanding global problem in both developed and developing countries [18]. *E. coli* and other uropathogens are becoming increasingly resistant to commonly used antimicrobial agents, reducing the effectiveness of some standard regimens.

In most cases of UTI, empirical antibiotic therapy is initiated before the laboratory results of urine cultures are received. This is due to the identification of bacteria using conventional methods take long time for a result. Therefore, early identification of uropathogens is necessary and it could help to select appropriate antibiotics for treatment. Currently, automated microbiological techniques like VITEK 2 Compact system are introducing to solve these problems. In line with this, the current study was designed to determine the bacterial etiological agents of UTI, as well as their antimicrobial susceptibility pattern of the bacteriuria using VITEK 2 compact system urines sample that will be requested for urine culture and sensitivity test at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia.

1.3. Significance of the study

Knowledge of the spectrum and drug susceptibility profile of bacteriuria with urinary tract infections using VITEK 2 Compact system provides up-to date relevant information on the local etiologic microorganisms and its antibiotic susceptibility pattern in bacterial uropathogens. Accurate identification of bacteria implicated causing UTI and determining drug susceptibility pattern of the etiologic agents is critical for efficient management of patients with UTI and is associated with significant clinical and financial benefits, via the reduction of mortality rates and overall hospitalization costs. However, in Ethiopia identification and drug susceptibility profile of bacterial uropathogens have been carried by conventional methods that appeared to be inferior to fully automated systems. So this study done by automated VITEK 2 Compact system gives us the real spectrum and drug susceptibility profile of bacteriuria at the study site. The study also help the concerned bodies to formulate guidelines for choosing an effective antibiotic therapy and serve as a base line information for further studies.

2. LITERATURE REVIEW

UTIs refer to the presence of microbial pathogens within the urinary tract. It may be caused by any pathogen that colonizes the urinary tract (bacteria, fungi, parasites, and viruses). Research studies have defined urinary tract infection as the most common form of bacterial infection [19]. The incidence of UTI as a result of viral or fungal infection is considered to be rare phenomena. The spectrum of bacteria causing UTI and their antibiotic susceptibility profiles have been studied by various researchers across the globe in different times. This chapter reviews these studies.

A study carried out in Kuwait by Sweih, et al, in 2004 by using the VITEK automated system, revealed that wide range of pathogens were identified and the six overall most common isolates were: *E. coli*, accounting for 47%, followed by *Candida* spp. (10.8%), *K. pneumonia* (9.6%), *S. agalactiae* (GBS; 9.5%), *E. faecalis* (4.2%) and *P. aeruginosa* (4.1%). Amikacin provided the widest coverage amongst all the antibiotics tested followed by ciprofloxacin, gentamicin and piperacillin-tazobactam. For the gram-negatives, high resistance (26–63%) to ampicillin, amoxicillin-clavulanic acid, cephalothin and cefuroxime observed. None of the enterococci was resistant to the glycopeptides, but 38–60% of the *S. haemolyticus* were resistant to vancomycin or teicoplanin [20].

Another study in Kuwait by Benwan et al, using conventional methods and the VITEK identification card system in the Al-Amiri Hospital, showed that uropathogens causing UTI were detected in 26.6% of urine samples. *E. coli* accounted for 54.9% from community-acquired UTI (CA-UTI) and 36.4% from hospital-acquired UTI (HA-UTI), followed by *S. agalactiae* 12.7% and *K. pneumoniae* 10.8% from CA-UTI cases. *Candida* spp. 15.7% and *K. pneumoniae* 12.1% were the second and third most prevalent isolates, respectively, in HA-UTI. And high resistance rates were observed among the Enterobacteriaceae against ampicillin, cephalothin, ciprofloxacin, piperacillin and trimethoprim-sulfamethoxazole. About 12% and 17% of *E. coli* and *K. pneumoniae*, respectively, were resistant to 64 antibiotics [21].

A retrospective study by Dugal and Purohit in Mumbai hospital indicate that of the 112 tested samples the most prevalent pathogens were *E. coli* (80%) followed by *Klebsiell*a spp (16.07%). Majority of the isolates (59%) were from females. Results indicated that ampicillin, ampicillin/sulbactam, and third generation cephalosporins like ceftriaxone and cefpodoxime should no longer be considered as first line of drugs for UTI. Instead, amikacin, carbapenem drugs, combinations of piperacillin/tazobactum, cefopar/tazobactum and cefoperazone-sulbactum could be preferred for the treatment of complicated UTI. 27.6% of isolates were found to produce ESBLs among which 70.9% were *E. coli* isolates. ESBL producers showed around 70 % resistance to cefepime, ampicillin, ampicillin /sulbactum and ciprofloxacin [22]. A study by Darbandi F, in Boras University collage, Swedish, indicates that 302 samples were evaluated and composed of 71% gram negative, and 24% gram positive. *E. coli* account 23.2% of total was the popular bacteria in the study collection [23].

A study conducted in India by Prakash D and Saxena RS, indicated that the UTI prevalence was 53.82% in patients; the prevalence was significantly higher in females than in males (females: 73.57%; males: 35.14%; P = 0.000). Females within the age group of 26–36 years and elderly males of ≥48 years showed higher prevalence of UTI. Gram negative bacteria (90.32%) were found in high prevalence than gram positive (9.68%). *E. coli* (42.58%) was the most prevalent gram negative isolate. Nitrofurantoin (78.71%) was found the most resistant drug among all uropathogens. The second most prevalent isolate was *K. pneumoniae* (18.71%) followed by *P. aeruginosa* (12.90%), *S. aureus* (9.68%), *Proteus* spp. (9.03%), and *Enterobacter* spp. (7.10%). Tested carbapenems (MRP and IMP) were found the most susceptible drug against isolated uropathogens which showed 92.26% and 84.52% susceptibility, respectively [24].

In Nigeria study done by Kolawole AS, et al, 2009 indicated that of the 300 specimens examined in this study, 180 (60%) showed significant bacteriuria; 120 (66.67%) were females while 60 (33.33%) were males. Gram negative bacteria had a higher frequency of occurrence than gram positive constituting 140 (77.7%) of the total isolates. These included: *E. coli* 55 (30.56%); *P. aeruginosa* 42 (23.33%); *Proteus mirabilis* 29 (16.11%) and *Klebsiella aerogenes* 14 (7.78%). Gram positive bacteria accounted for 40 (22.22%) of the isolates. They include *S. aureus* 27 (15%); *S. saprophyticus* 13 (7.22%). It was also found that the rate of isolates of *E. coli* and *P. aeruginosa* were higher in isolates exclusively from females. In-vitro antibiotic susceptibility

tests revealed that the gram negatives bacteria were sensitive to quinolones (ofloxacin, ciprofloxacin, pefloxacin) and erythromycin, while the gram positive isolates were sensitive to lincomycin, erythromycin and quinolones. Nitrofurantoin, Ampicillin and Cotrimoxazole were poorly effective [25].

A study in a university hospital of Northern Greece by *Mantadakis E* indicated that overall, 221 urinary isolates were identified from 218 children with a documented UTI, including 170 (76.9%) *Escherichia coli*, 17 (7.7%) *Proteus spp*., 15 (6.8%) *Klebsiella spp.*, 9 (4.1%) *Pseudomonas aeruginosa*, 4 (1.8%) *Enterococcus faecalis*, 2 (0.9%) *Enterobacter spp.*, 2 (0.9%) *Morganella morganii* and *2 (0.9%) Serratia fonticola.* Only 80 (49.1%) of the 163 tested *E. coli* isolates were found to be susceptible to ampicillin, whereas susceptibility to amoxicillin/clavulanic acid (AMC), ampicillin/sulbactam, trimethoprim/sulfamethoxazole and nitrofurantoin was 78.3%, 78.9%, 75.3% and 96.9%, respectively [26].

A retrospective analysis study by Moroh et al, conducted in Ivory Coast, period from 2000–2011 indicates of 12,175 urine samples was carried out. The presence of bacteria was detected in about 25% of samples in which 3071 bacterial belonging to 12 species were identified. *Escherichia coli* were the dominant species (28.7%). Other main detected species were *Staphylococcus aureus* (17.4%), *Klebsiella pneumoniae* (14.9%) and *Enterobacter aerogenes* (10%). Resistance tests to antibiotics indicated very high rates of resistance to amoxicillin (78.9%), tetracycline (76.4%), and trimethoprim/sulfamethoxazole (77.9%) [27].

In Ethiopia there is no any study conducted before by using VITEK 2 compact system but by conventional method there is a study conducted in Jimma University by Beyene G and Tsegaye W, a hospital based cross sectional study conducted from 228 clinically-suspected cases of urinary tract infections and tested bacteriologically using standard procedures. Significant bacteria were detected from 9.2% of the total patients. The most common pathogens isolated were *Escherichia coli (33.3%), Klebsiella pneumoniae (19%)* and *S. saprophyticus* (14.3%). *E. coli and K. pneumoniae* showed the highest percentage of resistance to ampicillin and amoxacillin (100%) however, all isolates of E. coli and K. pneumoniae were susceptible to ciprofloxacin. *S. saprophyticus* and *S. aureus* were resistant to ampicillin (100%) and amoxicillin

(66.7%). For all UTI isolates, least resistance was observed against drugs such as ceftriaxone, gentamycin and chloramphenicol [28].

A retrospective analysis by Kibret M, et al using conventional methods was done at Dessie Regional Laboratory in the period 2003 to 2010. Of the total 1404 samples, 319 (22.7%) were culture positive. *Escherichia coli* was the dominant isolate (63.6%) followed by *Klebsiella* spp. (8.5%) and *Proteus* spp. (8.2%). The overall resistance rates to erythromycin, amoxycillin, and tetracycline were 85.6%, 83.9% and 76.7%, respectively. The three most frequently isolated bacteria had resistance rates of 80.1%-90.0% to, amoxycillin, and tetracycline and sensitivity rates of 0 to 25% to nitrofurantoin, ciprofloxacin and gentamicin. Antibiogram of isolates showed that 152 (47.85%) isolates were resistance to two and more antimicrobials [29]. A study by *Tiruneh M, et al,* in Gondar two year study period by conventional methods. The commonest isolates were *E. coli*, *Klebsiella*, CoNS, *S. aureus*, *Proteus*, and *Citrobacter* species. Enterobacteriaceae isolates were developed 31% to 60% resistance to ciprofloxacin. Multi resistance isolates were predominant in study [30].

A study by Kabew G et al, an institution-based retrospective cross sectional population survey in Tikur Anbessa Specialized Teaching Hospital in 2011, using conventional methods. The overall prevalence of urinary tract infection was 23.32% and the highest prevalence was obtained among age groups 21-30 years (27.16%). The bacterial pathogens isolated were predominantly*, Escherchia coli:* 361 (44.62%), followed by *Klebsella* Spp: *136* (16.81%), Coaggulase negative *Staphylococci Spp:* 49 (6.06%) and *Entrococci* Spp: 41 (5.06%). The invitro drug sensitivity testing showed that both gram negative and gram-positive organisms were extremely resistant to Ampiciline: (83.93%), Amoxicillin: (78.87%) and Tetracycline: (77.75%) [31].

3. OBJECTIVES

3.1. General objective

To determine the spectrum of bacterial etiology and antibiotic susceptibility pattern of the bacteriuria that causes urinary tract infections in Arsho Advanced Medical Laboratory from March to July, 2016.

3.2. Specific objectives

- To determine the profile of bacteria implicated in causing UTIs and their drug susceptibility pattern by employing VITEK 2 compact system in AAML.
- To determine the drug susceptibility profile of bacteriuria.

4. MATERIALS AND METHODS

4.1. Study design, Study site and Study period

The present study was a single institutional cross-sectional study carried out in Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia from March to July 2016. Arsho Advanced Medical Laboratory is a private diagnostic advanced medical laboratory in Addis Ababa Ethiopia that delivers diagnostic testing in the country. Many clinical specimens are referred from all over the country to this advanced medical laboratory.

