PREVALENCE OF EXTENDED SPECTRUM BETALACTAMASE PRODUCING ENTEROBACTERIACEAE AND ASSOCIATED RISK FACTORS AMONG DIABETES MELLITUS PATIENTS WITH URINARY TRACT INFECTIONAT SHANAN GIBE GENERAL HOSPITAL, JIMMA TOWN,SOUTH WEST ETHIOPIA.

By

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A THESIES SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS MASTERS OF SCIENCE DEGREE IN MEDICAL MICROBIOLOGY.

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Abstract

Background: The worldwide emergence and spread of extended-spectrum beta-lactamases producing Enterobacteriaceae has become a threat to deal with an infections. This problem is more critical in low & middle income countries. Enterobacteriaceae family bacterial organisms are among bacterial isolates most frequentely encountered from different clinical samples. They are the most frequent causative agents of urinary tract infection among diabetic patients.

Objective: Todetermine prevalence of Extended Spectrum Betalactamase producing Entrobacteriaceae (ESBL-PE)& associated risk factors among Diabetes mellitus patients with urinary tract infection.

Methods: A hospital based cross-sectional study was conducted from July to October 2021.A total of272consecutive DM patients were included and their socio-demographic and risk factor-related data were collected using structured questionnaire. Mid-stream urine specimens were collected from study participants & analyzed using standard bacteriological methods.Antimicrobial Susceptiblity Testing (AST) & ESBLs production testing were doneby Disk diffusion & Double Disk Synergy Test methods respectively. Data were entered into Epidata version 3.1 & analyzed using SPSS ver. 26.

Results: The overall prevalence of urinary tract infection bacteria due to Enterobacteriaceae was 10.6% &most frequent isolates were *E.coli* 42.8%&*K. pneumoniae* 34.5%.The majority (98.3%)of isolated organisms were resistant to third generation cephalosporin (3GC)& Ampicillin & 96.3% were resistant to Cotrimoxazole.The prevalence of ESBL-PE isolates was 34.5% with *E.coli* & *K. pneumonia* being the dominant species contributing for 40% & 30% of the ESBL-PE prevalence respectively. Magnitude of multi-drug resistance level was 27/29(93.1%). Having history of previous antibiotic exposure & current UTI symptoms(PV=0.041) among study participants were found to be independent risk factors for acquiring UTI by ESBL-PE.

Conclusion& recommendation: Isolates of all analyzed species showed considerably high levels of resistance to commonly priscribed antibiotics as well as high frequency of multi drug resistant (MDR) and ESBL phenotypes among diabetic patients as compared with previous similar studies conducted at nearby health facility. Early detection of isolates and rational use of drugs as wel as culture and AST result are necessary before initiating antibiotics.

Key words: Antimicrobial resistance, Diabetes mellitus, ESBL-PE, risk factors and Urinary tract infection.

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List Of abbreviation and accronyms

AMR	Antimicrobial resistance
AOR	Adjusted Odds Ratio
ATCC	American Type Culture Collection
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CNS	Coagulase Negative Staphylococci
COR	Crude Odds Ratio
DDST	Double Disc Synergy Test
EMB	Eosin methylene blue
ESBL	Extended Spectrum Beta-lactum
ESBL-PE	Extended Spectrum Beta-lactumase producing Enterobacteriaceae
ESBLs	Extended Spectrum Beta-lactumase
FMOH	Federal Ministry of Health
JUMC	Jimma University Medical Center
MDR	Multi-drug resistant
MDR-TB	Multi-drug resistant tuberculosis
OPD	Out Patient Departement
MDRE	Multi-drug resistant Enterobacteriaceae
NCCLS	National Committee for Clinical Laboratory Standards
SGGH	Shanan Gibe General Hospital
UTI	Urinary tract infection
XLD	Xylose Lysine Deoxycholate
3GC	Third generation cephalosporin

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Chapter One: Introduction

1.1 Background

Urinary tract infectin is the second most common infection next to respiratory tract infection worldwide and the problem is further complicated while the infection is as a result of Extended Spectrum Beta-lactamase Producing Enterobacteriaceae (ESBL-PE)(1).It is the most common infection affecting patients with diabetes mellitus.Gram negative bacteria, especially the family Enterobacteriaceae are the common causes of both community &hospital acquired urinary tract infections(UTIs).The commonness of this problem inDM individualsis due to impaired body's natural defense mechanisms and is a majorproblem mainly in developing countries(2,3). UTIs are occurred as the result of growth of these microorganisms anywhere within the urinary tract.Its occurrence is characterized by presence of bacteria and white blood cells in the urine of a patient with symptoms of UTI. It is mainly due to bacteria from the digestive tract which climb the opening of the urethra & begin to multiply andinitiate infection(4).

These uropathogens colonize urinary tract by way of developing toxins, siderophores, and adhesins, among other mechanisms. In general, four factors have been more studied and are critical for virulence in at least one infection model: capsule, lipopolysaccharide, fimbriae, and siderophores(5).These characteristics help them colonize and conquer new regions(6). UTI is a common complication after kidney transplantation, frequently related to graft loss and unaffordable healthcare costs(7).

Both gram-positive and gram-negative microorganism are implicated as common causes of UTI. *E.coli* is one of the most common Enterobacteriaceae that cause UTIs in both DM and non-DM patients. Despite the fact that much less regularly than *E.coli*, UTIs in humans and companion animals also can be due to *K. pneumoniae*(8). *C*urrently it is the most important human pathogen in the Klebsiella family, causing a range of infections in hospitals, long-term care facilities, and populations around the world, including infections of the urinary tract, lungs, abdominal cavity, surgical sites, soft tissues, and even bacteremia(9,10).

A hyper-virulent version of *K. pneumoniae* that causes severe liver abscesses and bacteremia emerged during 1980s, which indicates the main characteristics of hyper-muco-viscosity and tendency to cause excessive community-acquired infection. This variantwas previously

isolated in Asian countries like Taiwan and South Korea, however has now spread to nations outside Asia. This new variant regularly harbors the K1 or K2 capsular polysaccharide(11).*E.colli andK.pneumonia* amongentrobacteriaceae are a major threat to public health with the emergence of isolates resistant to most, if not all, to useful antibiotics(12).

Enterobacteraceae are generally found in normal flora of the mouth, skin and gut or gastrointestinal tract (GIT) which serves them as a reservoir. But they can cause UTI depending on favorable conditions (13). The identification of types of species that cause UTI and their Antimicrobial resistance characterization in diabetic clients, as well as the selection of the suitable antibiotic against target organism is important to the successful remedy of those patients(14)**.**

Increased prevalence of the antimicrobial resistant Entrobacteriaceae is associated with different risk factors. Identification of risk factors for AMR may contribute toward improved empirical treatment of community acquired urinary infections (CA-UTIs). Those Previously reported risk factors for developing a UTI with a Community Acquired Extended Spectrum Beta Lactumase (CA ESBL)-producing Entrobacteriaceae include diabetes mellitus, old age, female sex, recurrent UTIs, invasive urological procedures, travel to high-prevalence countries, prior use of antibiotics, such as aminopenicillins, cephalosporins, and fluoroquinolones(15,16).

Since 1980s, beta-lactam antibiotics such as oxyimino-cephalosporins have been used for the treatment of Gram-negative bacterial infections. Unfortunately, nowadays, beta lactamase resistance has been growing among members of Enterobacteriaceae. Meanwhile, the most common cause of beta-lactam resistance is beta-lactamase enzymes, which inactivate beta-lactam drugs by breaking down their beta lactam rings(17).

AMR in Enterobacteriaceae family bacteria has been recognized by the WHO as one of the most significant problems challenging human health(18). Owing to the paucity of new antibiotics being developed, a raise in AMR limits treatment options and increases the risk of treatment failure, leading to increases in morbidity and mortality(19).

1.2 Statement Of The Problem

Urinary tract infection (UTI) is mainly due to invasion of the urethra, prostate, bladder or kidneys by uropathogens. It is the most common community-acquired infections in clinical practice and affects millions people worldwide each year due to a variety of etiological agents(20). For inistance in 2019, globally more than 404.6 million individuals had UTIs, nearly 236 786 people died of UTIs and 6 billion dollars indirect health care cost expended(21,22).

Due to their anatomic & physiologic nature, UTI ismore common in women than men. Nearly half of allwomen have been affected by this infection at least once intheir lifetime. Other important risk factor that enhanceUTI is diabetes mellitus (DM). It is one of the dominant non-communicable, chronic& endocrine diseases.DM patients have a higher prevalence of UTI than their non-diabetic counterparts with a higher severity UTI which may be a cause of complications, starting from dysuria to organ damage and on occasion even loss of life due to complicated UTI (pyelonephritis). On average as much as 35% of diabetic sufferers from a UTI(23).

DM has brought about 1.5 million deaths in 2012 and maintained to grow at an alarming rate from 4.7% in 1980 to 8.5% in 2014. According toestimates from the 2017 International Diabetics Federation,globally 451 million (8.4%) peoples in the age caterory of18–99 years were living with DM & 5 million people aged 20–79 years were died due to this disease. However, the number of people with DM is projected torise to 693 million (9.9%) by 2045 increasing the risk of acquiring various infections including UTI. The increased risk of acquiring UTI in DM is mainily due to frequent urination, high glucose lavel in urine and immunocompomized states of this population(24). In lower and middle-income countries the rate of DM has risen faster than high income countries during the last decade (36). From the Africanregion, in 2017, Ethiopia had the highest number of people(2.6 million) with diabetes, with a 5.2% nationalprevalence(25,26).Percentage of early deaths attributed to diabetes is also higher in lower and middle-income nations than in high income world countries(27).

Among organisms causing UTI in DM as well as non-DM individuals, Enterobacteriaceae are the most common organisms in both community and healthcare settings. However, the antibiotic susceptibility patterns of Enterobacteriaceae have been constantly changing due to

the continuous development of new resistance mechanisms, like the production of extended-spectrum beta-lactamases enzymes and the spread of genes on mobile elements(28). Extended-spectrum β-lactamase (ESBLs)–producing gram-negative microorganism are emerging pathogens. Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are worried about ESBL-producing microorganism because of the developing incidence of such infections, the regulations of powerful antimicrobial drug treatment, and detrimental affected individual consequences.

This antimicrobial resistance is a global public health concern contributing to increased morbidity and mortality.According to the Centers for Disease Control and Prevention (CDC), direct healthcare costs associated with antimicrobial resistance infections are estimated at $20 billion per annum in developed countries(29). This is a public health concern in the management of urinary tract infection especially in the developing countries.

The occurance of ESBL-PE strains of bacteria are major worldwide treat among drug resistant organisms in both community and hospitals settings(30). Extended Spectrum Beta-Lactamases enzymes can hydrolyze all penicillins and cephalosporins, including the extended-spectrum cephalosporins such as cefotaxime or ceftazidime. They are often encoded by the gene harbored by the same plasmids that determines the ESBL type and there are more than 1,600 known beta-lactamases(31). TEM- and SHV-type beta-lactamases, mainly produced by *K. pneumoniae*, have spread throughout hospital settings, and CTX-M enzymes, mainly produced by *E. coli*, have become predominant in the community(32).