4.2. Population

4.2.1. Source Population

The source population was all UTIs patients attending at Arsho Advanced Medical Laboratory during the study period.

4.2.2. Study Population

Study population was those patients of all age group from which urine were taken for urine culture within the study period.

4.3. Sampling Technique and Sample size

A convenient sampling technique was employed among 712 study participants who meet the inclusion criteria from March to July 2016. The minimum sample size was estimated using single population formula and since there is no study conducted on the prevalence of bacteriuria using VITEK 2 Compact system in our setting, we use the proportion of 50 %(p= 0.5) with 95% confidence interval. Thus, the study will include at least 384 subjects, but assuming 10 % non-response rate, the sample size will be: n=384+10 %= 384+38= 422. But in

the study area, more than 40 Microbiology tests have done per day and of these no less than 15 are urine culture test, that we collect 712 urine sample sizes at study period.

4.4. Measurement

4.4.1. Dependent Variables

- Bacterial profile
- Antibacterial susceptibility pattern

4.4.2. Independent Variables

- Age
- Sex

4.4.3. Inclusion and Exclusion Criteria

Inclusion criteria

- All patients clinically suspected of UTI and consent to participate in the study were included.

Exclusion criteria

- Patients under drug treatment with in the past 2 weeks were excluded from the study.
- delayed samples

4.5. Data collection and Processing

4.5.1. Sample collection and inoculation of primary isolation culture media

Urine samples from UTI patients were analyzed at the microbiology laboratory of Arsho Advanced Medical Laboratory during the specified period of time. Clean-catch midstream urine was collected from the study participants with sterile wide mouthed urine cups. Urine collected

from each patient was inoculated onto Blood Agar base (Oxoid, Basingstoke, Hampaire, UK) to which 10% sheep blood is incorporated and Cysteine Lactose Electrolyte Deficient medium (Oxoid, Basingstoke, Hampaire, UK) by using a calibrated loop with a capacity of 10µl. Preparation and performance evaluation of culture media were done as per the instruction of the manufacture. All inoculated plates were incubated at 37 ^{0}C for 18-24 hours aerobically and the number of colonies was counted. Colony counts yielding bacterial growth of $\geq 10^{5}$ cfu/ml of urine were regarded as significant for bacteriuria. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram-stain and catalase test before inoculating them into AST, GN72 and GP71 cards.

4.5.2. Inoculum size determination

Quality control bacteria and pure cultures of bacterial isolates were suspended in 3 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube to achieve a turbidity equivalent to that of a McFarland 0.50 standard (range, 0.50 to 0.63), as measured by the DensiChek™ (bioMe´rieux) turbidity meter. These suspensions were used for the inoculation of AST, GN72 and GP71 cards.

4.5.3. Card sealing and Inoculation of cards

The cards were automatically filled by a vacuum device and were automatically sealed and subjected to a kinetic fluorescence measurement in accordance with the manufacturer's instructions. In brief, identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The filled cassette was inserted manually into VITEK 2 compact reader-incubator module. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells and inoculated cards were automatically sealed prior to loading into the carousel incubator. All card types were incubated automatically at 35.5 $\pm$ 1.0ºC. Each card was removed from the carousel incubator once every 15

minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data were collected at 15-minute intervals during the entire incubation period and final identification results were obtained in approximately 18 hours or less. All cards used were automatically discarded in a waste container.

The GN card is used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting gram-negative bacilli based on established biochemical methods and substrates measuring carbon source utilization, enzymatic activities and resistance. There are 47 biochemical tests and one negative control well. Final identification results are available in approximately ten hours or less. The GP card is used for the automated identification of 115 taxa of the most significant non-spore-forming gram-positive bacteria (primarily cocci) based on established biochemical methods and newly developed substrates. There are 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Final identification results are available in approximately eight hours or less.

4.5.4. Identification and determination of antimicrobial susceptibility

Species identification and antimicrobial susceptibility testing of both gram positive and gram negative bacteria were determined by using the automated VITEK 2 compact system (bioMérieux, France). The VITEK 2 compact system (bioMe´rieux) is an integrated modular system that consists of a filling-sealer unit, a reader-incubator, a computer control module, a data terminal, and a multicopy printer. The system detects bacterial growth and metabolic changes in the microwells of thin plastic cards by using a fluorescence-based technology. GN and AST-GN72 cards were used for the identification and susceptibility testing of fermenting and non-fermenting gram-negative bacilli while the GP and AST-GP71 cards were used for the automated identification and susceptibility testing of non-spore-forming bacilli and gram-positive cocci bacteria. The cards were automatically filled by a vacuum device and were automatically sealed and subjected to a kinetic fluorescence measurement in accordance with the manufacturer's instructions.

A GN and AST-GN72 card consists of an array of biochemical method and substrates for identification and an array antibiotic for drug susceptibility testing. Substrates and biochemical tests used for identification of gram negative bacteria were: Ala-Phe-Pro-Arylamidase, Adonitol,

L-Pyrrolydonyl-Arylamidase, L-Arabitol, D-Cellobiose, Beta-Galactosidase, H_2S production, Beta-N-Acetyl-Glucosaminidase, Glutamyl ArylamidasepNA, D-Glucose, Gamma-Glutamyl-Transferase, Fermentation/Glucose, Beta-Glucosidase, D-Maltose, D-Mannitol, D-Mannose, Beta-Xylosidase, Beta-Alanine arylamidasepNA, L-Proline Arylamidase, Lipase, Palatinose, Tyrosine Arylamidase, Urease, D-Sorbitol, Saccharose/Sucrose, D-Tagatose, D-Trehalose, Citrate(Sodium), Malonate, 5-Keto-D-Gluconate, L-Lactate alkalinisation, Alpha-Glucosidase, Succinate alkalinisation, Beta-N-Acetyl-Galactosaminidase, Alpha-Galactosidase, Phosphatase, Glycine Arylamidase, Ornithine Decarboxylase Base, Lysine Decarboxylase, Decarboxylase Base, L-Histidine assimilation, Coumarate, Beta- Glucoronidase, O/129 Resistance (comp.vibrio.), Glu-Gly-Arg-Arylamidase, L-Malate assimilation, Ellman and L-Lactate assimilation.

Antibiotics with their different concentration used for determination of drug susceptibility profile of gram-negative bacteria in this investigation were: Ampicillin (4,8,32), Amoxicilin/Clavulanic Acid (4/2,16/8,32/16), Cefalotin (2,8,32), Cefazolin (4, 16, 64), Cefepime (2,8,16,32), Cefoxitin (8,16,32), Cefpodoxime (0.5, 1, 4), Ceftazidime (1,2,8,32), Ceftriaxone (1,2,8,32), Cefuroxime (2,8,32), Ciprofloxacin (0.5,2,4), Gentamicin (4,16,32), Levofloxacin (0.25,0.5,2,8), Nitrofurantoin (16,32,64), Piperacillin/Tazobactam (2/4,8/4,24/4,32/4,32/8), Tetracycline (2,4,8), Tobramycin (8,16,64), Trimethoprim/sulfamethoxazole (1/19,4/76,16/304).

Similarly, the GP and AST-GP71 card consists of an array of biochemical tests for species characterization and antibiotics for drug susceptibility testing of gram positive bacteria. Substrates and biochemical tests used for identification of gram bacteria were:- D-Amygdalin, Phosphatidylinositol Phospholipase C, D-Xylose, Arginine Dihydrolase 1, Beta-Galactosidase, Alpha-Glucosidase, Ala-Phe-Pro Arylamidase, Cyclodextrin, L-Aspartate Arylamidase, Beta-Galactopyranosidase, Alpha-Mannosidase, Phosphatase, Leucine Arylamidase, L-Proline Arylamidase, Beta-Glucuronidase, Alpha-Galactosidase, L-Pyrrolidonyl-Arylamidase, Alanine Arylamidase, Tyrosine Arylamidase, D-Sorbitol, Urease, Polymixin B Resistance, D-Galactose, D-Ribose, L-Lactate alkalinization, Lactose, N-Acetyl-D-Glucosamine, D-Maltose, Bacirtacin Resistance, Novobiocin Resistance, Growth in 6.5% NaCl, D-Mannitol, D-Mannose, Methyl-B-D-Glucopyranoside, Pullulan, D-Raffinose, O/129 Resistance (comp. vibrio.), Salicin, Saccharose/Sucrose, D-Trehalose, Arginine Dihydrolase 2, Optochin Resistance.

Antibiotics with their different concentration used for determination drug susceptibility profile of gram positive bacteria in this investigation were:- Benzylpenicillin (0.125, 0.25, 1), Cefoxitin Screen (6), Ciprofloxacin (1, 2, 4), Clindamycin(0.5,1,2), Daptomycin (0.5, 1, 2, 4, 16), Erythromycin (0.25,0.5,2), Gentamicin (8,16,64), Inducible Clindamycin Resistance (CM 0.5, CM/E 0.25/0.5), Levofloxacin (0.25,2,8), Linezolid (0.5,2,8), Minocycline (0.12,0.5,1), Moxifloxacin (0.25,2,8), Nitrofurantoin (16,32,64), Oxacillin (0.5,1,2), Quinupristin/Dalfopristin (0.25,0.5,2), Rifampicin (0.25,0.5,2), Tetracycline (0.5,1,2), Tigecycline (0.25,0.5,1), Trimethoprim/Sulfamethoxazole (2/38,8/152,16/304) and Vancomycin (1,2,4,8,16).

4.6. Data Quality Assurance

Data quality was ensured through use of standardized data collection materials, proper orientation and intensive supervision during data collection by the principal investigator. All data quality control tools (pre-analytical, analytical and post-analytical stages) of quality assurance that were incorporated in standard operating procedures (SOPs) of the microbiology laboratory were strictly followed. Adequate specimen was collected using appropriate equipment and method. The collected urine specimen was transported promptly to the microbiology laboratory. All the equipment was checked for their functionality and performance by using standard procedures. Sterility and performance of culture media was tested prior to the actual work. For the quality control of susceptibility tests *E. coli*ATCC 25922, *P. aeruginosa*ATCC27853, *S. aureus*ATCC 25923 and *E. faecalis*ATCC929212 strains were used. In addition, well-trained and experienced laboratory professionals were participate in the laboratory analysis procedure. The data were checked for completeness and representativeness prior to entry.

4.7. Data Processing and Analysis

Data entry and analysis was done using SPSS statistical software version 20 (Statistical Package for Social Sciences, SPSS). Results obtained were analyzed by counts and percentages using Statistical methods. During analysis frequencies of the different variables was determined, cross-tabulations was used to compare frequencies. Descriptive statistics was used to describe the study participants in relation to relevant variables. Variables that were show a significant association was selected for further analysis. In all cases P-value less than 0.05 was considered as statistically significant. Finally, the result was presented on words and tables.

4.8. Ethical Clearance

All ethical considerations and obligations were duly addressed and the study was conducted after gaining approval by the Department Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Then a letter informing to Arsho Advanced Medical Laboratory and permission was obtained from Arsho Advanced Medical Laboratory to access data from study population. Informed written consent was obtained from participants before data collection. The respondent was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the study subjects were coded to remain confidentially. When the participants were found to be positive for fungal pathogen, they were informed by the hospital clinician and received proper treatment.

4.9. Dissemination of results

The result of the study was submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences. Oral presentation of the thesis will be making. In addition, a copy of this material will be submitted to Arsho Advanced Medical Laboratory, annual conferences of professional societies and other concerned bodies. The finding of the study will also be presented to the medical scientific community and manuscript will be submitted to peer reviewed journals for publication.

5. RESULT

5.1. Study subjects

Of the total of 712 urine samples processed during the study period, 519 (72.9%) were collected from female patients and 193 (27.1%) were from male patients. Among urine samples processed, 256 (36%) yielded significant bacteriuria of which 208 (81.25%) were obtained from female patients and 48 (18.75%) from male patients. The association of UTIs with gender was statistically significant [AOR, 95%CI: 2.463(1.651, 3.674), $P = 0.001$]. Cases of 75 % UTIs were recorded among patients with an age group of 15-64 years. Pediatric patients (0-14 years) and elderly (≥ 65 years) patients accounted for 11.3 % and 13.7 % of the total number of UTIs (Table 1). Urinary tract infection was the highest (43.8%) in patients of age group 25-44 followed by age groups of 45-64 (19.9%). Age groups of 1-14 [(AOR=0.196, 95% CI, 0.084-0.457), (p=0.001)], 25-44 [(AOR = 0.391, 95% CI, 0.225 – 0.678) ($P = 0.001$)], and age groups of 45-64 [(OR = 0.443, 95% CI, 0.245-0.802) ($P = 0.007$)] were statistically significant affected with UTIs.

Table 1: Gender, age distribution and association of study participants

Variable	Categories	No.(%) of samples processed	No. (%) of samples with UTI	P- value	AOR	95% CI
Gender	Female	519 (72.9)	208(81.25)	0.001*	2.463	(1.651, 3.674)
	Male	193 (27.1)	48(18.75)			
	Total	712 (100)	256(100)			
Age group[1]	< 1	28 (3.9)	18(7.0)	0.742	0.866	(0.368,2.041)
	1-14	55 (7.7)	11(4.3)	0.001*	0.196	(0.084,0.457)
	15-24	74 (10.4)	29(11.3)	0.050	0.503	(0.253,0.999)
	25-44	330 (46.3)	112(43.8)	0.001*	0.391	(0.225,0.678)
	45-64	152 (21.3)	51(19.9)	0.007*	0.443	(0.245,0.802)
	65+	73 (10.3)	35(13.7)			
	Total	712 (100)	256(100)			

[1]-WHO age classification for health [32], *P-value <0.05, statistically significance, AOR: adjusted odds ratio

5.2. Spectrum of Bacteriuria

A total 256 (27 species) bacterial isolates belonging to 14 genera were recovered, of which 175 of the isolates (15 species) were gram negative and 81 isolates (12 species) were gram positive bacteria. Of the total isolates, gram negative bacteria were the most common accounting for 68.4% of the total isolates. *E .coli and K. pneumoniae*, were the two predominant gram negative bacteria consisting of 52.6% and 7% of the total isolates respectively. Of the total bacterial isolates, gram positive bacteria accounted for 31.6%. *S. haemolyticus* and *E. faecalis* being the first and second predominant gram positive bacteria respectively (Table 2 and Table 3).

Table 2: Isolated gram negative bacteria, no and %

Microorganisms	Genus	Species	Number (%)
Gram-negative bacteria	*Escherchia*	*E. coli*	135(52.6)
	Klebsiella	*K. pneumoniae*	18(7.0)
		K. oxytoca	1(0.4)
	Pseudomonas	*P. aeruginosa*	3(1.2)
		P. fluorescens	1(0.4)
		P. luteola	2(0.8)
	Moraxella	*M. nonliquefiens*	3(1.2)
	Citrobacter	*C. diversus*	2(0.8)
		C.freundii	1(0.4)
	Acinetobacter	*A.baumannii*	2(0.8)
	Providencia	*P. alcalifaciens*	1(0.4)
		P. rettgeri	1(0.4)
	Francisella	*F. tularensis*	1(0.4)
	Morganella	*M. morganii*	1(0.4)
	Sphingomonas	*S. paucimobilis*	3(1.2)
Total	10	15	175(68.4)

Table 3: Isolated gram positive bacteria, no and %

Microorganisms	Genus	Species	Number (%)
Gram-positive bacteria	*Staphylococcus*	*S. haemolyticus*	18(7.0)
		S. aureus	9(3.5)
		S. warneri	9(3.5)
		S. epidermidis	8(3.1)

		S. hominis	6(2.3)
		S. lentus	3(1.2)
		S. saprophyticus	2(0.8)
	Streptococcus	*S. agalactiae*	8(3.1)
		S. porcinus	1(0.4)
	Enterococcus	*E. faecalis*	14(5.5)
		E. gallinarum	1(0.4)
	Kocuria	*K. kristinae*	2(0.8)
Total	4	12	81(31.6)

5.3. Antibacterial susceptibility pattern of gram-negative bacteria isolates.

The overall drug susceptibility profile of gram negative bacteria against the nineteen antibacterial drugs tested is summarized in table 4. Ampicillin had the highest overall resistance rate (78.3%) against gram negative bacteria followed by trimethoprim/sulfamethoxazole (SXM) (66.3%) and tetracycline (62.3%). Gram negative bacteria showed sensitivity towards Piperacillin/Tazobactam combination, cefoxitin, gentamicin and nitrofurantoin with the overall resistance rates 17.7%, 24%, 25.7%, and 29.1% respectively.

As far as species specific antimicrobial resistance rates are concerned, *E. coli*, the most frequently isolated bacterium, showed 77.8%, 70.4% and 69.6% resistance rates to ampicillin, SXT and tetracycline respectively. The least resistance rate (20%) of the bacterium was observed against nitrofurantoin. *K. pneumoniae*, the second most commonly isolated gram negative bacterium exhibited a resistance rate of 100% against ampicillin and 66.7% to SXT. The least resistance rate (5.6%) of the bacterium was observed against Piperacillin/Tazobactam combination and cefoxitin. *Pseudomas aeruginosa, Moraxella nonliquefiens* and *Sphingomonas paucimobilis* were the 3rd commonly isolated gram negative bacteria. *Moraxella nonliquefiens* and *Pseudomas aeruginosa* were 100% resistant to fifteen and nine drugs respectively. *Sphingomonas paucimobilis* on the other hand was 100 susceptible to all drugs tested. *Acinetobacter baumannii* the 4th most frequently isolated bacteria was 100% resistant to ten drugs.

Table 4: Antibacterial susceptibility pattern of gram-negative bacteria isolates**.**

Species	Patter	Antibacterial drugs (n, %):																		
	n	AM	AMC	CF	CZ	CXM	CXM A	FOX	CPD	CAZ	CRO	FEP	CIP	GM	LEV	FT	TM	TE	SXT	TZP
E. coli	S	30	74	55	78	76	74	104	84	87	88	76	67	97	60	108	82	41	40	106
(135)		(22.2)	(54.8)	(40.7)	(57.8)	(56.3)	(54.8)	(77)	(62.2)	(64.4)	(65.2)	(56.3)	(49.6)	(71.9)	(44.4)	(80.0)	(60.7)	(30.4)	(29.6)	(78.5)
	R	105	61	80	57	59	61	31	51	48	47	59	68	38	75	27	53	94	95	29
		(77.8)	(45.2)	(59.3)	(42.2)	(43.7)	(45.2)	(22.9)	(37.8)	(35.6)	(34.8)	(43.7)	(50.4)	(28.1)	(55.6)	(20.0)	(39.3)	(69.6)	(70.4)	(21.5)
K. pneumoniae	S	0(0)	14	8	9	10	10	17	10	10	10	9	15	14	16	7	11	10	6	17
(18)			(77.8)	(44.4)	(50)	(55.6)	(55.6)	(94.4)	(55.6)	(55.6)	(55.6)	(50)	(83.3)	(77.8)	(88.9)	(38.9)	(61.1)	(55.6)	(33.3)	(94.4)
	R	18	4	10	9	8	8	1	8	8	8	9	3	4	2	11	7	8	12	1
		(100)	(22.2)	(55.5)	(50)	(44.4)	(44.4)	(5.6)	(44.4)	(44.4)	(44.4)	(50)	(16.7)	(22.2)	(11.1)	(61.1)	(38.9)	(44.4)	(66.7)	(5.6)
P. aeruginosa	S	0(0)	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2	0(0)	2	2	3	3	0(0)	3	1	1	2
(3)			(33.3)							(66.7)		(66.7)	(66.7)	(100)	(100)		(100)	(33.3)	(33.3)	(66.7)
	R	3	2	3	3	3	3	3	3	1	3	1	1	0	0	3	0	2	2	1
		(100)	(66.7)	(100)	(100)	(100)	(100)	(100)	(100)	(33.3)	(100)	(33.3)	(33.3)	(0)	(0)	(100)	(0)	(66.7)	(66.7)	(33.3)
M. nonliquefiens	S	0(0)	3	0(0)	0(0)	0(0)	0(0)	3	0(0)	3	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3
(3)			(100)					(100)		(100)										(100)
	R	3	0(0)	3	3	3	3	0(0)	3	0(0)	3	3	3	3	3	3	3	3	3	0(0)
		(100)		(100)	(100)	(100)	(100)		(100)		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	
C. diversus	S	0(0)	0(0)	0(0)	0(0)	1	0(0)	0(0)	1	1	1	1	1	2	2	2	2	2	0(0)	2
(2)						(50)			(50)	(50)	(50)	(50)	(50)	(100)	(100)	(100)	(100)	(100)		(100)
	R	2	2	2	2	1	2	2	1	1	1	1	1	0(0)	0(0)	0(0)	0(0)	0(0)	2	0(0)
		(100)	(100)	(100)	(100)	(50)	(100)	(100)	(50)	(50)	(50)	(50)	(50)						(100)	
A. aumannii	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2	0(0)	2	1	2	2	0(0)	2	2	2	2
(2)										(100)		(100)	(50)	(100)	(100)		(100)	(100)	(100)	(100)
	R	2	2	2	2	2	2	2	2	0(0)	2	0(0)	1	0(0)	0(0)	2	0(0)	0(0)	0(0)	0(0)
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)		(100)		(50)			(100)				

Continues from table 4, antibacterial susceptibility pattern of gram-negative bacteria isolates…

Species	Pattern	Antibacterial drugs (n, %):																		
		AM	AMC	CF	CZ	CXM	CXMA	FOX	CPD	CAZ	CRO	FEP	CIP	GM	LEV	FT	TM	TE	SXT	TZP
P. luteola	S	1	2	1	1	1	1	1	1	2	2	2	2	2	2	1	2	2	2	2
(2)		(50)	(100)	(50)	(50)	(50)	(50)	(50)	(50)	(100)	(100)	(100)	(100)	(100)	(100)	(50)	(100)	(100)	(100)	(100)
	R	1	0(0)	1	1	1	1	1	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	0(0)	0(0)	0(0)	0(0)
		(50)		(50)	(50)	(50)	(50)	(50)	(50)							(50)				
K. oxytoca	S	0(0)	1	1	1	1	1	1	1	1	1	1	1	1	1	0(0)	1	1	1	1
(1)			(1000	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)		(100)	(100)	(100)	(100)
	R	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	0(0)	0(0)	0(0)	0(0)
		(100)														(100)				
P. fluorescens	S	0(0)	0(0)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0(0)	1	1
(1)				(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)		(100)	(100)
	R	1	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	0(0)	0(0)
		(100)	(100)															(100)		
M. morganii	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	1	0(0)	1	1	0(0)	1	1	0(0)	1
(1)											(100)	(100)		(100)	(100)		(100)	(100)		(100)
	R	1	1	1	1	1	1	1	1	1	0(0)	0(0)	1	0(0)	0(0)	1	0(0)	0(0)	1	0(0)
		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)			(100)			(100)			(100)	
C. freundii	S	1	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	1	1	0(0)	1	0(0)	0(0)	1	0(0)	0(0)	1
(1)		(100)								(100)	(100)	(100)		(100)			(100)			(100)
	R	0(0)	1	1	1	1	1	1	1	0(0)	0(0)	0(0)	1	0(0)	1	1	0(0)	1	1	0(0)
			(100)	(100)	(100)	(100)	(100)	(100)	(100)				(100)		(100)	(100)		(100)	(100)	
P. alcalifaciens	S	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0(0)	1	1	1	1
(1)		(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)	(100)		(100)	(100)	(100)	(100)
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1	0(0)	0(0)	0(0)	0(0)
																(100)				