Majority of ESBL-producing strains among Enterobacteriaceae are *E. coli*&*K. pneumonia*. Both were diagnosed as an urgent threat to human health based on their developing AMR to the beta-lactam antibiotics. Previous report also indicates that approximately 1/3 of such bacterial isolates *E.colli* and *K. pneumoniae* are resistant to current commonly used antibiotics(33). These pathogens have grown to be a hazard to both patients and healthcare providers as their prevalence and their AMR patterns are raising(34). Particularly this is true when the infections with MDR pathogens impose a huge and increasing burden on every patients and healthcare facilities. Among MDR pathogens, *K. pneumoniae* is one of the world's most risky superbugs; and becoming resistant to nearly every antibiotic available these days

(35). Indiscriminate use of antibiotics frequently results in the increased resistance of these uropathogens to most commonly used antimicrobial drugs(36).

Global AMR reported that both *E.colli* and *K. pneumoniae* had touched a frightening levels of resistance in various parts of the world including resistant to third-generation cephalosporins and carbapenems of up to 54% (37).

Powerful antibiotic therapy for treating those infections is constrained to a small range of medicine like carbapenems; as a result, the chance of resistance to carbapenems among the Enterobacteriaceae increases. Acquisition and shifting of antibiotic resistance genes within or between different species of Gram-negative bacteria via mobile plasmids and transposons are reported to be the principal cause of the producing of β-lactamases(38)**.**

The resistance patterns of microorganisms vary from country to country, state to state, large hospital to small hospital & hospital to community.Africa is a continent in which financial sources for the health care system are low in many nations, particularly in sub-Saharan Africa. Consequently, antibiotics can be missing and the provision of health care can be suboptimal.Despite the fact that statistics concerning the prevalence of ESBL-PE are limited in African nations such as Ethiopia, those few study conducted on these area has shown that ESBL-PE are common in health facility setting and community. For inistance systematic evaluation and meta-analysis done in 2021 shows that pooled prevalence of ESBL-PE is in Sub-saharan Africa (9.3), Ghana (9%), Ethiopia 18 %(39), & specifically in jimma south west Ethiopia it was 23%(40).

The success in management of urinary tract infections in diabetic patients by ESBL-PE or non-ESBL-PE uropathogens relies up on the organisms': identification, characterization of their AMR pattern, evaluation of predisposing factors and the selection of an effective antibiotic towards target organisms isolated.This understanding of spectrum and resistance patterns of isolates may help to guide effective empirical antibiotic therapies, decrease treatment failure and health care costs(41)**.**So the research of the AMR among Enterobacteriaceae uropathogens within the developing countries of Africa would possibly optimize the remedy alternatives used for patients with UTI (42).

Information about bacterial pathogens isolated from urinary tract infection in diabetic patients, characterization of their AMR patterns (ESBLs production), and risk factors associated to this specific problem is limited in deveveloping countries including Ethiopia.

Hence this hospital based cross-sectional study was conducted at the study area where such AMR pattern is not currently assessed and patients are simply treated by empirical treatment wich is based on general guide line and suffering from different morbidity and mortality possibly due to treatment failure.The finding of this study is important in indicating most common uropathogens and their AMR patterns at the study area as well as in providing information about risk factors contributing to acquiring UTI by ESBL-PE among the study participants.

1.3 Significance Of The Study

The success in managment of patients affected by UTI in DM or non-DM patients relies up on the organisms identification, and the selection of an effective antibiotic based AST result of target organism .The finding of this study provide updated information about the burden of UTI,antimicrobial resistance, specifically beta-lactum drugs resistanceprofile of common UTI causing Enterobacteriaceae species among diabetes mellitus patients with urinary tract infection. The finding also indicate risk factors associated with diabetes mellitus patientsacquiring of urinary tract infection by Extended Spectrum Beta Lactamase Producing Enterobacteriaceae.

Chapter Two: Litrature Review

2.1 Enterobacteriaceae

Enterobacteriaceae is a large family of gram-negative bacteria and it was first proposed by Rahn in 1936 and currently taxonomically, Enterobacteriaceae family has 53 genera and over 170 named species. The nomenclature of the Enterobacteriaceae is complicated and has been based on biochemical and antigenic characteristics. Recently, the application of new technologies such as DNA hybridization resulted in numerous changes in classification of the Enterobacteriaceae(43).

Enterobacteriaceae are distributed worldwide and may be found in soil, water, plants, humans and animals. Bacteria belonging to the family Enterobacteriaceae are the most commonly encountered organisms isolated from clinical samples. Most species grow well at 37°C, although some species grow better within temperature range of 25-30°C.Are commonly called the fermentative, gram-negative, enteric bacilli, showing that they are gram-negative rods which can ferment sugars(44).

Generally bacterial organisms categorized under this family are facultative anaerobes, oxidase negative, straight rod-shaped, have variable catalase reactions, nitrates reducer to nitrites except by some strains of Erwinia, glucose-fermenters producing differentend products, having simple nutritional requirements and vary widely in their biochemical characteristics(45). Some of the more common medically important genera of the family Enterobacteriaceae include: *Escherichia, Klebsiella, Proteus, Serratia,Enterobacter, Citrobacter, Salmonella, Shigella, Morganella, Yersinia, Edwardsiella,* and *Providencia*(46).

The most common genera of Enterobacteriaceae causing opportunistic infections in humans are *Escherichia coli*, *Proteus, Enterobacter, Klebsiella, Citrobacter,* and *Serratia*(47). They act as opportunistic pathogens when they get entry into body locations where they are not normally found, especially if the host is debilitated. The most common infection caused by these opportunistic Enterobacteriaceae is UTI. Its occurrence is characterized by presence of bacteria and white blood cells in the urine of a patient with symptoms of infection of the urethra, urinary bladder, or the kidney. Infections of the urinary tract include urethritis, cystitis, pyelonephritis, and prostatitis.

These infections are very common, and every year cause over 7 million physician office visits and about 1 million hospitalizations. *E. coli* is the most common cause of these urethritis, cystitis, prostatitis, and pyelonephritis. With the presence of risk factors that cause functional & structural abnormalities of the urinary tract, everyone can get UTI. These abnormalities increase the volume of residual urine & interfere with the normal clearance of bacteria through urination.

In diabetic patients, it is generally agreed that UTI are frequent and is being the most common bacterial infections(48). UTI in diabetic can be more severe leading to complications, ranging from dysuria to organ damage and sometimes even death due to pyelonephritis.The prevalence of UTI among diabetic patients was 51.3% in study conducted on Egyptian Diabetic patients in 2019. Still this study also indicates that Entrobacteriaceae family members are the dominant cause of UTI in which *E.colli* (39%) and Klebsiella (21%) are the 1st and 2nd followed by CNS (Coagulase Negative Staphylococcuys) (15%) and protous (12.5%).In study conducted on similar topic in Harar, Eastern Ethiopia: significant bacteriuria was detected in 15.4% (37/240) in study participants (DM patients). The majority (70%) of the isolates were Gram-negative bacteria(49). According to the study conducted as Prevalence and associated factors of urinary tract infections among diabetic patients in Arba Minch Hospitalthe prevalence of UTI is higher in diabetic patients. These results also revealed that the predominant pathogens of UTI were Gram-negative bacilli (Enterobacteriaceae), particularly *E. coli.* Study done on Adult Diabetic PatientsatMetuKarlHeinzReferralHospital in 2018 also reported that the predominant isolates were members ofEnterobacteriaceae, *E.colli* (25.6%) followed by *Klebsiella spps* (20.5%) (50).

2.2 Extended-Spectrum β-Lactamase producing Enterobacteriaceae(ESBL-PE)

Bacterial resistance to β-lactam antibiotics occurs by three mechanisms: failure of the β-lactam antibiotics to reach the penicillin-binding proteins (PBPs), low binding affinity to the PBPs and inactivation of the drug by β-lactamases. Among theseβ-Lactamases are the commonest cause of bacterial resistance to β-lactamantimicrobials(51).

ESBL-PE are Enterobacteriaeae organisms producing Extended-Spectrum β-Lactamase which is produced by bacteria to become resistant to extended-spectrum penicillin, cephalosporins,

and monobactams except for cephamycins and carbapenems.They are often encoded by acquisition and transferring of antibiotic resistance genes within or between different species of Gram-negative bacteria through mobile plasmids and transposons.This mechanism is reported to be the principal cause of the β-lactamases production (38).There are more than 1,600 known beta-lactamases, a list that is rapidly expanding (31).

Extended-Spectrum β-Lactamase -producing Enterobacteriaceae were identified in the early 1980s following the introduction of oxyimino-β-lactam agents. Majority of Extended-Spectrum β-Lactamase-producing strains among Enterobacteriaceae are *E.coli* and *K.pneumoniae*. This Extended-Spectrum β-Lactamase enzymecan be inhibited by beta-lactamase inhibitors like clavulanic acid(52).Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE), particularly the Gram-negative bacilli such as *E. coli* and *K. pneumoniae*, are the major global health threat due to their pattern of multidrug resistance. Poor drug regulation and control systems in many parts of the world have led to an extensive misuse and overuse of antibacterial drugs in both humans and animals settings. Such activities collectively favor the spread of resistant bacterial strains into the community and the clinical settings that subsequently increase the treatment failure rate due to raise of resistance pattern among these group of organisms(53)**.**

Beta-lactam drugs such as extended-spectrum penicillins, cephalosporins, monobactams, carbapenems, fluoroquinolones (e.g. ciprofloxacin) and aminoglycosides (e.g. gentamicin) are among the most ordered antibiotics to treat infections caused by Enterobacteriaceae. The widespread use of β-lactam antibiotics has led to the spread of resistant Enterobacteriaceae.

The most important mechanism of resistance to beta-lactam antibiotics is by production of beta-lactamases that inactivate beta-lactam antibiotics & this continue to be the leading cause of β-lactam antibiotics resistance among Enterobacteriaceae throughout the world(54).

2.3Isolation& identification of Enterobacteriaceae/ESBL-PE from DM-UTI patients

The enterobacteria grow easily on simple media, on which they will survive for years in tubes sealed with paraffin wax. The majority of species, being easy and 'safe' to handle, provide students, biochemists, geneticists, and even bacteriologists with suitable experimental material(55). To isolate Enterobacteriaceae and Pseudomonas, specimens from the infected site are plated out on any one of a large number of selective and differential media such as

EMB agar, Endo agar, Deoxycholate agar, MacConkey agar, Hektoen Enteric agar, and XLD agar(56). XLD agar is selective for gram-negative bacteria. In addition, different gram-negative bacilli, due to their biochemical reactions, produce different appearing colonies(57).The laboratory culture standard for a UTI is the presence of more than 100,000 CFUs /ml of midstream urine or any CFUs from a catheter obtained urine sample (46).

Even though historically, the differentiation of members of this family has been based on biochemical features now more recently it is based on the analysis of the sequence of the 16S RNAr gene. However, in some cases, the low discriminatory ability of 16S RNAr sequence analysis as a result, the taxonomy of the Enterobacteria have undergone frequent changes in recent decades, this phenomenon make it necessary to use additional identification techniques(58).