Continues from table 4, antibacterial susceptibility pattern of gram-negative bacteria isolates…

Species	Pattern	Antibacterial drugs (n, %):																		
		AM	AMC	CF	CZ	CXM	CXMA	FOX	CPD	CAZ	CRO	FEP	CIP	GM	LEV	FT	TM	TE	SXT	TZP

P. rettgeri (1)	S	1 (100)	1 (100)	1 (100)	1 (100)	0(0)	1 (100)	1 (100)	1 (100)	1 (100)	0(0)	1 (100)	0(0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	R	0(0)	0(0)	0(0)	0(0)	1 (100)	0(0)	0(0)	0(0)	0(0)	1 (100)	0(0)	1 (100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
S. Paucimobillis (3)	S	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
F. tularensis (1)	S	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	R	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
All Isolates	**S**	**38 (21.7)**	**101 (57.7)**	**72 (41.1)**	**96 (54.9)**	**95 (54.3)**	**93 (53.1)**	**133 (76)**	**104 (59.4)**	**116 (66.3)**	**110 (62.9)**	**102 (58.3)**	**95 (54.3)**	**130 (74.3)**	**94 (53.7)**	**124 (70.9)**	**112 (64)**	**66 (37.7)**	**59 (33.7)**	**144 (82.3)**
	R	**137 (78.3)**	**74 (42.3)**	**103 (58.9)**	**79 (45.1)**	**80 (45.7)**	**82 (46.9)**	**42 (24)**	**71 (40.6)**	**59 (33.7)**	**65 (37.1)**	**73 (41.7)**	**80 (45.7)**	**45 (25.7)**	**81 (46.3)**	**51 (29.1)**	**63 (36)**	**109 (62.3)**	**116 (66.3)**	**31 (17.7)**

AM=Ampicillin, AMC=Amoxicilin/Clavulanic Acid, CF= Cefalotin, CZ=Cefazolin, FEP=Cefepime, FOX=cefoxitin, CPD=cefpodoxime, CAZ=ceftazidime, CRO=ceftriaxone, CXM=cefuroxime, CXMA=Cefroxime axetil, CIP=Ciprofloxacin, GM=Gentamicin, LEV=Levofloxacin, FT=Nitrofurantoin, TZP=piperacillin/Tazobactam, TE=tetracycline, TM=Tobramycin, SXT=Trimethoprim/sulfamethoxazole, S= sensitive, R= resistance

5.4. Antibacterial susceptibility pattern of gram-positive bacteria isolates.

Table 5 summarizes the overall drug susceptibility profile of gram positive bacteria against sixteen antibacterial drugs tested. The highest overall resistance rate of gram positive bacteria was observed against erythromycin (82.2%), followed by tetracycline (75.6%) and clindamycin (68.4%)while all isolates showed sensitivity towards daptomycin with a sensitivity rate of (98.1%) followed by nitrofurantoin(97.1%), gentamicin(93%) and linezolid(92.1%). *S. haemolyticus*, the most frequently isolated gram positive bacterium was 100% sensitive to minocycline and tigocycline. As depicted in table 5, *E. faecalis,* the 2nd most frequently isolated gram positive bacterium was 100% susceptible to six drugs.

Table 5: Antibacterial susceptibility pattern of gram-positive bacteria isolates**.**

Species	Pattern	Antibacterial drugs (n, %):															
		CIP	CM	E	GM	LEV	MNO	MXF	FT	QDA	RA	TE	TGC	SXT	LIN	VA	DAP
E. faecalis	S	13(92.9)	13(92.9)	2(14.2)	14(100)	14(100)	2(14.3)	14(100)	14(100)	0(0)	14(100)	3(21.4)	13(92.9)	13(92.9)	13(92.9)	8(57.1)	14(100)
(14)	R	1(7.1)	1(7.1)	12(85.8)	0(0)	0(0)	12(85.8)	0(0)	0(0)	14(100)	0(0)	11(78.6)	1(7.1)	1(7.1)	1(7.1)	6(42.9)	0(0)
S. aureus	S	6(66.7)	3(33.3)	3(33)	7(77.8)	7(77.8)	8(88.9)	9(100)	9(100)	7(77.8)	6(66.7)	3(33.3)	6(66.7)	4(44.4)	9(100)	5(55.6)	9(100)
(9)	R	3(33.3)	6(66.7)	6(66.7)	2(22.2)	2(22.2)	1(11.1)	0(0)	0(0)	2(22.2)	3(33.3)	6(66.7)	3(33.3)	5(55.6)	0(0)	4(44.4)	0(0)
S. epidermidis	S	3(37.5)	0(0)	2(25)	8(100)	3(37.5)	7(87.5)	8(100)	8(100)	4(50)	4(50)	3(37.5)	6(75)	6(75)	5(62.5)	1(12.5)	-
(8)	R	5(62.5)	8(100)	6(75)	0(0)	5(62.5)	1(12.5)	0(0)	0(0)	4(50)	4(50)	5(62.5)	2(25)	2(25)	3(37.5)	7(87.5)	-
S. haemolyticus	S	13(72.2)	2(11.1)	1(5.6)	15(83.3)	11(61.1)	18(100)	11(61.1)	17(94.4)	11(61.1)	9(50)	5(27.8)	18(100)	8(44.4)	17(94.4)	3(16.7)	17(94.4)
(18)	R	5(27.8)	16(88.9)	17(94.4)	3(16.7)	7(38.9)	0(0)	7(38.9)	1(5.6)	7(38.9)	9(50)	13(72.2)	0(0)	10(55.6)	1(5.6)	15(83.3)	1(5.6)
S. agalactiae	S	8(100)	0(0)	-	-	8(100)	-	-	-	8(100)	-	0(0)	6(75)	-	8(100)	6(75)	-
(8)	R	0(0)	8(100)	-	-	0(0)	-	-	-	0(0)	-	8(100)	2(25)	-	0(0)	2(25)	-
S. saprophyticus	S	2(100)	1(50)	2(100)	2(100)	2(100)	2(100)	2(100	2(100	2(100)	1(50)	2(100)	2(100	2(100	-	1(50)	2(100)
(2)	R	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	-	1(50)	0(0)
S. lentus	S	0(0)	2(66.7)	1(33.3)	3(100)	1(33.3)	1(33.3)	2(66.7)	3(100)	1(33.3)	2(66.7)	0(0)	3(100)	0(0)	3(100)	1(33.3)	3(100)
(3)	R	3(100)	1(33.3)	2(66.7)	0(0)	2(66.7)	2(66.7)	1(33.3)	0(0)	2(66.7)	1(33.3)	3(100)	0(0)	3(100)	0(0)	2(66.7)	0(0)
S. hominis	S	2(33.3)	0(0)	0(0)	6(100)	2(33.3)	3(50)	6(100)	6(100)	2(33.3)	2(33.3)	1(16.7)	6(100)	3(50)	6(100)	1(16.7)	6(100)
(6)																	

	R	4(66.7)	6(100)	6(100)	0(0)	4(66.7)	3(50)	0(0)	0(0)	4(66.7)	4(66.7)	5(83.3)	0(0)	3(50)	0(0)	5(83.3)	0(0)
S. warneri	S	5(55.6)	3(33.3)	2(22.2)	9(100)	7(77.8)	7(77.8)	8(88.9)	8(88.9)	8(88.9)	5(55.6)	2(22.2)	9(100)	4(44.4)	9(100)	6(66.7)	-
(9)	R	4(44.4)	6(66.7)	7(77.8)	0(0)	2(22.2)	2(22.2)	1(11.1)	1(11.1)	1(11.1)	4(44.4)	7(77.8)	0(0)	5(55.6)	0(0)	3(33.3)	-
K. kristinae	S	0(0)	1(50)	0(0)	2(100)	-	-	-	-	-	-	-	-	-	-	-	-
(2)	R	2(100)	1(50)	2(100)	0(0)	-	-	-	-	-	-	-	-	-	-	-	-
S. porcinus	S	1(100)	-	0(0)	-	-	-	-	-	-	-	-	-	-	-	-	-
(1)	R	0(0)	-	1(100)	-	-	-	-	-	-	-	-	-	-	-	-	-

Continues from table 5, antibacterial susceptibility pattern of gram-positive bacteria isolates…

Species	Pattern	Antibacterial drugs (n, %):															
		CIP	CM	E	GM	LEV	MNO	MXF	FT	QDA	RA	TE	TGC	SXT	LIN	VA	DAP
E. gallinarum	S	0(0)	-	0(0)	-	0(0)	0(0)	-	1(100)	-	-	0(0)	-	-	0(0)	0(0)	-
(1)	R	1(100)	-	1(100)	-	1(100)	1(100)	-	0(0)	-	-	1(100)	-	-	1(100)	1(100)	-
All isolates	S	**53**	**25**	**13**	**66**	**55**	**48**	**60**	**68**	**43**	**43**	**19**	**69**	**40**	**70**	**32**	**51**
		(65.4)	**(31.6)**	**(17.8)**	**(93.0)**	**(70.5)**	**(68.6)**	**(86.9)**	**(97.1)**	**(55.8)**	**(62.3)**	**(24.4)**	**(89.6)**	**(58)**	**(92.1)**	**(41.0)**	**(98.1)**
	R	**28**	**54**	**60**	**5**	**23**	**22**	**9**	**2**	**34**	**26**	**59**	**8**	**29**	**6**	**46**	**1**
		(34.6)	**(68.4)**	**(82.2)**	**(7.0)**	**(29.5)**	**(31.4)**	**(13.1)**	**(2.9)**	**(44.2)**	**(37.7)**	**(75.6)**	**(10.4)**	**(42)**	**(7.9)**	**(59.0)**	**(1.9)**

CIP=ciprofloxacin, CM=clindamycin, E=erythromycin, GM=Gentamicin, LEV=Levofloxacin, MN0=Minocycline, MXF=Moxifloxacin, FT=Nitrofurantoin, QDA=Quinupristin/Dalfopristin, RA=Rifampicin, TE=Tetracycline, TGC=Tigecycline, LIN=Linezolid, SXT=Trimethoprim/sulfamethoxazole, VA=vancomycin, DAP=Daptomycin, S= sensitive, R= resistance.

5.5. Multiple antibiotic resistances pattern of isolated bacteria

5.6. In this study, the overall resistance rate to two and more antibacterial of gram negative bacteria was 75.4%. The overall resistance to two and more antimicrobial agents of *E. coli* and *K. pneumonia*, the most commonly isolated gram negative bacteria were 77% and 72.2% respectively (Table 6). Similarly, the overall resistance rate to two and more antibacterial of gram positive bacteria was 91.3%. The resistances to two and more antimicrobial agents of *S. haemolyticus* and *E. faecalis*, the most commonly isolated gram positive bacteria were 100% and 92.9% respectively (Table 7).