2.4Drug resistance of Enterobacteriaceae family bacterial isolates.

Organisms like *E.colli* and *K. pneumoniae* among Enterobacteriaceae family have been identified as an urgent threat to human health based on their increasing AMR to the beta-lactam antibiotics. Previous study indicates that approximately one third of such bacterial isolates *E.colli* and *K. pneumoniae* are resistant to current commonly used antibiotics (33).

The bacterial uropathogens, isolated from the urine specimen of DM patients, showed different degree of resistance to commonly used antimicrobial agents in different study setting. Forinistance in study conducted in NRH American bacterial uropathogens isolated from DM patients revealed the presence of high levels of single & multiple antimicrobial resistances against commonly used drugs. *K. pneumoniae* & *E.coli*, are the most predominant uropathogens, among gram-negative bacteria, & they displayed highest level of resistance (100%) to amoxicillin, ampicillin, & penicillin G; & lowest level of resistance to ceftriaxone & nitrofurantoin(25% vs 0%)(50)

All bacterial isolates were resistant to at least one antibiotic, & 92.5% of the isolates from the study participant were resistant to multiple drugs as reported in Study conducted in Harar, Eastern Ethiopia(14).

Concerning ESBL resistance, Extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-PE) infections are a growing threat to human health, and the treatment of these infections becomes more and more challenging(1). In study conducted as ‘Risk Factors for

Colonization with Extended-Spectrum β-Lactamase– Producing Bacteria and Intensive Care Unit Admission' in Europe, Of 5,209 patient admissions, 117 (2%) patients were colonized by an ESBL-producing *E. coli* or *Klebsiella* species bacterium on ICU admission. Specifically, 76 (65%) patients were colonized by an ESBL-producing *E. coli*, 55 (47%) were colonized by an ESBL-producing *Klebsiella species*, and 14 (12%) patients were colonized by both. According to study conducted at Mekelle University, northern Ethiopia, Of 47 Gram-negative isolates, 12(25.5%) were positive for ESBLs production with10/12 of them *E.coli* isolates & 2/12*K.pnuemoniae*(59).

2.5 Risk factors

Different study result shows that there are risk factors that predispose both diabetic and non-diabetic patients to UTI infection with organisms that are susceptible or resistant to different categories of antimicrobials including Extended-Spectrum β-Lactam. For e.g. it is already reported that up to 35% of diabetic patients experience a UTI. The worldwide dissemination of ESBL-PE, and their subset carbapenimase producing Enterobacteriaceae (CPE), is alarming(60).

Predisposing factors for UTI among patients with and without DM have been identified as: female sex, lower education level, low immunity, glycosuria, employment status, poor diabetic control, obesity, incomplete bladder emptying due to autonomic neuropathy, bladder dysfunction and prostate syndrome in men(50).Study conducted in NRH American revealed among socio-demographic characteristics and clinical history of the study participants: level of education, history of UTIs and glucosuria was found to be significantly associated with UTIs. Illiterate diabetic patients had an odds of 9.3 (AOR = 9.3, 95 % CI (1.1–79.2) for being UTIs positive than those who had university/college level of education. Moreover, the study participants with history of UTIs and glucosuria had odds of 3.2 (AOR = 3.2, 95 % CI (1.2–8.7) and 3.2 (AOR = 3.2, 95 % CI (1.2–8.1) for being UTIs positive, respectively, than those who did not have history of UTIs and glucosuria(50,59).

Studies conducted in Egypt in 2019 also remind that patients with diabetes have a 10-fold increased risk of UTI when compared to non-diabetics as shown in a previous study.Some hospital-based studies conducted in the central, northwest and southern part of Ethiopia reported a varying prevalence of UTI ranging from 10.4% to 17.8% and a high rate of multidrug resistance varying between 59.8%, and 93.9% among diabetic patients(14).

Significant bacteriuria had an association with the consumption of alcohol, gender and glucose level as reported in study conducted in Arbaminch province.

With regard to Extended-Spectrum β-Lactamase; the study results obtained from 'The Prevalence and Characterization of Extended-Spectrum β-Lactamase- and Carbapenemase-Producing Bacteria from Hospital Sewage, Treated Effluents and Receiving Rivers' showed that ESBL (blaCTX-M) is widely detected in a number of different bacterial species. These resistance genes were mainly harbored in Enterobacteriaceae, followed by Acinetobacter and Aeromonas isolates(61).

Antibiotic use within the previous 3 months ($p = 0.001$) and admission to hospital within the previous 3 months ($p = 0.03$) are found to be associated with infection with Extended-Spectrum β-Lactamase producing Entrobacteriaceae. According to study conducted as Risk Factors for and Outcomes of Bacteremia Caused by Extended-Spectrum ß-Lactamase–Producing *E.coli* and *Klebsiella* species at a Canadian Tertiary Care Hospital, antibiotic use within the previous 3 months was found to be an independent risk factor for acquisition of ESBL-PE bacteremia (odds ratio 5.2, 95% confidence interval 1.6–16.9)(62).

Conceptual frame work

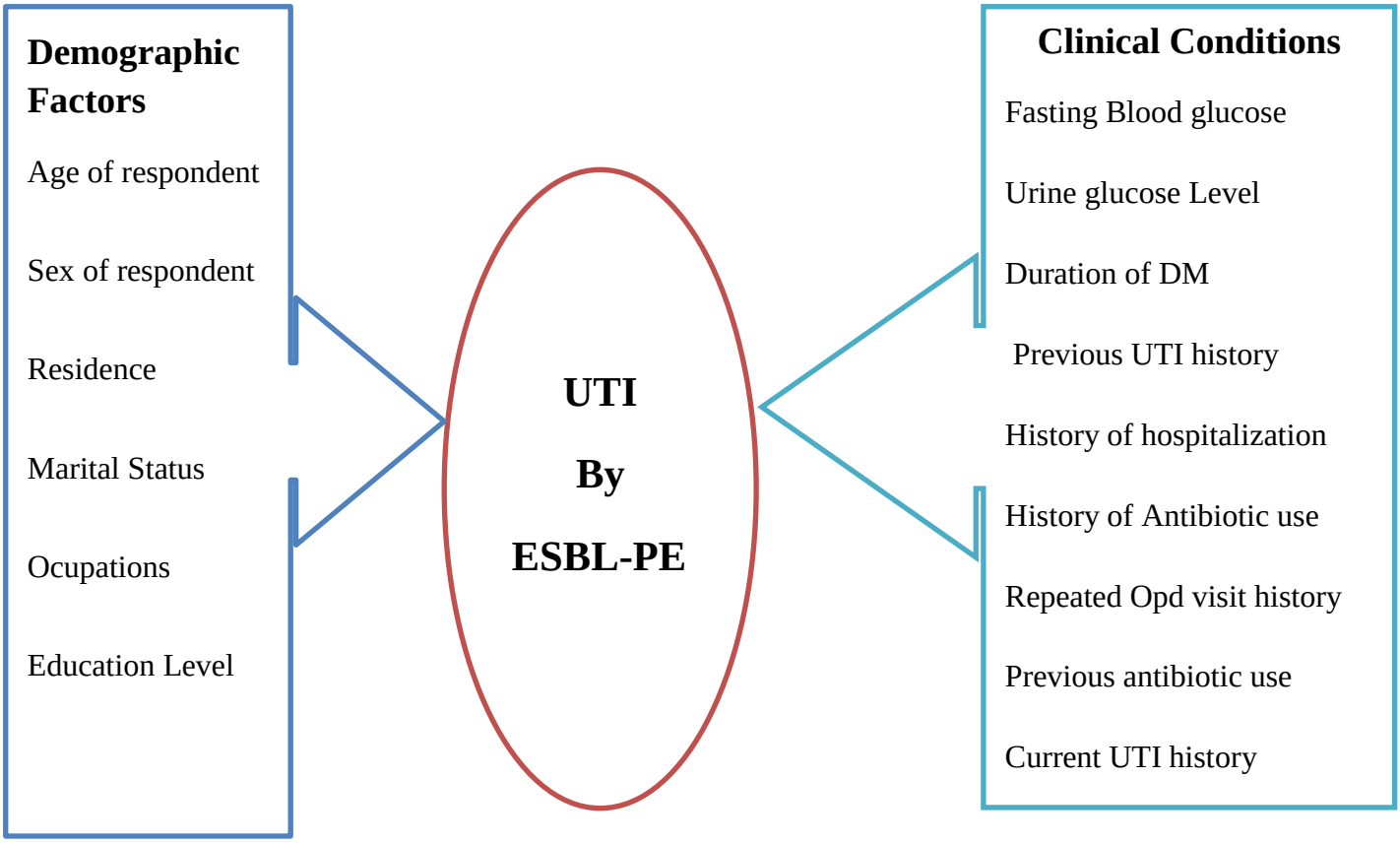


Figure 1 Conceptual frame work elucidating possible relationship between Dependent & independent variables.

Chapter Three: Objectives

3.1 General Objective

To determine the Prevalence of Extended Spectrum Betalactamase producing Entrobacteracae and associated risk factor among Diabetes mellitus patients with urinary tract infectionat Shanan Gibe General Hospital, Jimma town,south west Ethiopia 2021.

3.2 SpecificObjectives

- To determine the prevalence of UTI caused Enterobacteriaceae uropathogens among diabetic patients with UTI atSGGH
- To determine the Prevalence of Extended Spectrum Beta-Lactamase produsing Enterobacteriaceae among diabetic patients with UTI atSGGH.
- To determine Antimicrobial Resistance profile of Enterobacteriaceae among diabetic patients with UTIat SGGH.
- To assess the risk factors associated with UTI by ESBL-PE among diabetic patients with UTI at SGGH.

Chapter Four: Materials and Methods

4.1Study area

This study was conducted at Shanan Gibe General Hospitals which is located in Oromia region, Jimma town south west Ethiopia. It is 352 Km away from Addis Ababa which is the capital city of Ethiopia. It is one of the recently established public hospitals in the Oromia region.

This hospital has 1.5 million Catchment population and 80 beds for inpatient service. Currently it has a total of 288staffs(147 health professionals and131 administration staffs). In addition to other routine services the hospital is serving as COVID-19 treatment center, MDR-TB treatment center, and as leading cluster Hospital of 8 nearby district and General Hospitals. As routine service this hospital started by focusing mainly on disease prevention like vaccination and maternal health service. But now a days the hospital is forced to also focus on chronic care services since the number of clients with chronic disease like diabetic mellitus are increasing from time to time and almost all of the health centers are referring this clients to this hospital.

4. 2Study design and period

An institution based cross-sectional study designwasconductedfrom July 15 to October 2021 todeterminethe prevalence of ESBL-PE andassociated risk factors among diabetic patients with UTI at SGGH.

4.3Population

4.3.1 Source population

The source populations of the study were all diabetic patients visiting the study setting during the study period.

4. 3.2Study population

All diabetic patients who visited SGGH during the study period &fulfilled the inclusion criteria.

4.4 Inclusion and Exclusioncriteria

4.4.1 Inclusion criteria

All DM patients who came to SGGH during the study period w and are volunteer to participate in the study.