5.7. Table 6: Multiple antibiotic resistances pattern of gram-negative bacteria

5.8. Isolates	**5.9.** No. (%)	**5.10.** Combination of antibacterial agent/ Antibiogram pattern:					
	5.12.	**5.13.** R_0 (%)	**5.14.** R_1(%)	**5.15.** R_2(%)	**5.16.** R_3(%)	**5.17.** R_4(%)	**5.18.** ≥ R_5 (%)
5.19. *E. coli*	**5.20.** 135(52.6)	**5.21.** 21(15.6)	**5.22.** 10(7.4)	**5.23.** 30(22.2)	**5.24.** 7(5.2)	**5.25.** 4(2.9)	**5.26.** 63(46.7)
5.27. *K. pneumoniae*	**5.28.** 18(7)	**5.29.** 0(0)	**5.30.** 5(27.8)	**5.31.** 2(11.1)	**5.32.** 1(5.6)	**5.33.** 2(11.1)	**5.34.** 8(44.4)

5.35. *P. aeruginosa*	**5.36.** 3(1.2)	**5.37.** 0(0)	**5.38.** 0(0)	**5.39.** 0(0)	**5.40.** 0(0)	**5.41.** 0(0)	**5.42.** 3(100)
5.43. *M. nonliquefaciens*	**5.44.** 3(1.2)	**5.45.** 0(0)	**5.46.** 0(0)	**5.47.** 0(0)	**5.48.** 0(0)	**5.49.** 0(0)	**5.50.** 3(100)
5.51. *S. Paucimobilis*	**5.52.** 3(1.2)	**5.53.** 3(100)	**5.54.** 0(0)	**5.55.** 0(0)	**5.56.** 0(0)	**5.57.** 0(0)	**5.58.** 0(0)
5.59. *C. diversus*	**5.60.** 2(0.8)	**5.61.** 0(0)	**5.62.** 0(0)	**5.63.** 1(50)	**5.64.** 0(0)	**5.65.** 0(0)	**5.66.** 1(50)
5.67. *A. baumannii*	**5.68.** 2(0.8)	**5.69.** 0(0)	**5.70.** 0(0)	**5.71.** 0(0)	**5.72.** 0(0)	**5.73.** 0(0)	**5.74.** 2(100)
5.75. *P. luteola*	**5.76.** 2(0.8)	**5.77.** 1(50)	**5.78.** 0(0)	**5.79.** 0(0)	**5.80.** 0(0)	**5.81.** 0(0)	**5.82.** 1(50)
5.83. *K. oxytoca*	**5.84.** 1(0.4)	**5.85.** 0(0)	**5.86.** 1(100)	**5.87.** 0(0)	**5.88.** 0(0)	**5.89.** 0(0)	**5.90.** 0(0)
5.91. *P. fluorescens*	**5.92.** 1(0.4)	**5.93.** 0(0)	**5.94.** 0(0)	**5.95.** 0(0)	**5.96.** 1(100)	**5.97.** 0(0)	**5.98.** 0(0)
5.99. *M. morganii*	**5.100.** 1(0.4)	**5.101.** 0(0)	**5.102.** 0(0)	**5.103.** 0(0)	**5.104.** 0(0)	**5.105.** 0(0)	**5.106.** 1(100)
5.107. *C. freundii*	**5.108.** 1(0.4)	**5.109.** 0(0)	**5.110.** 0(0)	**5.111.** 0(0)	**5.112.** 0(0)	**5.113.** 0(0)	**5.114.** 1(100)

5.115. *P. alcalifaciens*	**5.116.** 1(0.4)	**5.117.** 0(0)	**5.118.** 1(100)	**5.119.** 0(0)	**5.120.** 0(0)	**5.121.** 0(0)	**5.122.** 0(0)
5.123. *P. rettgeri*	**5.124.** 1(0.4)	**5.125.** 0(0)	**5.126.** 0(0)	**5.127.** 0(0)	**5.128.** 1(100)	**5.129.** 0(0)	**5.130.** 0(0)
5.131. *F. tularensis*	**5.132.** 1(0.4)	**5.133.** 1(100)	**5.134.** 0(0)	**5.135.** 0(0)	**5.136.** 0(0)	**5.137.** 0(0)	**5.138.** 0(0)
5.139. Total	**5.140.** 175(68.4)	**5.141.** 26(14.9)	**5.142.** 17(9.7)	**5.143.** 33(18.9)	**5.144.** 10(5.7)	**5.145.** 6(3.4)	**5.146.** 83(47.4)

5.147. R_0- No antibiotics resistance, R_1- Resistance to one, R2-Resistance to two , R3-Resistance to three, R4- Resistance to four, ≥R5-resistance to five and more antibiotics

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5.149. **Table 7: Multiple antibiotic resistances pattern of gram-positive bacteria**

5.150. Isolates	**5.151.** No. (%)	**5.152.** Combination of antibacterial agent/ Antibiogram pattern:					
	5.154.	**5.155.** R_0(%)	**5.156.** R_1(%)	**5.157.** R_2(%)	**5.158.** R_3(%)	**5.159.** R_4(%)	**5.160.** ≥ R_5(%)
		5.163.	**5.164.**	**5.165.**	**5.166.**	**5.167.**	**5.168.**

5.169. *S. haemolyticus*	**5.170.** 18(7)	**5.171.** 0(0)	**5.172.** 0(0)	**5.173.** 0(0)	**5.174.** 5(27.8)	**5.175.** 2(11.1)	**5.176.** 11(61.1)
5.177. *E. faecalis*	**5.178.** 14(5.5)	**5.179.** 0(0)	**5.180.** 1(7.1)	**5.181.** 0(0)	**5.182.** 7(50)	**5.183.** 4(28.6)	**5.184.** 2(14.3)
5.185. *S. aureus*	**5.186.** 9(3.5)	**5.187.** 1(11.1)	**5.188.** 0(0)	**5.189.** 3(33.3)	**5.190.** 2(22.2)	**5.191.** 0(0)	**5.192.** 3(33.3)
5.193. *S. warneri*	**5.194.** 9(3.5)	**5.195.** 1(11.1)	**5.196.** 1(11.1)	**5.197.** 3(33.3)	**5.198.** 0(0)	**5.199.** 1(11.1)	**5.200.** 3(33.3)
5.201. *S. epidermidis*	**5.202.** 8(3.1)	**5.203.** 0(0)	**5.204.** 0(0)	**5.205.** 0(0)	**5.206.** 2(25)	**5.207.** 2(25)	**5.208.** 4(50)
5.209. *S. agalactiae*	**5.210.** 8(3.1)	**5.211.** 0(0)	**5.212.** 0(0)	**5.213.** 8(100)	**5.214.** 0(0)	**5.215.** 0(0)	**5.216.** 0(0)
5.217. *S. hominis*	**5.218.** 6(2.3)	**5.219.** 0(0)	**5.220.** 0(0)	**5.221.** 0(0)	**5.222.** 0(0)	**5.223.** 0(0)	**5.224.** 6(100)
5.225. *S. lentus*	**5.226.** 3(1.2)	**5.227.** 0(0)	**5.228.** 0(0)	**5.229.** 0(0)	**5.230.** 1(33.	**5.231.** 1(33.	**5.232.** 1(33.3

						3)	3)	)
5.233.	*S* . *saprophyticus*	**5.234.** 2(0.8)	**5.235.** 1(50)	**5.236.** 0(0)	**5.237.** 0(0)	**5.238.** 1(50)	**5.239.** 0(0)	**5.240.** 0(0)
5.241.	*K* . *kristinae*	**5.242.** 2(0.8)	**5.243.** 0(0)	**5.244.** 1(50)	**5.245.** 1(50)	**5.246.** 0(0)	**5.247.** 0(0)	**5.248.** 0(0)
5.249.	*S* . *porcinus*	**5.250.** 1(0.4)	**5.251.** 0(0)	**5.252.** 1(100)	**5.253.** 0(0)	**5.254.** 0(0)	**5.255.** 0(0)	**5.256.** 0(0)
5.257.	*E* . *gallinarum*	**5.258.** 1(0.4)	**5.259.** 0(0)	**5.260.** 0(0)	**5.261.** 0(0)	**5.262.** 0(0)	**5.263.** 0(0)	**5.264.** 1(100)
5.265.	T otal	**5.266.** 81(31.6)	**5.267.** 3(3.7)	**5.268.** 4(4.9)	**5.269.** 15(18.5)	**5.270.** 18(22.2)	**5.271.** 10(12.3)	**5.272.** 31(38.3)

5.273. R_0- No antibiotics resistance, R_1- Resistance to one, R2-Resistance to two , R3-Resistance to three, R4- Resistance to four, ≥R5-resistance to five and more antibiotics.

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5.286. DISCUSSION

5.287. A change in the trends of bacterial uropathogens and their drug resistance development to most of the existing antimicrobial agents has become a global concern. Prolonged hospitalization, inadequate personal and environmental hygiene, indwelling of medical devices (catheters) functional or anatomical abnormalities and underlying diseases that compromise the immune mechanism of the host have been identified as major factors that contribute to the increasing number bacterial uropathogens [33]. Among many factors, drug abuse has been incriminated as a leading cause of drug resistance development in bacterial uropathogens in the developing world [7]. As the result of these, nosocomial and community acquired UTIs are still the commonest infections in the developing world. To this effect, accurate identification of bacteria implicated causing UTIs and determining drug susceptibility pattern of the etiologic agents for efficient management of patients with UTI is still active field of research. In view of this, accurate identification and antimicrobial susceptibility testing (AST) of clinical isolates by means of fully automated system is of one of the highest priorities.

5.288. Of the 712 urines collected from patients with cases of UTIs referred to the Arsho Advanced Medical Laboratories in the period of March 2016 to July 2016, UTIs have been identified in 256 patients giving a prevalence of 36%. Though the prevalence of UTIs in the present study was well within the reported range, it was relatively higher than the prevalence rates of UTI (between 9.5% and 22.7) obtained in similar local studies [29, 34-38], and lower than prevalence of 53.82% that has been reported in study conducted in India by Prakash and Saxena (24) and 60% study conducted in Nigeria [25]. Disparity in the prevalence rates of UTIs in different studies could result from difference in the definition of bacteriuria, methodology, the length of the study period and size and type of population.

5.289. Urinary tract infections are caused by both gram negative and gram positive bacteria. However, the most commonly encountered bacteria are gram negative in which *E. coli* consisting of the largest proportion of bacterial uropathogen worldwide [7]. This is evident by the present study in which, out of 256 (27 species) bacterial isolates recovered, 175(68.4%) of them were gram negative bacteria. Our finding of gram negative bacteria as the predominant species in patients with UTIs was consistent with similar studies conducted locally [28-31, 34-38] and internationally [7, 23, 26, 28, 30]. In the present study, 77.1% (135/175) of gram negative bacterial isolates and 52% of the total bacterial isolates were compromised by *E. coli. E. coli* has been reported as the main bacterial uropathegen accounting for 75 to 90% of bacterial isolates among patients with UTIs [22, 39, 40]. The prevalence of other predictable bacterial uropathogens varies from region to regions and from one study to another study [41, 42]. *S. saprophyticus and K. pneumoniae* were the 2nd predominate isolates each consisting of 7 % of the total bacterial isolates.

5.290. Although our finding of *E. coli* as the predominant species was consistent with similar studies conducted locally [34-38] there were some striking differences. For instance, the culture positivity rate obtained in our study (36 %) was comparatively higher than the culture positivity rates reported in previous studies. Isolation of 27 bacterial species belonging to 14 genera in the present study was higher than previous studies. Similarly, their study apparently could not isolate *Francisella tularensis, Moraxella nonliquefiels, Pseudomonas fluorescens, Pseudomanas luteala, Sphingomonas paucimobilis, Klebsiella oxytoca, Staphylococcus warneri, Staphylococcus hominis, Staphylococcus lentus,* and *Kocuria kristinae* that made 12.2% of bacterial isolates in our study.

5.291. The significance of this finding could be bacteria that were not commonly isolated from UTIs may replace the ones that have been commonly isolated from UTIs under selective pressure of drugs, resulting in infections refractory to the current drug based treatment in Ethiopia. Isolation of *Moraxella nonliquefiels, P. aeruginosa* and *A. baumannii* that were 100% resistant to nine to ten drugs tested in good number in the present study than the previous local studies [34-38] may support our suggestion. The present guideline of the Ethiopian Ministry of Health (MOH) for the management of

urinary tract infections includes trimethoprim/sulfamethoxazole combination as a first choice drug, and norfloxacin and amoxicillin as alternative drugs [43].