4. 4.2 Exclusion criteria

Diabetic patients whocannot speak or listen,who had mental health problems,all emergencies or critically ill patients and those on antibiotic treatment within at least two weeks including the day of data collectionwere excluded from the study.

4.5 Sample size determination and sampling techniques

4.5.1Sample size determination

The sample size for this study was determined by using a single population proportion equation.

Previous study indicates that the prevalence of ESBL-PE in Jimmasouth west Ethiopia was23%(63).So P = 0.23, margin of error (d) = 0.05 and 95% confidence-interval critical value (Zά/2=1.96) was used.

$$n = \frac{(Z_{\frac{\alpha}{2}}^{2})P(1-P)}{d^2} = n_o = \frac{(1.96)^2\ 0.23(1-0.23)}{(0.05)^2} = 0.6803/0.0025 = \mathbf{272}$$

Where: n = the number of study subjects from which samples will be taken

P = anticipated population proportion. Prevalence of ESBL taken as 0.23

Z = Standard normal distribution value at 95% CI, which is 1.96

d = Degree of precision taken as 5%.

4.5.2 Sampling techniques

During selection of study participant'sconvenient sampling method was used and study participants came for DM follow-up wereall consecutively included until the desired minimum sample size (272) wasachieved.

4.6 Variable of the study

4.6.1Independent Variable

- ✓ Socio-demographic characteristics (age, sex, address, occupation, level of education) of the participants
- ✓ Clinical characteristics (Duration of DM, Blood glucose level, History of UTI, Urine glucose level, symptom of UTIs) of the participants

4.6.2 Dependent Variables

- ✓ Prevalence of UTI by ESBL-PE
- ✓ AMR pattern of Enterobacteriaceae urine isolates

4. 7 Data collection method and laboratory diagnosis

4. 7.1 Data collection method

Socio-demographic and other clinical data: Respondents Socio-demographic and other clinical data were collectedusing structured questionnaire developed for this specific study. During this data collection physician assigned to chronic care OPD who is responsible for clerking and examining the respective DM patientswas consulted to get preper clinical data of study participant.

4.7.2 Laboratory Examination of Urine

a. Midstream urine sample collection

A total of 272 midstream urine samples were collected with a sterile, wide mouthed, dry and leak-proof containers. Once the request paper was chacked for its' completeness for essential informations like patients' full name, source/method of sample collection (catheter obtained, simply collected by voiding, etc), date and tme of collection; study participants are provided with appropriate container as well as inistructed to collect sufficient, not contaminated midstream urine sample. All specimens were collected based on standard operational procedure and stored in proper storage condition. During sample collection processes the study participants were instructed as per the standard procedure (SOP) for general urine sample collection as detailed in annex three. Due to high probability of contaminations at vulva female study participants were provided with especial inistructions. Forinistance they were told to hold appart or spread labia majora (outer folds) and swipe each two sides of labia minora

(inner folds) with sterile separate towelettes using a single downward stroke. With the third towelette, cleanse meatus (center area) with a single downward stroke.Then void first few(20-25) milliliters of urine into the toilet and in between collect ½ to 2/3 full of provided urine cup & pass the rest urine into the toilet again. (62)

b. Transportation and storage of the urine samples

Collected urine sampleswere transported at room temperature and delivered to JU microbiology laboratory for culturing, isolation, biochemical test and drug-resistance test and processed within 2 hours of collection.Alternatively specimens were refrigerated and processed within 24 hours of collection when delliance was experienced .

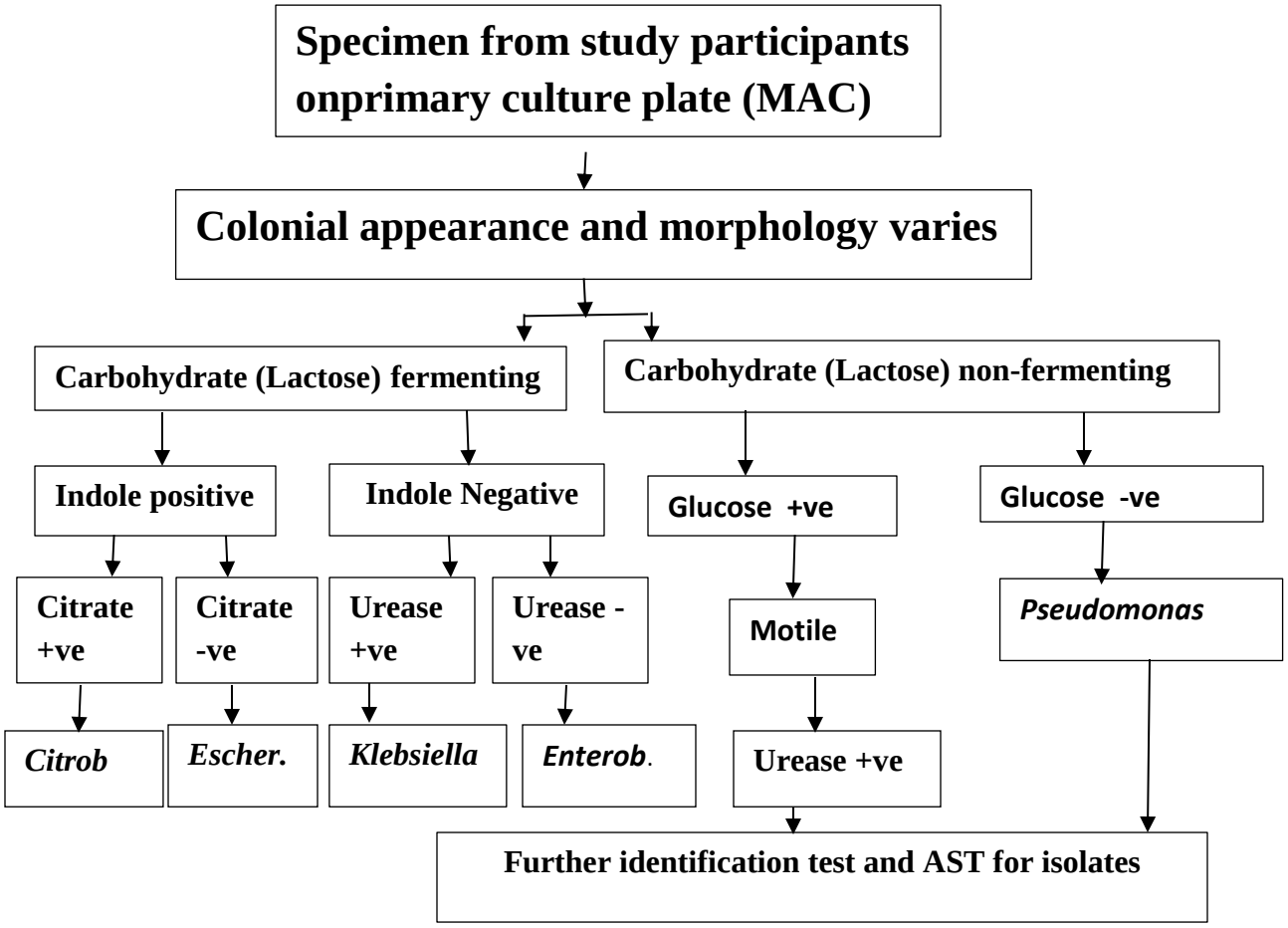


Figure 2 Flowchart for Isolation, Identification and AST of Enterobacteriaceae urine isolate.

c. Screening of UTI

1µl urine sample from 10ml well-mixed urine sample was inoculated on MacConkey agar (Oxoid, UK) using a standard calibrated wire loop (1µL) and incubated at 37°C for 18-24 hours. On the next day, the bacterial growth on the respective media was observed.Those samples with colony count of at least 10^5CFU/ml single isolate for single midstream urine weretaken as positive urine culture (UTI). For samples with clonies less than 10^5CFU/ml only it can be considered significant based on some situations like method of sample collection (catheter collected urine),symptom of the patient etc(64) (Table 1)

Table 1 Interpretations of bacterial colonies observed from urine culture.

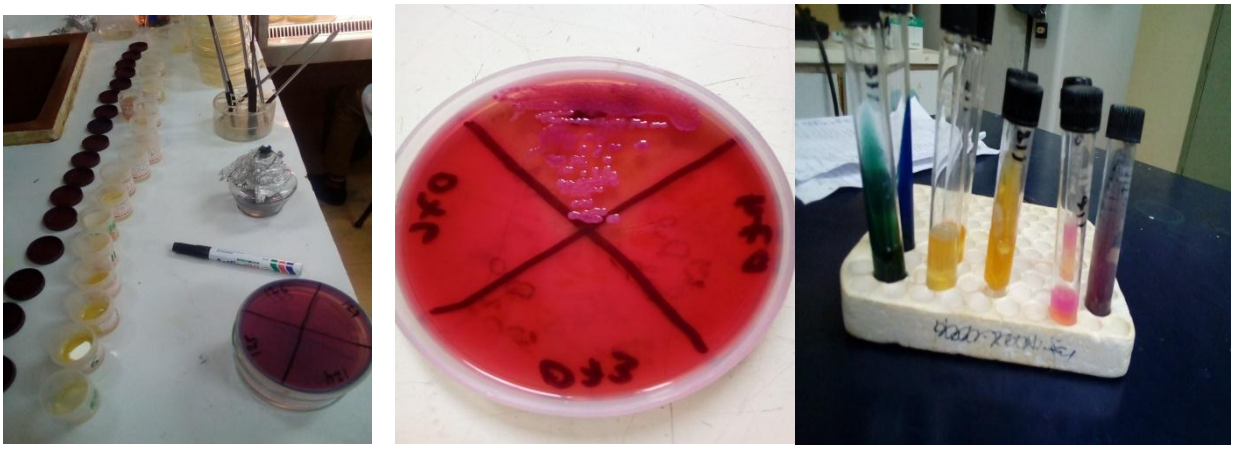
SN	No of colony count	Expected no of bacteria	Interpretation
1	≥100 colonies of one type bacteria	$\geq 10^5$CFU/mL	UTI
2	1-100 colonies of one type bacteria	10^4-10^5CFU/mL	Depends on clinical feature
3	≤10 colonies of one type bacteria	$\leq 10^4$	UTI is less probable
4	2 types of colonies with at least one of them with ≥100 colonies		UTI is likely
5	If <100 colonies both types		UTI is unlikely
6	If>2 types of colonies are observed		Contaminated

d.Isolation and Identification of Enterobacteriaceae uropathogens

All the isolates were preliminarily screened on MacConkyagar which is selective and differential medium by their colony morphology, pigment production (pink to colorless flat or mucoid colonies).This was done by inoculation of 1µl urine sample taken from well mixed 10ml sample of respective study participant on MacConkey and further identifications of isolates were made with relevant biochemical tests(1).

These biochemical tests were TSI for sugar fermentation test, SIM agar for sulfide & indole production as well as for motility test, citrate agar for testing of organism's ability to use citrate as sole source of energy, and urea agar medium for testing organism's ability to produce urease enzyme. For example, an isolate was considered as *E. coli* when it is indole positive (dark pink ring), citrate negative (no change or remained green), urea negative, gas and acid producer, and motile and considered as *K. pneumoniae*when it is indole negative, citrate

positive, urea slow producing, and non-motile. All media and biochemical testsused for screening and identification were from OXOID Ltd. England. In case of delay to perform AST, the isolated bacteria were kept at 2–8°C in the nutrient broth for not more than 24hrs or longer time in tryptone soya broth with 15% glycerol until the AST test was done.