5.292. Another point to be noted in line with this finding is that, since there are geographical variations in the spectrum of uropathogens and drug resistance properties depending on local antimicrobial prescription practices, accurate identification of bacterial uropathogens down to a species level is important because: (a) different species have different antibiotic susceptibilities (b) serious bacterial infections caused by accepted "pathogens" have decreased in recent years in proportion to those caused by opportunistic bacteria that once were considered to be of low virulence (i.e. the incidence of opportunist infections is increasing) and (c) such infections cannot be traced epidemiologically or documented without identification of bacteria to a species level.

5.293. In our study, statistically significant difference in UTIs was witnessed between male and female study subjects as the majority of UTIs were recorded from females indicating that women are 2.5 times more likely to develop UTIs than men. Our result in this regard was in concordance with the findings of similar studies [42, 44, 45, 46]. Women are more prone to develop UTIs than men probably due to their anatomical and physiological changes. Shorter urethra in woman, the proximity of the urethra to the anus, antibacterial effect of prostate secretion, failure of bacteria to grow in a drier environment of male urethra, urethral trauma during intercourse, dilatation of the urethra and the stasis of urine during pregnancy have been identified as possible explanations for differences in UTIs in male and female patients [25, 44, 47]. Age groups of 1-14years ($p=0.001$), 25-44years ($P = 0.001$) and 45-64years ($P = 0.007$) were statistically significantly affected with the infection. Our finding in this regard was in agreement with the results of a study done locally [29] and internationally [48].

5.294. Given that the majority of therapy for UTIs is empiric and that urinary tract pathogens are demonstrating increasing antimicrobial resistance, continuously updated data on antimicrobial susceptibility patterns would be beneficial to guide empiric treatment. In the current study, drug susceptibility of all isolates of gram negative and gram positive bacteria was performed against a panel of nineteen and sixteen antibacterial drugs respectively. The number of drugs tested against urinary isolates in the present study was greater than the number of drugs tested in previous studies in Ethiopia [18-23]

and this is important to maximize the option for the selection of drugs for the empirical treatment of urinary tract infections.

5.295. The overall drug resistance rates of gram negative bacterial isolates ranged from 17.7% to piperacillin/tazobactam combination and 78.3% to ampicillin demonstrating that ampicillin was the least effective drug to treat patients with UTIs. Resistance rates of bacterial uropathogens to ampicillin vary from country to country and/or from one study to another. Lower resistance rates of gram negative bacteria to ampicillin than the present study have been reported in studies conducted in Italy (36%) [49], UK (23%) [50], USA (43%) [51], Canada (33%) [52] and Norway (25%) [53]. However, a resistance rate of 87%, which is higher than ours, has been reported in India [54]. The resistance rates of bacterial uropathogens to ampicillin have been found out to be 45%, 50% and 100% in children from Canada, Europe and Africa, respectively [55, 56, 57]. Our study showed that the use of ampicillin as a single agent for empirical treatment of a suspected UTI would not cover the majority of gram negative urinary tract pathogens in the study area. A notable observation was that majority of the isolates showed a higher sensitivity pattern towards nitrofurantoin, gentamicin, cefoxitin and piperacillin/tazobactam with a sensitivity of 70.1%, 74.3%, 76% and 82.3% respectively. The results of our study, therefore, support the use of either of these drugs as a reasonable choice for empiric therapy for uncomplicated UTIs.

5.296. In this study, resistance of gram negative bacteria to two and more antimicrobial agents was 85.4% which was higher than reported by previous study in Ethiopia [58]. Availability of antimicrobials without prescription and inappropriate dosing schedule may explain the isolation of high level of multidrug resistance gram negative bacteria. Nitrofurantoin was the most effective drug (80% sensitivity) against *E. coli,* the most frequently isolated gram negative bacterium. Our finding in this regard was similar to the previous local study [29]. *P. aeruginosa* and *A. baumannii* the 3rd and the 4th most frequently isolated bacteria were resistant to greater than nine drugs tested. Similar result was obtained in a study conducted by Lu et al [59].

5.297. The overall drug resistance rates of gram positive bacterial isolates ranged from 1.9% to daptomycin and 82.2% to erythromycin followed by tetracycline (75.6%) and clindamycin (68.4%). An overall resistance rate of 85.6% and 76.7% of uropathognes to erythromycin and tetracycline respectively has been reported in a similar study conducted

in Ethiopia [29]. However, the majority of gram positive bacterial isolates showed a higher sensitivity pattern towards daptomycin, nitrofurantoin, gentamicin and linezolid with a sensitivity rates of, 98.1%, 97.1%, 93.0% and 92.1% respectively. *S. haemolyticus*, the most frequently isolated gram positive bacterium was 100% sensitive to minocycline and tigocycline and 94.4% to linozilid and daptomycin. *E. faecalis, the* 2nd most frequently isolate gram positive bacterium was 100% susceptible to six drugs. In this study, resistance of gram positive bacteria to two and more antimicrobial agents was 91.3. Development of multiple antibiotic resistances in gram positive bacteria was higher than gram negative bacteria.

5.298. In general, observation of high level of resistance in both gram negative (66.3%) and gram positive (42%) bacterial isolates to SXT and 70.4% resistance rate to SXT in *E. coli*, given that *E. coli* is the principal pathogen in urinary tract infections in the present study are important indicators of whether SXT should continue to be used for empirical treatment of urinary isolates. This is a serious concern to Ethiopia, in which trimethoprim/sulfamethoxazole(SXT) combination has been used as a first line drug for treatment of uncomplicated UTI [43]. High resistance rates of bacterial uropathogens against SXT are seen as a global problem and this is particularly true in developing countries like Ethiopia where unregulated prescription of antibiotics is more likely [60, 61]. In line with this, our study clearly indicated that to reduce the disease burden of UTIs and there by lower its consequences and costs a switch to another therapeutic regime is inevitable.

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5.319. LIMITATIONS OF THE STUDY

- Lack of clinical information to confirm whether urinary tract infections were community or hospital-acquired and complicated or uncomplicated. As well the study was institutional based which may decrease/increase the detection rate.

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5.345. CONCLUSION AND RECOMMENDATION

5.346. *E. coli* remained as predominant gram negative bacilli in UTIs, isolation of many unpredictable bacteria and a prevalence of 36% urinary tract infection were documented. Development of high level of resistance by urinary isolates particular of *E. coli*, the predominate uropathogens to trimethoprim/sulfamethoxazole (SXT) that has been used as a first line drug for the treatment of urinary isolates. 75.4% and 91.3% rates of multiple antibiotic resistances in gram negative and gram positive bacteria respectively were also documented. To this effect a switch to another therapeutic regime is inevitable. In light of this, although among antimicrobial tested peperacillin/tazolactam and daptomycin were found to be the most effective drugs against gram negative and gram positive urinary isolates, continuous nationwide study on the spectrum and drug sensitivity pattern of bacterial uropathogens is recommended.

5.347. In addition we suggest that empiric antibiotic selection for urinary isolates should be based on knowledge of local prevalence of etiological agents and antibiotic sensitivity rather than universal guide lines. Furthermore, given that the majority of therapy for UTIs is empiric and that urinary tract pathogens are demonstrating increasing antimicrobial resistance, continuously updated data on antimicrobial susceptibility patterns urinary isolates would be beneficial for effective management of UTIs.

5.348. The following recommendations are made based on the findings of the present study:

- ✓ Multiple antibiotic resistances bacteria were common, so periodic and continuous follow up are mandatory.
- ✓ It is necessary to establish an antimicrobial susceptibility surveillance system to improve current infection control programs in institutions to prevent the spread of MDR.
- ✓ A predictable bacterial profile and antibiotic sensitivity will be of great help for clinicians to start empirical treatment. Hence periodical monitoring of bacterial profile and their antibiotic susceptibility pattern is important.

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1. Schappert SM, Rechtsteiner EA, Hyattsville MD, Hyattsville. Ambulatory medical care utilization estimates for 2006. National health statistics reports and national center for health statistics 2008; 8.
2. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. Annual Epidemiology 2000; 10:509–515.
3. Griebling TL, Litwin MS, Saigal CS. Urinary tract infection in women, *Urologic Diseases in America*, department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Washington DC. NIH publication 2007; 07:587–619.
4. Foxman B, Frerichs RR. Epidemiology of urinary tract infection: Diaphragm use and sexual intercourse. America Journal of Public Health 1985; 75:1308–13.
5. Kozak LJ, DeFrances CJ, Hall MJ. National hospital discharge survey: 2004 annual summary with detailed diagnosis and procedure data. Vital Health Stat 2006; 13:1–209.
6. Bethesda MD. National Kidney and Urologic Diseases Information Clearinghouse, kidney and urologic diseases statistics for the United States. National Institute of Diabetes and Digestive and Kidney Diseases, 2010.
7. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. American Journal of Medicine 2002; 113:5–13.
8. Vorland LH, Carlson K, Aalen O. An epidemiological survey of urinary tract infections among outpatients in Northern Norway. Scand Journal of Infectious Disease, 1985; 17: 277–283.
9. Gupta K, Hooton TM, Wobbe CL, Stamm WE. The prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in young women. International Journal of Antimicrobiology Agents 1999; 11: 305–308.

10. Barrett SP, Savage MA, Rebec MP, Guyot A, Andrews N, Shrimpton SM. Antibiotic sensitivity of bacteria associated with community acquired urinary tract infections in Britain. Journal of Antimicrobiology and Chemotherapy 1999; 44: 359–365.
11. Vromen M, van der Ven AJ, Knols A, Stobberingh EE. Antimicrobial resistance patterns in urinary tract isolates from nursing home residents, fifteen years of data reviewed. Journal of Antimicrobiology and Chemotherapy 1999; 44: 113–116.
12. Manges AR, Natarajan P, Solberg OD, Dietrich PS, Riley LW. The changing prevalence of drug-resistant *Escherichia coli* clonal groups in a community: evidence for community outbreaks of urinary tract infections. Epidemiology of Infection 2006; 134: 425–431.
13. Doern GV, Vautour R, Gaudet M, Levy B. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. Journal of Clinical Microbiology 1994; 32:1757–1762.
14. Donay JL. Evaluation of the automated phoenix system for potential routine use in the clinical microbiology laboratory. Journal of Clinical Microbiology 2004; 42:1542–1546.
15. Hoberman A, Charron M, Hickey RW, Baskin M, Kearney DH, Wald ER. Imaging studies after a first febrile urinary tract infection in young children. England Journal of Medicine 2003; 348(3):195–202.
16. Trautner BW. Management of catheter-associated urinary tract infection. Current Opinion Infection Disease 2010; 23:76-8.
17. Gul N, Mujahid TY, Ahmed S. Isolation, Identification and Antibiotic Resistance Profile of Indigenous Bacterial Isolates from Urinary Tract Infection Patients. Pakistan Journal of Biological Science 2004; 12:2051–2054.
18. Finch RG. Antibiotic resistance. Journal of Antimicrobial Chemotherapy 1998; 42: 125–128.
19. Alzohairy M, Khadri H. Frequency and Antibiotic Susceptibility Pattern of Uro-Pathogens Isolated from Community and Hospital-Acquired Infections in Saudi Arabia: A Prospective Case Study. British Journal of Medicine 2011; 1(2):45–56.
20. Sweih NA, Jamal W, Rotimi VO. Spectrum and Antibiotic Resistance of Uropathogens Isolated from Hospital and Community Patients with Urinary Tract Infections in Two Large Hospitals in Kuwait. Department of Microbiology, Faculty of Medicine, Kuwait University, and Mubarak Teaching Hospital, Kuwait 2005;14:401–407.
21. Benwan KA, Sweih NA, Rotimi VO. Etiology and Antibiotic Susceptibility Patterns of Community- and Hospital-Acquired Urinary Tract Infections in a General Hospital in Kuwait, Department of Microbiology, Al-Amiri Hospital, and Faculty of Medicine, Kuwait University, Kuwait 2010;19:440–446.