A **B** **C**

Figure 3Isolation & Identification of Enterobacteriaceae uropathogensamong DM-UTI patients at SGGH, 2021.

A=Urine sample B=groth of LF Enterobacteriaceae on MacConkey agar C= Biochemical tests

4.7.3Antimicrobial susceptibility testing(AST)

The antimicrobial susceptablity testing was performed Mueller-Hinton agar(Oxoid, UK) using Kirby bauer disk diffusion technique following the guidelines Clinical and Laboratory Standard Institute (CLSI)(65). About 3-5 similar colonies of bacteria were taken from pure colony and

transferred to a tube containing 5ml sterile normal saline (0.85% NaCl) and mixed gently until it will form a homogeneous suspension. The turbidity of the suspension was adjusted to the optical density of McFarland 0.5 tubes in order to standardize the inoculums size. Next the sterile cotton swab was dipped into the suspension and the excess suspension (solution) was removed by gentle rotation of the swab against the surface of the tube. Then the swab wasused to distribute the bacteria suspension evenly over the entire surface of Mueller-Hinton agar (OXOID Ltd. England).

By using a sterile forceps, the antibiotic discs were placed on the plates inoculated with respective isolates and incubated at 37^{o}C for 18-24 hours. After 18-24 hours or on the next day of inoculationthe diameter of the zone of inhibition around the diskswere measured to the nearest millimeter using a ruler and the isolate were classified as sensitive and resistant according to CLSI guide line(50).

The following antibiotic disks were used to determine the AST profile of identified pathogens:Penicillin (10 µg)Ampicillin (10 µg),Amoxicillin-clavulanate (20+10) µg, Norfloxaccin(10µg), ciprofloxacin (10µg), ceftriaxone (30µg), Gentamycin (10µg), Nitrofurantoin (300µg),Nalidixic acid(30 µg), amikacin (30µg),tetracycline (30µg), cefotaxime (30µg), ceftaziime (30µg),& trimethoprime-sulfamethoxazole (1.25/23.75 µg) (ROSCO Ltd. Danish). These antibiotics were selected based on CLSI guide line, consultion of concerned physicians & pharmacy professionals currently serving the community at different public as well as private health facilities and current availability the respective disks on the market.*E. coli* ATCC®25922,*Pseudomonas aerogenosa* ATCC® 27853 and *S.auras* ATCC® 27923were used as quality control strain during culture and sensitivity testing precess.

Figure 4 AST Antimicrobial Susceptibility Testing for Enterobacteriaceae uropathogens isolated from urine samples of DM-UTI patients at SGGH, 2021.

4.7.4Detection of Extended Spectrum Beta Lactamase producing Enterobacteriaceae

Of various phenotypic methods recommended in the routine practice to detect the ESBL production in Enterobacteriaceae,Double Disc Synergy Test (DDST) which uses the 3GC with amoxicillin-clavlanic acidwas used in this study.For screening a0.5 McFarland's standard suspension of the test isolates werestreaked on the surface of Muller Hinton Agar plates (MHA) (OXOID Ltd. England) by using a sterile cotton swab.3GCceftazidime (CAZ:30µg),Cefotaxime (CTX:30µg) &ceftriaxone(CTR:30µg) were placed onthe MHA and lightly pressedon to make sure they are firmly placed on to the media. These plates were incubatedat 37˚C for 18-24 hours. Inhibition zone of <21mm forceftazidime (CAZ: 30µg),<26mm forCefotaxime (CTX: 30µg) and <23mm forceftriaxone (CTR:30µg) were considered as suspect for ESBL production based current CLSI guide-line (2021). The ESBL production was tested by the Double Disc Synergy Test by using a disc of Amoxicillin-clavulanate (20/10 µg) along with three3GC (Cefotaxime, Ceftriaxone and Ceftazidime)(66). A disc which contained Amoxicillin-clavulanate (20/10 µg) was placed in the centre of the plate. The discs of third generation cephalosporin were placed 15 mm apart centre to centre to that of the Amoxicillin-clavulanatedisc . Any distortion or increase in the zone of inhibition towards the disc of Amoxicillin-clavulanatewas considered as positive for the ESBLs production.

4.8 Data Quality Assurance

- ✓ To ensure data quality the questionarie was translated to local languages (Amharic & Afan Oromo) before data collection &collected data were again re-translated to English before data entry & analysis
- ✓ The quality of reagents was checked as per SOP of Jimma University medical microbiology Laboratory before main laboratory based activities were done.
- ✓ Quality control (QC) strains;

E. coli, ATCC 25922: Gram negative for gram staining as well as for culture &AST

S. aureus, ATCC 25923: Gram positive and

*P. aeruginosa*ATCC 27853: Oxidase positive were used as reference during the isolation andAST activity.

Data and samples wereethically maintained in pre-analytical, analytical and post analytical phases of the research process.

4.9Statistical analysis

The completed questionnaires were checked for completeness, consistency and coded by the principal investigator. Any error identified were corrected immediately by tracing back with the systematic chain of communicationbetween principal investigator, data collectors, data sources and/or respondents. Data entry was done by using Epidata version 3.1 and analysis wasdoneusingSPSSversion26.Frequencyofvariableswasdeterminedanddescriptivefindings were presented using tables and graphs. Crude odds ratio (COR) and adjusted odds ratio (AOR) with 95 % confidence interval (CI) was also calculated. Toassess the associations between dependentand independent variable binary logistic regression wasdone and those independent variables with p value less than 0.25 by binary logistic regression were selected for multivariate logistic regression analysis. Independent variables with P value less than 0.05 by multiple logistic regressions wereconsidered statically significant.

4.10 Ethical statement

Ethical clearance and approval for the study was obtained fromInstitutional Ethics ReviewBoard of Health Institute, Jimma University. The study area (SGGH) wascommunicatedby legal letters written for this specific purpose from Jimma University, Institute of health, Faculty of health Science and School of medical laboratory science to get administrative permission for data collection. At study area consent form was signed by each and every study participants included in the study before starting interview and collecting samples. Positive resuls (comfirmed UTI cases) were ethically communicated soon specifically to physician who was working on chronic follow-up OPD with the permission of respondent.

4.11 Operational definition

Current UTI symptom = Presence of UTI symptom at the moment of of data collection.

Diabetic Mellitus = a chronic disease associated with abnormally high levels of the sugar glucose in the bloodof study participants (fasting blood glucose level >126mg/dl).

Duration with DM= It is the total length of stay in years the study participants live DM.

Extended Spectrum Beta Lactamase = are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam.They hydrolyze extended spectrum cephalosporins with oxymino side chain.

Fasting Blood glucose = is early morning blood glucose level in mg/dl of the study participants ..

History of hospitalization= is a respondent who had already hospitalized to any health inistitution within last 12 months preceeding dy of data collection.

Previous OPD history = A respondents who ever came to OPD for any health services as new or follow-up within the three months preceeding data collection.

Preveous Antibiotic use= Antibiotic use history of study participants within the three months preceeding data collection.

Previous UTI history= A respondents who aquired UTI within the 3 months preceeding data collection.

Significant bacteriuria = the presence in a midstream urine sample of 10^5 colony forming unit of bacteria in one milliliter of urine sample on pure culture.

Multi-Drug Resistant (MDR) = are Enterobacteriaceae uropathogen isolates found to be resistant to at least drugs from three antibacterial drug families.

Midstream urine (MSU) = it is a urine sample obtained by first voiding few milliliters of urine into toilet and in-between into urine cup and finally the rest is voided to the toilet again by study participant.

Urinary Tract Infection (UTI) = it is infection urinary tract by microorganisms (uropathogens) or study participants having of UTI symptoms at urethra, blader , kidney and etc.

Third Generation Cephalosporins= are antibiotics that are active againist both gram positive and gram negative uropathogens, but are relatively more effective against gram negative (Enterobacteriaceae).

Beta-Lactum antibiotics = are bactericidal antibiotics that those excert their bactericidal effect through binding covalently to and inhibit penicillin binding proteins (PBPs) of uropathogens and make them lysed.

Double Disk Synergy Test = is synergistic bactericidal effects of clavulanic acid in AMC & 3GC antibiotic disks towards Enterobacteriaceae uropathogens.

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Chapter Five: Result

5.1 Socio-emographic and clinical characteristics of study participants

A total of 272 known Diabetes mellitus patients within the age range of 18-94 years were included in this study. Of the total study participants 145(53.3%)were female,156 (57.3%) were urban resident and 85.7% were married. Regarding the educational status of study participants, 89 (32.7%) had no education followed by primary education 75 (27.6%) and secondary education 56 (20.6%).

Moreover, we also assessed some clinical status of the study participants. As a result respondents with previous UTI history and antibiotic exposure in last 3 months and current UTI symptoms were 95 (34.9%), 22 (8.1%) and 39 (14%) respectively. Study participants with repeated OPD visit in last one month were 42 (15.4%). Furthermore, study participants were assessed for if their blood and urine glucose level was controlled or not during data collection. Consequently, 197 (72.4%) and 52 (19.1%) of study participants had fasting blood glucose ≥ 126mg/dl and different urine glucose level ranges from trace to 4+(Table2).

Table 2Socio-demographic and clinical characteristics of study participants atSGGH, Jimma town, south-west Ethiopia, July 15- Oct. 2021.

Variables	Categories of variables	Frequencies	percent
Sex of study participants	Female	145	53.3%
	Male	127	46.7%
Age of study participants	18-24 years	1	0.4%
	25-34 years	17	6.3%
	35—44 years	17	6.3%
	45-54 years	63	23.2%
	55-64 years	79	29.0%
	> 64 years	95	34.9%
Marital Status of study participants	Single	13	4.8%
	Married	233	85.7%
	Divorced	19	7%
	Widowed	7	2.6%
Occupation of study participants	House wife	89	32.7%
	Gov't Employee	6	2.2%
	Non-gov't employee	61	22.4%

	Student	7	2.6%
	Merchant	75	27.6%
	Daily labor	5	1.8%
	Farmer	29	10.7%
Educational Level of study participants	No education	89	32.7%
	Primary education	75	27.6%
	Secondary Education	56	20.6%
	Higher Education	52	19.1%
Residence of study participants	Urban	156	57.4%
	Rural	116	42.6%
Fasting blood glucose level	≤126mg/dl	75	27.6%
	>126mg/dl	197	72.4%
Urine glucose level	Negative	220	80.9%
	Positive (Trace - 4+)	52	19.1%
Times in years since diagnosed with DM	Less than 1 year	20	7.3%
	1-5 years	128	47.1%
	6-10 years	86	31.6%
	Greater than 1 year	38	14%
UTI history of respondent in last three months	History of UTI	95	34.9%
	No history of UTI	177	65.1%
Respondents history of antibiotic use in the past three months	Antibiotic was used	22	8.1%
	Antibiotic was not used	250	91.9%
Respondents history of repeated OPD visit in last one month	Repeated OPD visit	42	15.4 %
	No repeated OPD visit	230	84.6 %
Presence of UTI symptom during the data collection	UTI symptom available	39	14.3%
	UTI symptom not available	233	85.7%

5.2 Frequency of Enterobacteriaceae Urine Isolates

Screening of UTI causedby a member of Enterobacteriaceae family uropathogens was done for all 272 samples collected from study participants and twenty nine samples were found to be positive for significant bacteriuria for these uropathogens. Thus, prevalence of UTI caused by Enterobacteriaceae among DM patients was 10.6% in this study.Those Enterobacteriaceae organisms isolated were, *E.coli*, *Klebsiella* spp., *Proteus* spp, *Enterobacter* spp., and *Citrobacter* spp. The frequencyof Enterobacteriaceae organisms isolated were, *E.coli*41.4%, (n=12) followed

by *Klebsiella* spp.34.5% (n=10), *Proteus* spp 10.3% (n=3),*Enterobacter* spp 10.3% (n=3) & *Citrobacter* spp. 3.4% (n=1). (Fig.5)

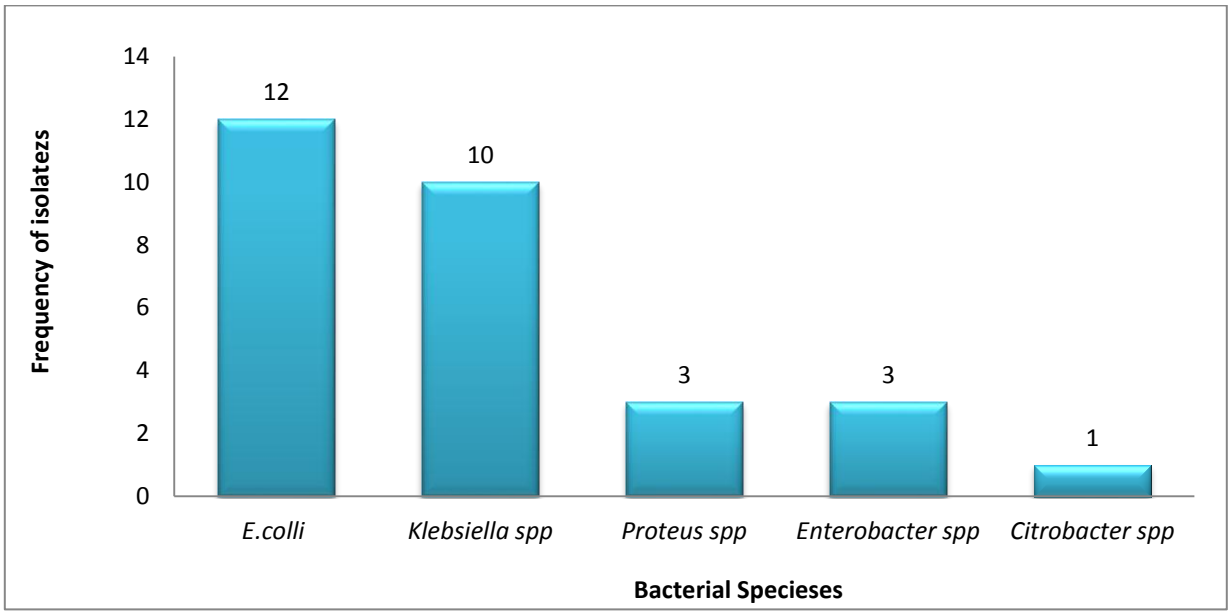

Figure 5 Frequency of Enterobacteriaceae uropathogens species isolated from urine of DM patients with UTI at SGGH, Jimma town, south-west Ethiopia, July 15- Oct 22, 2021.

5.3 Antibiotic resistance profile of Enterobacteriaceae isolates

Enterobacteriaceae bacterialisolates showed varied resistance patterns againist 14 selected antibacterial drugs within the range of relatively minimum resistance 6.9% to nitrofurantoin and maximum resistance 100% to ceftazidime:in addition to ceftazidime the isolates specially showed increased resistance, with 96.5% of isolates resistant toother 3GC (CTR &CTX) and penicillin,93.1% to ampicillin, 86.2% to Ciprofloxacin (CIP) & Amoxicillin/clavulanic acid(AMC), 82.8% to trimethoprim-sulfamethoxazole(STX), 79.3% toTetracycline,75.9% to Gentamycin (G),41.4% to Norfloxacin(NOR)&Naldixic acid (NA), &34.5% to Amikacin (AMK).During antibiotic susceptibility testingthe results of intermediatesusceptibility were considered as resistant.

Among isolated Enterobacteriaceae ,resistanceprofiles of *E. coli*to differentselectedantibiotics indicated big variation with 100% of isolates resistant to CTZ and penicillin, 91.6% to CTR, CTX, and ampicillin, as well as 83.3% to ciprofloxacin, Tetracycline&Amoxicillin/clavulanic acid.But 100%, 66.7%, & 41.7 % of *E.colli*isolatesweresusceptible to Nitrofurantoin, Amikacin and Naldixic acid/ Norfloxacinrespectively.

Similarly*Klebsiella* species had also shown different level of resistancetowards those selected antibiotics by beingall of them resistantto tested 3GC, 90% to Penicillin andAmpicillin,80%to Amoxicillin/clavulanic acid, Ciprofloxacin and,Gentamycin(Table3). Butall of them were susceptible to nitrofurantoin, 90% to Norfloxacin&80% to Amikacin and Naldixic acid.Out of 29Enterobacteriaceae isolates, 27(93%) of them were MDR (resistant to3 or more drugs belonging to different antibiotics classes).

Table 3 Antibiotics susceptiblity profile of Enterobacteriaceae isolated from urine specimen of the study participants at SGGH, Jimma town, south-west Ethiopia, July 15- Oct. 2021.

Antibiotics	***E.coli* (n=12) n(%)**	***Klebsiella* spp (n=10) n(%)**	***Enterobacter* spp (n=3) n(%)**	***Proteus* spp (n=3) n(%)**	***Citrobacter* spp (n=1) n(%)**	**Totaln(%)**
AMC	10(83.3)	8(80)	3(100)	3(100)	1(100)	25/29(86.2)
AMP	11(91.6)	9(90)	3(100)	3(100)	1(100)	27/29(93.1)
AMK	4(33.3)	2(20)	2(66.7)	2(66.7)	0(0%)	10/29(34.5)
CIP	10(83.3)	8(80)	3(100)	3(100)	1(100)	25/29(86.2)
CTR	11(91.6)	10(100)	3(100)	3(100)	1(100)	28/29(96.5)
CTX	11(91.6)	10(100)	3(100)	3(100)	1(100)	28/29(96.5)
CTZ	12(100)	10(100)	3(100)	3(100)	1(100)	29/29(100)
GEN	8(66.7)	8(80)	3(100)	3(100)	0(0)	22/29(75.9)
PEN	12(100)	9(90)	3(100)	3(100)	1(100)	28/29(96.5)
NA	7(58.3)	2(20)	2(66.7)	1(33.3)	0(0)	12/29(41.4)
NIT	0(0%)	0(0)	1(33.3)	1(33.3)	0(0)	2/29((6.9)
NOR	7(58.3)	1(10)	2(66.7)	2(66.7)	0(0)	12/29(41.4)
SXT	11(91.6)	6(60)	3(100)	3(100)	1(100)	24/29(82.8)
TTC	10(83.3)	7(70)	2(66.7)	3(100)	1(100)	23/29(79.3)
MDR	11/12(91.6)	9/10(90%)	3/3(100%)	3/3(100%)	1/1(100%)	27/29(93.1%)

AMK=Amikacin, GEN=Gentamycin, CTR= Ceftriaxone, CTX= Cefotaxime, CTZ=Ceftazidime, AMP=Ampicillin, CIP=Ciprofloxaccin, NOR=Norfloxacin, NIT=Nitrofurantoin, NA=Naldixic acid, TTC=Tetracycline STX=trimethoprim-sulfamethoxazole, Spp = specieses

5.4 Prevalence of ESBL-PE

Among all (29) Enterobacteriaceae uropathogens isolated from the urine sample of study participants (DM-patients) became resistant to 3GC (cefotaxime, ceftriaxone and ceftazidime)and unfortunately all were candidate ESBL production test.

For ESBL confirmition Double Disk synergy test method was used and 10/29(34.5%) of the isolates were found to beExtended Spectrum Beta-lactamase Producers (Table4.).*E. colli* 4/10(40%) and *Klebsiella* spp 3/10(30%) were the dominant ESBL producers among Enterobacteriaceae urine isolate in this study.The rest 3 (30%) ESBL-PE were contributed by proteus, enterobacter & Citrobacter specieses 1/10(10%) each.

Table 4Frequency of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae among DM-UTI patients, at SGGH Jimma town, South West Ethiopia, July-Oct. 2021.

ESBL production	Frequency	Percent
ESBL-PE	10	34.5
Non-ESBL-PE	19	65.5
Total	29	100.0

ESBL-PE= Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae ,
Non- ESBL-PE=Non Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae

5.5 Risk factors associated with urinary tract infection by ESBL-PE

In this studydifferentindependent variable from socio-demographic and clinical condition of study participants were assessed for their association with the dependent variable.

Fromall 14 independent variablesassessed for their association with acquiring UTI with ESBL-PE, only three: previous history of antibiotic use (CI; 1.751-71.637, PV=0.011), repeated OPD visit (CI; 0.740-2.678, PV=0.103) andcurrent UTI symptoms(CI; 1.751-71.637, PV=0.011)became a candidate for multiple logistic regression (Table 6). Of these three independent variable taken to Multivariable Logistic Regression Analysis only two,current UTI symptoms (CI=1.103-97.560, PV=0.041) and previous antibiotic use history in last three months(CI= 1.103-97.560, PV=0.041) were found to be significantely associated with study participants acquiring UTI by ESBL-PE.

So the finding of this study indicates that repeatedly taking antibiotic and having current UTI symptom increase the risk of study participants acquiring UTI by ESBL-PEby ten times when compared with study participants who do not have history of antibiotic use and current UTI symptoms in last three months (Table 5). All the rest independent variables assessed were not found to be significantly associated with the dependent variable of the study.

Table 5Bivariate&multivariable Logistic Regression Analysis to determine association of dependent & independentvariables, SGGH,Jimma town, South West Ethiopia, July-Oct. 2021.