22. Dugal S, Purohit H. Antimicrobial Susceptibility profile and detection of Extended Spectrum Beta-Lactamase production by Gram Negative Uropathogens, Department of Microbiology, Sophia College, University of Mumbai, Maharashtra, India 2013;5.
23. Darbandi F. Parallel Comparison of Accuracy in Vitek2 Auto analyzer and API 20 E / API 20 NE Microsystems, University College of Boras, School of Engineering 2010; 2.
24. Prakash D, Saxena RS. Distribution and Antimicrobial Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infection in Urban Community of Meerut City, India. ISRN Microbiology 2013; 13: 1-5.
25. Kolawale AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicine and Medical Sciences 2009; 1: 163-167.
26. Mantadakis E, Vouloumanou EK, Panopoulou M, Tsouvala E, Tsalkidis A, Chatzimichael A, et al. Susceptibility patterns of Uropathogens identified in hospitalized Children with community-acquired urinary tract infections in Thrace, Greece. Journal of global antimicrobial resistance 2015; 3: 85-90.
27. Moroh JLA, Fleury Y, Tia H, Bahi C, Lietard C, Coroller L, et al. Diversity and antibiotic resistance of uropathogenic bacteria from Abidjan, University of Felix Houphouet-Boigny ex University of Cocody, Abidjan, Cote d'Ivoire. African Journal of Urology 2014; 20: 18-24.
28. Beyene G, Tsegaye W. Bacterial Uropathogens in Urinary tract infection and antibiotic susceptibility pattern in Jimma university specialized hospital, Southwest Ethiopia, Department of Laboratory Sciences and Pathology, College of Public Health and Medical Sciences, Jimma University, 2011; 21:No.2.
29. Kibret M, Abera B. Prevalence and antibiogram of bacterial isolates from urinary tract infections at Dessie Health Research Laboratory, Ethiopia. Asian Pacific Journal of Tropical Biomedicine 2014; 4(2): 164-168.
30. Tiruneh M, Yifru S, Gizachew M, Molla K, Yeshambel B, Moges F, et al. Changing Trends in Prevalence and Antibiotics Resistance of Uropathogens in Patients Attending the Gondar University Hospital, Northwest Ethiopia, University of Gondar. Hindawi Publishing Corporation, International Journal of Bacteriology 2014; 7.
31. Kabew G, Abebe T, Miheret A. Prevalence and antimicrobial susceptibility patterns of bacterial isolates from urinary tract infections in Tikur Anbessa Specialized Teaching Hospital, Addis Ababa University, College of Medical Sciences, Department of

Microbiology, Immunology and Parasitology. Ethiopian Journal of Health Development, 2013; 27(2).
32. Series M. World Health Organization. Provisional Guidelines on Standard International Age Classifications: Statistical Papers, WHO, United Nations, New York 1982; 74: 4-11.
33. Davoodian P, Nematee M, Sheikhvatan M. The inappropriate use of urinary catheters and its common complications in different hospital wards. Saudi Journal of Kidney Diseases and Transplantation 2012; 23(1):63.
34. Assefa A, Asrat D, G/Hiwot W, Abdella A, Melesse T. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia**.** Ethiopian Medical Journal 2008; 46(3):227-35.
35. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma University Specialized hospital, Southwest Ethiopia**.** Ethiopian Journal of Health Science 2011; 21: 2.
36. Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw BA. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC Research Notes 2012; 5:197. http://www.biomedcentral.com/1756-0500/5/197 (accessed 13 February 2016).
37. Ferede G, Yismaw G, Wondimeneh Y, Sisay Z. The Prevalence and Antimicrobial Susceptibility pattern of Bacterial Uropathogens Isolated from pregnant women. European Journal of Experimental Biology 2012; 2 (5):1497-1502.
38. Demilie T, Beyene G, Melaku S, Tsegaye W. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia**.** Ethiopian journal of health science 2012; 22: 2.
39. Gupta K, Hooten TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Annual International Medicine, 2001; 135: 41–50.
40. Nicolle LE. Epidemiology of urinary tract infection, journal of Infection Medicine 2001; 18: 153–162.
41. Bean DC, Krahe D, Wareham DW. Antimicrobial resistance in community and nosocomial *Escherichia coli* urinary tract isolates, London 2005–2006. Annual Clinical of Microbiological Antimicrobial 2008; 7:13.
42. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital Aligarh, India. Annual Clinical of Microbiology Antimicrobial 2007; 6:4.

43. Standard treatment guideline for general hospitals, Drug Administration and Control Authority of Ethiopia Contents, 2010.
44. Oladeinde BH, Omoregie R, Olley M, Anunibe JA. Urinary tract infections in a rural community of Nigeria, North American Journal of Medical Sciences, 2011; 3(2):75-77.
45. Manjunath GN, Prakash R, Vamseedhar A, Kiran S. Changing trends in the spectrum of the antimicrobial drug resistance pattern of the uropathogens which were isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore, International Journal of Biological and Medical Research, 2011; 2(2):504-07.
46. Barate DL, Ukesh C. The bacterial profile and the antibiotic resistance pattern of urinary tract infections. DAV International Journal of Science 2012; 1(1), 21-24.
47. Hooton TM. Pathogenesis of urinary tract infections: an update. Journal of Antimicrobial Chemotherapy, 2000; 46(1): 1-17.
48. Desai P, Ukey PM, Chauhan AR, Malik S, Mathur M. Etiology and antimicrobial resistance patterns of uripatyhogens in a hospital from suburb of Mumbai. International Journal of Biology and Medical Research, 2012; 3: 2007-2012.
49. Bonadio M, Meini M, Spitaleri P, Gilgi C. Current microbiological and clinical aspects of urinary tract infections. European Journal of Urology, 2001; 40: 439–445.
50. Farrell DJ, Morrissey I, De Rubeis D, Robbins M, Felmingham D. A UK multicentre study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infection. Journal of Infection, 2003; 46: 94–100.
51. Mathai D, Jones RN, Pfaller MA. SENTRY Participant Group of North America Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). Diagnostic Microbiological Infection of Disease, 2001; 40(3): 129–136.
52. Jones RN, Kugler KC, Pfaller MA, Winokur PL. Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY Antimicrobial Surveillance Program. Diagnostic Microbiological Infection of Disease, 1999; 35: 55–63.
53. Grude N, Tveten Y, Kristiansen B-E. Urinary tract infections in Norway: bacterial etiology and susceptibility: A retrospective study of clinical isolates. Clinical Microbial Infection, 2001; 7: 543–547.
54. Navaneeth BV, Belwadi S, Sughanthi N. Urinary pathogens' resistance to common antibiotics: a retrospective analysis. Tropical Documents, 2002; 32: 20–22.

55. Adjei O, Opoku C. Urinary tract infections in African infants. International Journal of Antimicrobial Agents, 2004; 24(1):S32–4.
56. Haller M, Brandis M, Berner R. Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. Journal of Pediatric Nephrology, 2004; 19:982–986.
57. Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. Arch Dis Child 2003; 88:215–218.
58. Yismaw G, Asrat D, Woldeamanuel Y, Unakal CG. Urinary tract infection: Bacterial etiologies, drug resistance profile and associated risk factors in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. European Journal of Experimental Biology, 2012; 2: 889-898.
59. Lu PL, Liu YC, Toh HS, Lee YL, Liu YM, Ho CM, et al. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). International Journal of Antimicrobial Agents. *2012; 40S1: S37–S43.*
60. Bours PHA, Polak R, Hoepelman AIM, Delgado E, Jarquin A, Matute AJ. Increasing resistance in community-acquired urinary tract infections in Latin America, five years after the implementation of national therapeutic guidelines. International Journal of Infectious Diseases, 2010; 14: 770–774.
61. Matute AJ, Hak E, Schurink CA, McArthur A, Alonso E, Paniagua M, et al. Resistance of uropathogens in symptomatic urinary tract infections in Leo´n, Nicaragua. International Journal of Antimicrobial Agents 2004; 23:506–519.

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5.373. Annex I: Operational Definitions

5.374. Antimicrobial Resistance (AMR): The ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once sensitive. It is the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth.

5.375. Bacteriuria: The presence of bacteria in the urine.

5.376. Mid-stream urine specimen: a specimen obtained from the middle part of urine flow.

5.377. Multiple antibiotic resistances (MARs): a resistant to more than one antimicrobial agent.

5.378. Significant bacteriuria: the presence of $\geq 10^5$ colony forming units (cfu) per milliliter of urine.

5.379. Urinary tract infection (UTI): is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract.

5.380. Sensitive (S): bacterial isolates are inhibited by the usual achievable concentration of antimicrobial agent.

5.381. Resistant (R): bacterial isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedule.

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5.394. Annex II: Materials, Equipments and Culture media

5.395. Equipments available at the study site;

5.396. Vitek 2 compact instrument
5.397. Bio safety cabinet (BSC II)
5.398. Autoclave
5.399. Incubators
5.400. Microscope
5.401. Hot air oven
5.402. Balance
5.403. Refrigerator
5.404. Water Bath
5.405. Vortex Shaker
5.406. Digital PH meter

5.407. Culture media and Items available;

5.408. Sheep blood agar (SBA)
5.409. MacConkey agar (MAC)
5.410. CLED agar
5.411. Vitek GP card
5.412. Vitek AST-GP card
5.413. Vitek GN card
5.414. Vitek AST-GN card
5.415. Normal saline (0.45% NaCl)
5.416. DensiChek turbidity meter

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5.422. Annex III: Participant Information Sheet (for Adult)

5.423. My name is Tamirat. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled "**Spectrum and Antibiotic profile bacterial uropathogens isolated from patients attending Arsho Advanced Medical laboratory with Urinary Tract Infections by using VITEK 2 Compact system".** You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical urine specimen.

5.424. Introduction

5.425. The topic of this study is Spectrum and Antibiotic profile of bacterial uropathogens isolated from patients attending Arsho Advanced Medical laboratory with Urinary Tract Infections by using Vitek 2 Compact system*, Addis Ababa, Ethiopia*. Since *Urinary Tract Infection* is one of the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the problem. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the Laboratory. If you decide to participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet.

5.426. Expected from participants

5.427. As a participant of this study, you are expected to give urine. Being asked to give sample does not neccessarly mean that you have the disease. When you are found to be positive for the bacterial micro-organism, you will be informed by the health worker and receive proper treatment. But your name, address will not be disclosed rather an identification code will be used in such conditions.

5.428. Time required for participating

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

5.430. Risks of participant

5.431. Specimen collection will have no effect and you will not get any risk.

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5.434. Confidentiality

5.435. The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional will have access to the information. The information will be encoded in a computer and saved with password protection.

5.436. Benefits of participation

5.437. By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better understanding of the problems of ***Urinary Tract Infection***. You will also obtain all the results of the analysis and communicated to your physician for the appropriate management.

5.438. Rights of participants

5.439. Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

5.440. Communication

5.441. In case if you have any questions, unclear ideas and doubt about the project, contact addresses are: **Investigator: Tamirat Molalign** (BSc, Msc student), DMLS; AAU, +251913457049 Email- tamiratmolalign@gmail.com

5.442. **Advisor:** Adane Bitew (Associate prefacer), DMLT, AAU +251911039162

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

5.444. Department of Medical Laboratory Sciences at: Telephone +251112755170

5.445. 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 any question.

5.446. 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.