Variable	Category	ESBL		Binary logistic regression			Multivariate logistic regression		
		Yes	No	COR	95 %CI	P-Value	AOR	95 %CI	P-Value
Age	18-29	1	1	1.00					
	30-39	1	2	0.500	0.013-19.562	0.771			
	40-49	3	4	0.75	0.032-17.506	0.858			
	50-59	3	6	.500	0.023-11.08	0.661			
	≥ 60	2	6	0.333	0.014-8.182	0.501			
Sex	Male	3	4	1.00					
	Female	7	15	0.662	0.109-3.564	0.594			
Marital Status	Single	1	1	1.000					
	Married	6	15	0.400	0.021-7.484	0.540			
	Divorced	2	2	1.000	0.034-29.807	1.00			
	Widowed	1	1	1.000	0.020-50.397	1.00			
Educational Status	No Educ.	5	4	2.500	0.292-21.399	0.403			
	Primary	1	8	0.250	0.017-3.660	0.311			
	Secondary	2	3	1.333	0.113-15.704	0.819			
	Higher	2	4	1.000					
Occupational status	Housewife	2	5	1.000					
	Gov't E	1	1	0.800	0.044-14.643	0.880			
	N-gov't	1	5	2.000	0.05178.25	0.711			
	Merchant	5	6	0.400	0.016-10.017	0.577			
	Farmer	1	2	1.667	0.115-24.256	0.708			
Residence	Urban	6	11	1.000					
	Rural	4	8	0.917	0.193-4.357	0.913			
FBS Level in mg/dl	< 126	2	2	1.00					
	≥ 126	8	17	0.471	0.056-3.970	0.488			
Urine glucose level	Trace	5	12	1.000					
	1+	2	3	1.600	0.202-12.694	0.656			
	2+	1	1	2.400	0.124-46.391	0.562			
	3+	1	1	2.400	0.124-46.391	0.562			
	4+	1	2	1.200	0.088-16.439	0.891			
Duration Of DM in Year	< 1	1	1	1.000					
	1-5	3	11	.273	0.013-5.768	0.404			
	6-10	3	4	.750	0.032-17.506	0.858			
	> 10	3	3	1.000	0.041-24.547	1.000			
Prev.UTI History	Yes	9	13	4.154	0.424-40.661	0.221			
	No	1	6	1.000					
Antibiotic Use history	Yes	8	5	11.200	1.751-71.637	**0.011***	10.373	1.103-97.560	**0.041****
	No	2	14	1.00					
OPD Visit History	Yes	8	9	4.444	0.740-2.678	**0.103***			
	No	2	10	1.000					
Current UTI Symptoms	Yes	8	5	11.200	1.751-71.637	**0.011***	10.373	1.103-97.560	**0.041****
	No	2	14	1.00					

*-P-value <0.25, **=P-value <0.05, 1.00-Reference, COR= Crude Odds Ratio,AOR= Adjusted Odds RatioCL = Confidence Interval

Chapter Six: Discussion

In this study; 272 urine specimens were collected from study participants. Among these 29sampleswereculture positive or found to have significant bacteriuria for Enterobacteriaceae uropathogens.The finding indicated thatthe prevalence of UTI caused by Enterobacteriaceae among DM patients is 10.6%.This result is consistent with the finding of hospital-based studies conducted in the central, northwest and southern part of Ethiopia which reported a varying prevalence of UTI ranging from 10.4% to 17.8%(14), and at Algeria 2018 (12%)(43).

This finding is significantly higher than the finding of similar study conducted atSurabaya, Indonesia (3.93%)(67). But lower than the finding of the study conducted at Nigeria (78%)(68), Ethiopia(18.75%)(69). This difference may be related with geographical differencesince the distribution of some Enterobacteriaceae (*E.coli*) varies with salinity and rain amount of the environment (70), sample size& methods, local antibiotic use and regulation.

Those Enterobacteriaceae organisms isolated in this study were, *E.coli*41.4%(n=12), *Klebsiella* .spp 34.5% (n=10), *Proteus* spp 10.3% (n=3), *Enterobacter* spp 10.3%, (n=3), &*Citrobacter* spp 3.4%(n=1). Thus in this study*E.colli*was the leading isolate followed by *Klebsiella* species.

This order of domination agrees with most studies conducted on UTI causing gram negative organisms. For instance in study conducted in Hawasa University college of medicine, *E.coli* , 44.4% (32/72) followed by *K. pneumoniae*, 27.8% (20/72)(71) and at, Dessie Referral Hospital, Northeastern Ethiopia *E. coli* 12/39 (30.8%), followed by *K. pneumoniae* 11/ 39 (28.2%)(72)which were the same in order of domination as compared to this study.The predominance of this organisms especially *E.colli* is due to that this organisms commonly resides as commensal of the bowel were they normally not cause an infection even if they are commonly involved in the UTI due to its anatomical proximity to the genito-urinary area (73).

The present finding is different from those findings at Addis Ababa(74)and Nigeria(75) in which *proteus* species were reported as2ndand 1stdominant spp.isolated from the urine sample

of study participants. This defference might be due to difference in study population, climate & geographic location.

Regarding antibiotic susceptibility testing, Enterobacteriaceae showed varied resistance pattern to selected 14 antibacterial drugs: most of isolated Enterobacteriaceae uropathogens has shown increased resistance, 96.5% and above to 3GC, Penicillin and Ampicillin, 86.2% to Ciprofloxacin & Amoxicillin-clavulanic acid, 82.8% to Trimethoprim-sulfamethoxazole (STX) , and have shown relatively less resistance 41.4% to naldixic acid as well to Norfloxacin, 34.5% to Amikacin & only 6.9% to nitrofurantoin.

The profile of Enterobacteriaceae drug resistance pattern towards commonly prescribed antibiotic is consistent with study conducted at Hawassa University College of Medicine and Health Sciences except Nitrofurantoin is relatively more effective in this study and otherwise, ampicillin (95.8%), trimethoprim-sulfamethoxazole (86.1%), ciprofloxacin (47.2%)& Norfloxacin (45.8%)(71).Rather the high susceptablity of isolates in this study to Nirtfurantoin (92.1.3%) agrees with the finding at Arbaminch Hospital 100% (20).

The resistance profile of these organisms to commonly prescribed antibiotics like ampicillin(96.6%), Tetracycline(84%) and Cotrimoxazole(92.3%) in this study is relatively higher as compared to the study done at Gondar University where: ampicillin (68.1%), tetracycline (83%), and cotrimoxazole (42%). While they were sensitive to ceftriaxone (84.6%), ceftazidime (84.6%), and gentamycin (84.6%)(76). This difference may probably due to difference in adhering to local and institutional drug prescribing guides, year to year increasing prevalence of AMR, and culture based test utilization (rational use antibiotics)(36).

Prevalence of ESBLs producers within the Enterobacteriaceae uropathogens in this study was 10/29 (34.5%). It is consistent with the similar study conducted at at JUSH (38.4%)(77) & Hawasa University college of medicine 41.7%(71). The finding is much greater than the finding of the similar study conducted at Adama 25%(78), Debretabor University 18%(79), Dessie Referral Hospital, Northeastern Ethiopia 2% (41), Morocco 25.5% (80), and Iran 28.4%(81). But this result is lesser than finding at Addis Abeba Ethiopia 57.7%(82), Bahir-Dar-Ethiopia 57.6% (83),Burkina Faso 58.0%(60), Uganda 62.0%(84). This variation might be due to probable difference in study populations access to use of culture based diagnostic services , rational use of drus, sampling methods and size, level of health facilities(85), time (year) of study (86).

Out of the 29 Enterobacteriaceae isolates tested for AST, 27(93%) of them were proved to be resistant to 3 or more drugs belonging to different antibiotics classes indicating increased rate of MDR Enterobacteriaceae organisms among isolates. This finding is consistent with similar study conducted at Harar (92.5%)(14), and Hawassa (93.9%)(87). But this finding is much higher than those findings at Debre Tabor (56.7%)(4), Gondar (59.8%)(36), and Addis Ababa (79.1%) . This difference might be due that communities who were participated in this stuy have increased chance of repeatedly using similar antibiotics as a result of scarsity of

alterative antibiotic and lack of crucial diagnostic services like culture and sensetivety testing for patient service at current study setting or facility.In this studyall ESBL-PE isolates were all found to be multi-drug resistant.

In this study different socio-demographic and clinical factors (Age, Sex, Marital status, Educational level, Occupation, Residence, Duration with DM, FBS, UTI history, History of Antibiotic use, current UTI symptoms, repeated OPD visit) were assessed for their significant association with dependent variable of the study.Even though three independent variables (repeated OPD visit in last month, previous antibiotic exposure in last three months, and current UTI symptoms) has shown some degree of association at bivariate logistic regression level and most of otherfactors assessed have no association with the patients acquiring of UTI with ESBL-PE, at at multi-variate logistic regression level our study validated that antibiotic exposurein last three months and current UTI symptom (CI=1.103-97.560, AOR=10.373 PV=0.041)were remained significant risk factors for acquiring a new ESBL-PE urinary tract infection.Therefore DM-Patients with history of previous antibiotic use and current UTI symptoms in last three months have ten times risk of acquiring UTI with ESBL-PE than those with the absence antibiotic usein last three months and current UTI symptoms.

The finding of our study is comparable with the study done at Addis Abeba(82),and Switzerland which indicates that study particpants exposure to antibiotic within the previous 3 months (OR 2.96, 95% CI 1.37–6.41, p = 0.006) and at Dessie Referral Hospital (41) which reported that current UTI symptom is significantely associated with UTI by ESBL-PE were independently associated with colonization with ESBL-PE species(88,89).But it is different from the study conducted at Malawi which reported that there was no relationship between ESBL-PE carriage in community patients and their demographic or clinical characteristics (90).

Limitations

The time of data collection for this study was during when the society movement was restricted due to COVID-19 pandemic and it was also conducted at hospital which was specifically serving as COVID-19 testing treatment center. This could affected the diversity of study participants since probably those who were highly ill patients might dominate as those stable patients might protect themselves from repeatedly going for follow-up physically.

Similarly some antibiotics were already out of market or too costy as aresult of emergency state lock down due to COVID-19 and was difficult to increase the scope of antibiotics tested for resistance by identified organisms.

Chapter Seven:Conclusion and Recommendation

7.1 Conclusion

The prevalence of bacteriuria among 272 study participant were 29(10.6%) and the predominant Enterobacteriaceae species isolated were *E.coli* 12(41.3%) followed by *Klebsiella* species 10 (34.55%). Whereas the prevalence of ESBLs producers Enterobacteriaceae among isolated uropathogens was 10/29 (34.5%). Still *E.coli* and *Klebsiella* spp contributed a total of seventy percent, 4(40%) and 3(30%) each respectively.

Most of isolated Enterobacteriaceae uropathogens had shown increased resistance, 96.5% andabove to 3GC, Penicillin and Ampicillin, 86.2% to Ciprofloxacin and Amoxicillin-clavulanic acid, and 82.8% to Trimethoprim-sulfamethoxazole (STX) . But they had shown relatively less resistance to naldixic acid & Norfloxacin 41.4%, Amikacin 34.5% and Nitrofurantoin 6.9%. Furthermore 27(93.1%) of isolates were muti-drug resistant.

Study participants with repeated exposure to antibiotics and current urinary tract infections symptom (AOR=10.373, CI= 1.103-97.560, PV=0.041)were10 times at risk to acquire Urinary tract infection with Extended Spectrum Beta-lactamase enzyme producing Enterobacteriaceae when compared with those who do not have antibiotic exposure in last three months and do not have have current UTI symtoms.