5.447. Agree to participate? Yes---------- No----------

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5.451. Annex IV: የተሳታፊ ስምምነት ቅጽ

5.452. ይህ ገጽ "Spectrum and Antibiotic profile of bacterial uropathogens isolated from patients attending Arsho Advanced Medical laboratory with Urinary Tract Infections by using Vitek 2 Compact system*, Addis Ababa, Ethiopia*" ማለትም የሽንት ቧንቧ በሽታ የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ በሚል ርዕስ የተሳታፊ ስምምነት ቅጽ ነዉ። በመሆኑም እባክዎን በዚህ በታች የተዘረዘሩትን ነጥቦች ይረዱና፤ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃለሁ።

5.453. የሽንት ቦንባ በሽታ የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ የሚለው ጥናት ዓላማ በደንብ ተገንዝቤአለሁ።

5.454. ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ። ሁሉም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ። በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ። በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም ጊዜ የማቋረጥ መብት እንዳለኝ አዉቄአለሁ። ሁሉም መረጃዎች በአስተባባሪዉ/ዎች ተግልጾልኝ በደንብ ተረድቻለሁ።

5.455. የተሳታፊ ፊርማ፡ ----------

5.456. የተሳታፊ አድራሻ፡----------

5.457. ቀን፡----------

5.458. ይህን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ።

5.459. ታምራት ሞላልኝ ፤ሞባይል፡+251913457049 ኢ-ሜይል፤ tamiratmolalign@gmail.com

5.460. የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፤ የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

5.461. አማካሪ፡ አዳነ ቢተዉ (PhD), ሞባይል፡ +251911039162

5.462. ለተጨማሪ መረጃ፡ አዲስ አበባ ዩኒቨርሲቲ ፤ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ። ስልክ+251112755170

5.463.

5.464. Annex V: English Versions of Participant Information Sheet (for mothers or guardians)

5.465. 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.

5.466. Introduction

5.467. The topic of this study is "Spectrum and Antibiotic profile of bacterial uropathogens isolated from patients attending Arsho Advanced Medical laboratory with Urinary Tract Infections by using VITEK 2 Compact system". 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.

5.468. 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 institution 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.

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

5.470. 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 your child health and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals out of this institution. But the name of you or your child and address will not be disclosed and rather than identification code will be used in such conditions.

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

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

5.473. What are the risks of participating in this study?

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

5.475. How our information is to be kept in secret?

5.476. 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.

5.477. What are the benefits from participation?

5.478. 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.

5.479. What are our rights as a participant of this study?

5.480. You have the right to withdraw your child from the study at any time and all the services provided in the institution 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.

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

5.482. In case if you have any questions, unclear ideas and doubt about the project, contact addresses are: **Investigator:** Tamirat Molalign (BSc, Msc student), +251913457049 Email- tamiratmolalign@gmail.com

5.483. **Advisor:** Adane Bitew (Associated professor), DMLT, AAU, +251911039162

5.484. For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170.

5.485. 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.

5.486. Agree to participate? Yes----------

No----------

5.487. Annex VI: Amharic Versions of Participant Information Sheet (for mothers /guardians)

5.488. አዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ, የአላይድ ጤና ሣንይስ ት/ቤት, የሕክምና ላቦራቶሪ ሣንይስ ክፍል የሽንት ቧንቧ በሽታ የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ 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 እንዲሳተፉ ተጋብዘዋል።እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ወይም ያዳምጡና ግልጽ ያልሆነዉን/ትን ማንኛዉም ሃሳብ/ጥያቄ ይጠይቁ።

5.489. *መግቢያ*

5.490. የጥናቱ ርዕስ የሽንት ቧንቧ በሽታ የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም የሚበጀውን መድሃኒት ለመምረጥ የሽንት ፈሳሽ ተወስዶ ለሚሰራው ጥናት ነው ። አላማውም የሽንት ቧንቧ በሽታ የተባለዉን 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 ተቋም የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል። ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ።

5.491. ልጄ የጥናቱ ተሳታፊ በመሆኑ የሚጠበቅበት ምንድን ነው?

5.492. 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 ተቋም 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 ተጨ ማሪ ጥያቄዎች መልስ መ ስጠት ይጠበቅብዎታል።

5.493. በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

5.494. የተዘጋጀዉን መጠይቅ ለመ ሙ ላት፤የስምምነት ቅጹ ላይ ለመፈረምና ናሙ ና ለመስጠት ከ 20-25 ደቂቃ ያስፈልጋል።

5.495. በዚህ ጥናት መሳተፍ የሚ ያስከትላቸዉ ቸግሮቸ ምንድን ናቸዉ?

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

5.497. የልጄ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

5.498. ስለራሶና 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 እርሶና ስለ ልጅዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

5.499. በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

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

5.501. የልጄ በዚህ ጥናት ተሳታፊ መሆኑ መብቱ ምንድን ናቸው?

5.502. በጥናቱ ውስጥ ያላችሁን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ ልጅን ከጥናቱ በማግለል ምክንያት የሚቀርበት ምንም አይነት የተቋሙ አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱ በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለበት።

5.503. ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

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

5.505. ታምራት ሞላልኝ (ቢኤስሲ) +251913457049

5.506. Email- tamiratmolalign@gmail.com

5.507. አማካሪ: አዳነ ቢተው (ፒኤችዲ), DMLT, AAU +251911039162

5.508. ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርስቲ የጤና ትምህርት የህክምና ላቦራቶሪ ክፍልን በ ስልክ ቁጥር፡ +251112755170 አባክዎ ያነጋግሩን ።

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5.512. Annex VII: English Versions of Consent form (for mothers/guardians)

5.513. Code number----------

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

5.515. I have been informed about the study which is aimed to assess "Spectrum and Antibiotic profile uropathogens isolated from patients attending Arsho Advanced Medical laboratory with Urinary Tract Infections by using Vitek 2 Compact system." For this study urine will be collected from my child who 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.

5.516. 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.

5.517. 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.

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

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

5.520. Name of deponent ----------signature----------

5.521. (For mothers unable to read)

5.522. Name of counselor ----------Signature----------

5.523. Date ----------

5.524.

5.525. **Annex VIII: Amharic Versions of Consent form (for mothers/guardians)**

5.526. የተሳታፊወች ስምምነት ማረጋገጫ ቅጽ

5.527. የሚስጥር ቁጥር ----------

5.528. የተሳታፊዉ ልጅ እናት/ አሳዳጊ ስም ----------

5.529. እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የሽንት ቧንቧ በሽታ የተባለዉን በሽታ የሚፈጥረውን ደቂቅ ህዋስ ስርጭት ለማወቅና ህዋሱንም ለማከም 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 መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።

5.530. 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/ ተረድቻላዉ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

5.531. የተሳታፊዉ ወላጅ ወይም አሳዳጊ ፊርማ / የጣት አሻራ **----------**

5.532. የምስክር ስም፡1---------- ፊርማ----------

5.533. 2---------- ፊርማ----------

5.534. **(**የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች**)**

5.535. የአማካሪ ረዳት ስም፡---------- ፊርማ ----------

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5.539. Annex IX: VITEK 2 compact system guidelines

5.540. History: It was a byproduct of US, space exploration system in 1960s. Originally manufactured by McDonnell Douglas Corporation for aerospace projects. First test UTI from astronauts. It is modified in 1970s for clinical laboratory use primarily for UTI, both identification and qualitative AST (S, I, R) modes. In 1980s MIC cards were introduced for semiqantitative tests for most rapidly growing bacteria.

5.541. Principle: Very small plastic reagent cards were designed to contain micolitres of biochemical tests and selective growth media for detection and identification of organism.

5.542. Procedure: The Vitek 2 AST innoculum is automatically introduced via a filling tube into a miniaturized plastic 64 wells closed card containing specific concentration of antibiotics. Cards are incubated in a temperature controlled compartment. The antibiotic is rehydrated when the organism suspension is introduced in to the card during the automated filling process. Optical readings are performed every 15 minutes to measure the amount of light transmitted through each well including a growth control well.

Algorithmic analysis of the growth kinetics in each well is performed by the system's soft wares to drive the MIC data. The MIC data is validated with the advanced expert system (AES) soft ware a category interpretation is assigned and the organism antimicrobial patterns are reported. It will take 6-8 hrs. It allow us Rapid screening of MDR (ESBL, MRSA, VRE)

5.543. Component: The vitek 2 instrument houses:- Sample processor and reader, Incubator, Computer work station: Allow data entry and analysis, storage, epidemiology reports, Smart carrier station : direct interface between personnel in the bench and the instrument, Barcode scanner: facilitate data entry.

5.544. VITEK 2 Compact and its Expert software, AES™, offer significant benefits for the microbiologist, the clinician and ultimately, the patient. In addition to optimized result reliability, the microbiologist can be confident of detecting even weakly expressed resistance mechanisms. The clinician obtains a rapid, accurate and validated test report, and is alerted in the event of antibiotic resistance. This report helps the clinician to make the correct diagnosis and, when necessary, adapt the antibiotic therapy at the earliest possible stage. The patient benefits from timely treatment with the most relevant antibiotics.

5.545. VITEK® 2 Compact is powered by the same Advanced Expert System (AES) software for the validation and interpretation of susceptibility test results as VITEK 2. This expert software ™® is capable of identifying antibiotic resistance mechanisms, even emerging and low-level resistance. It also contributes to the detection of nosocomial infections, enabling proactive measures to be implemented.

5.546. Furthermore, identification performance has been enhanced on VITEK® 2 systems through the introduction of "Advanced Colorimetric" technology. An expanded database of over 330 species of microorganisms can be identified using the new VITEK 2 Colorimetric Identification cards. This database covers over 95 % of routine microbiology tests.

5.547. The VITEK® 2 Compact system answers QC laboratory testing needs for fast and accurate microbial identification. The innovative VITEK® 2 technology includes an expanded identification database, the most automated platform available, rapid results, improved confidence, with minimal training time. The VITEK® 2 Compact next-generation platform provides greater automation while increasing user-safety and eliminating repetitive manual operations. The rapid response time means results can be

provided more quickly than with manual techniques. VITEK® 2 Compact offers: bi-directional interface, data management software, a new generation of test cards, 21CFR Part 11 compliant software, a comprehensive database. The VITEK® 2 Compact can: Reduce time to identification results, offer an extensive identification menu, reduce waste with a miniaturized card-format that measures 10 cm x 6 cm x 0.5 cm and weighs only 16 grams.

5.548. VITEK® 2 Compact offers significant advantages over other microbial identification techniques:

5.549. **Traceability:** Electronic linkage of the sample ID with the barcode on the test cards are loaded into the software via the barcode reader and then compared again as the cards are loaded into the reader. All discrepancies are immediately noted and voided. All user operations are automatically logged into the audit trail.

5.550. **Accuracy:** New Advanced Colormetric Technology™ enables use of additional wavelengths, resulting in an increased number of existing and novel substrates in each card. The expanded database consists of internal and external organisms with multiple isolates of each, culminating in 93% accuracy for the GP card, 94% for the GN card, 87% for BCL and 84% for YST.

5.551. **Speed:** Time to result has decreased substantially. Cards are now read every 15 minutes, and average time to result is 2-8 hrs for GP and GN, including the non-fermenters. BCL are read at 14 hours and YST at 18 hours. The ANC and NH cards are read at six hours, and the CBC card is read at eight hours.

5.552. **Safety:** The VITEK® 2 Compact continues the VITEK® tradition of user safety and minimal biohazardous waste. The test card is automatically filled, sealed, read and disposed of in a final waste container.

5.553. Two modules are available: The VITEK® 2 Compact 30, which can accept up to 30 cards at one time, and the VITEK® 2 Compact 60, which can hold up to 60 cards at one time. Test cards can be added in batches of mixed card product types or as single units. Up to two units can be connected to one operating system, increasing your capacity to 120 tests at one time.

5.554. The system includes software that is 21CFR Part 11 Compliant and delivers accurate identifications for a wide range of organisms from environmental sources or final products.

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5.562. Annex X: Assurance of Principal Investigator
5.563. I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I was providing timely progress report to my advisor and seek the necessary advice and approval from my primary advisors in the course of the research. I was communicating timely to my advisors all stakeholders involved in the study including any source of funding for this research.
5.564. Name of the Investigator: **Tamirat Molalign (BSc.)**
5.565. Signature: _____
5.566. Date: _____
5.567. Approval of the primary Advisor
5.568. Name of the Primary Advisor: **Adane Bitew (PhD)**
5.569. Signature: _____
5.570. Date: _____
5.571.