7.2 Recommendation

Based on the findings of this study the following recommendations were forwarded;

- Concerned health professionals working at the study facility has to consider this Enterobacteriaceae uropathogens prevalence, species distribution, drug resistance patterns especially ESBL-PE & MDR, and associated risk factors when planning resources and managing UTI among the stuy participants.
- Local, regional, and national health planners and administrators should work on expanding availability of culture and AST based diagnostic services, rational use of drugs, advocating of adherence to appropriate guide-lines while priscribing as well as using antibiotics
- Scientific communities should also be concerned with the increased prevalence of AMR in Enterobacteriaceae family of bacteria to look for discovery of new alternative antibiotics.

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Annexes

Annex One: Participant Information Sheet

A, Participant Information Sheet English Version

Jimma University, institute of health, faculty of Health Sciences, school of medica laboratory science

Study title

Prevalevceof Extended Spectrumβ-lactamase producing Enterobacteriaceae andassociated risk factor for UTI by ESBL-PE in DM patients, Jimma SGGH, South West Ethiopia.

Invitation paragraph

You have invited to take part in this study. Before you decide whether to participate or not it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask question if there is anything that is not clear or if you would like more information.

Introduction of the disease

Urinary Tract Infections (UTIs) are more common in people with Diabetes Mellitus because of possible decrease in body's natural defense mechanisms and is a major problem especially in developing countries. UTIs are caused by growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection. It is one of the most common human diseases, affecting 250 million people worldwide each year due to a variety of etiological agents.

The purpose of the study

The study was aimed to determine Prevalence of ESBL-PE and associated risk factors byUTI by ESBL PE family of bacteria among UTI-DM Patients at Jimma SGGH, South West Ethiopia.

Why you have been chosen

You are invited to participate in this study as suspect of UTIwhich might be caused be ESBL-PE if any, based on your Risk factor (DM).

Participant right

Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to withhold information, decline to cooperate in the study and refuse running of specimens.

Duration of the study

The duration of this study depend upon the availability of study subjects. It might take about two months or more. But each participant do not waste more than maximum of 30 minutes in avarage to give the important information and sample.

Study procedures

For this study to be successful we need your participation. If you are voluntary to participate in this study, you are expected to understand and sign the informed consent. Then, socio demographic condition related to UTI infections which are important for this study will be taken. Samples will be collected by your self by strictly following instruction forwarded to you by the experienced laboratory proffessional assigned for data collection. Collected samples are transported to JU microbiology laboratory within 2hours of collection and analyzed for the presence of Entrobacteriaceaeand drug susceptibility test are done as perCLSI & standard operating procedures (SOPs).

Risk probably happen to the study participant

There is no risk associated with the specimen collection and participants are only expected to not contaminate the sample during collection by following standard procedures as inistructed.

Expected benefits

We will inform the presence of drug resistant isolates basedon laboratory culture results to chronic care OPD focal person in charge at Jimma SGGH for the better management of UTI.

Confidentiality issue

All your personal information collected for the purpose of this study will be kept confidential.

Payment: No payment will be provided by participating in this study.

Approval: This research project has got ethical clearance from the Institutional Ethics Review Board of Health Institute, Jimma University.

Whom to contact

If you have any question or need description about this study, you can communicate on the following address:

JU, institute of Health, faculty of Health Sciences, school of Medical Laboratory Sciences

Tel: +251-4-111-1457/60 **Fax:** +251-4-111-1450 E-mail: P.o.Box: 337, Jimma, Ethiopia

The address of investigator: Tesfaye Sutuma Mobile: +251-941-52-04-32 E-mail: sutumanegeri@gmail.com

B. Participant Information Sheet Amharic Version

ጅማዩኒቨርሲቲ ሜድካልላብራቶሪ ሣይንስትምህርት ክፍል

የጥናት ርዕስ፡

በጅማ በሸነን ግቢጀነራል ሆስፒታል መደበኛ መደሃኒቶች ጋር የተላመዱ ህዋሳት ልየታ ፡ ስርጭት ፡ ና፡ጅማ ደ.ምዕራብ ኢትዮጵያ

ማብራሪያ፡

ይህ የጥናት መረጃ የተዘጋጀው በጅማ በሸነንግቤ ጀነራል ሆስፒታል በህክምና ክፍሉ ለስካር በሽታ ህክምና ለመጡ በጥናት ላይለሚሳተፉ የተዘጋጀነው፡፡

መግቢያ፡ይህ የጥናት መረጃ የተዘጋጀው መደበኛመደሃኒቶች ጋር የተላመዱ ህዋሳት ልየታ፡ስርጭት፡ና፡ ተጋላጭነት(Determination of ESBL producing prevalence, and associated risk factors among Enterobacteriaceae isolates from DM-UTI patients) ለማጥናት አልሞ ነው፡፡ከጥናቱም የሚገኘው መረጃ የባክቴሪያዎቹን ስርጭት ለመቆጣጠር የመፍትሔ እርምጃ ለመውሠድያገለግላል፡፡በጥናቱም ላይ አንድ የጥናቱ ዋናተመራማሪ፡፡ ሁለት የመረጃሠ ብሳቢዎች (በሙያነርስ) እንዲሁም ሁለት ላቦራቶሪ ቲክኖሎጅስት አንድ ሱፐርቫይዘር እና ለት አማካሪዎች ይሣተፉሉ፡፡

የጥናቱ ማብራሪያ፡ጥናቱት ኩረት የሚያደርገው መደበኛ መደሃኒቶች ጋርየተላመዱ የሸንት ቱቦ ፊኛ ና ኩላሊትን የሚያጠቁ ህዋሳቶችንና፡ ተጋለጭነትን መለየትነዉ

እነዚህ ተዋስያን በህሙማኑ ላይ፡ በተለይም የስካር ህሙማን ላይ የተለያዩ ችግሮችን ያስከትላሉ፡በመሆኑም የነዚህን ባክቴሪያዎች በህሙማኑ ላይ ያላቸውን ስርጭት ማወቅ አስፈላጊውን የመከላከያ መንገድ ለመቀየስ ዋነኛግብአት ነው፡፡

Tel: +251-4-111-1457/60 **Fax:** +251-4-111-1450 E-mail: P.o.Box: 337, Jimma, Ethiopia

The address of investigator:

Tesfaye Sutuma Mobile: +251-941-52-04-32 E-mail: sutumanegeri@gmail.com

C. Participant Information SheetIn Afan Oromo Version

Yuuniivarsii Jimmaatti, Inistitiyuutii fayyaa, Faakaaltii Saayinsii Fayyaa, Kutaa barnootaa Saayinsii Mediikaal Laaboraatoorii

Waraqaa Odeeffanoon Hirmaataaf Ittiin kennamu Afaan Oromoon

Mata-duree Qorannoo:

Jarmiiwwan (Baakteeriyaa) dhukkuboota keessoo adda addaa kan akka Ujummoo fincaanii, Afuuffee fincaanii, Kalee, Ujummoo qilleensaa , Ujummoo nyaataa fi KKF irraan miidhaa guddaa geessisan qorichoota garee ESBL jedhamanii wajjin ammam akka walbaran, wantoota saaxilamummaa jarmoota kanaan qabamuu namatti fidanuu fi qorichoota kanniin kamiin yaalamuu akka danda'aman saamuudota dhukkubsattoota shukkaaraa qaban irraa fuudhame irratti qorannoo geggeessuu. Hoospitaala Waliigalaa Shanan Gibee, Jimmaa Kibba-dhihaa Ityoophiyaa.

Keewwata Affeerraa :

Akka qorannoo kana keessatti hirmaattanuuf Affeeramtaniittu. Qorannoo kana keessatti hirmaachuuf ykn hirmaachuu dhiisuu, murteessuu keessaniin dura . Yeroo xiqqoo fudhaatoo Odeeffannoowwan armaan gadii suuta hubannoon dubbisaa. Odeeffannoo dabalataa argachuu yoo barbaaddan ykn waan ifa isiniif hinta'in yoo qanaattan gaafachuu ni dandeessu.

Seensa waa'ee dhibee ujummoo caanii (UTI)

Dhibeen dhukkuba ujummoo fincaanii sababa jarmiiwwan (Bacteria)'n dhufan nama kamiinuu qabuu kan danda'anu yoo ta'es, namoota sababa adda addaa kan akka dhibee shukkaaraan dandeettiin dhukkuba ofirraa ittisuu isaanii gadi bu'e irratti haalaan baramaa dha. Keessumattuu biyyoota guddataa jiran keessatti immoo dhibeen kun baayyee baballatee mullata.

Dhibeen kun sababa garaa-garaatiin jarmoonni ujummoo fincaanii, afuuffee fincaanii, kalee keessatti carraa argataniin walhoruu irraa kan maddudha. Innis adduunyaa kana irratti dhibeewwan dhala namaa haalaan huban keessaa isa tokko kan ta'ee fi waggaatti hanga namoota miliyoo 250 kan hubuudha.

Kaayyoo Qorannoo Kanaa

Kaayyoon qorannoo kanaa hanga tatamsa'iinsa (prevalence), sadarkaa qorichoota "ESBL" n walbaruu, fi wantoota jarmoota qorichaan walbaran kanaan akka saaxilamnu nu godhan kana qorachuu ta'a.

Maaliif qorannoo kanaaf akka filatamtan

Qorannoo kanaaf wanti filatamtaniif ulaagaa filannoo waan guuttaniifi, jechuunis dhibee shukkaaraa qabaachuun keessan akka saaxilamummaa qabdanitti waan fudhatameefi.

Mirga Hirmaataa

Akka waliigalaatti qorannoo kana irratti hirmaachuu fi dhiisuun fedhii hirmaataa (dhibamaa) ti . Hirmaachuu dhiisuun addabbii kan hin qabnee fi waan sila argachuu dandeessanu kamiinuu akka dhabdanu isin gochuu hin danda'u. Hirmaatichi erga hirmaachuu jalqabee booda odeeffannoo kennuu hin barbaanne kennuu dhaabuu, atooma gochuu dhiisuu fi saamuuda qorannoof barbaachisu kennuu diduuf illee mirga guutuu qaba.

Yeroo turtii Qorannichaa

Qorannoon kun dhaabbata (Hoospitaala) kana keessatti kan adeemsifamu yoo baay'ate ji'oota sadii (jechuunis waxabajjii 01 irraa hanga Hagayya 30 bara 2021 A.L.A) tti yommuu ta'u, hirmaataan tokko waliigalaan odeeffannoofii saamuuda kennuuf giggugaleessaan daqiiqaa soddoma caalmaa kan itti hin fudhanneedha.

Wal-duraa duuba adeemsa qorannoo

Milkaa'ina qorannoo kanaatiif hirmaannaan keessan baay'ee barbaachisaadha. Hirmaachuuf fedhii kan qabdanu yoo ta'e garuu adeemsa qorannochaa hubachuu akkasumas fedhii keessaniin hirmaachuu keessan mirkaneessuuf mallattoon nuuf mirkaneessuun keessan baay'ee barbaachisaa dha. Sanaan booda dhimmoota waliigalaa kallattii fi alkallattiin dhibee kanaan walqabatan irratti odeeffannoo fi qorannichaaf kan oolu samuuda (fincaan/Boolii qal'aa) nuuf kennitu. Saamuudni kunis gara Yuuniiversiitii jimmaa geeffamuun yeroo sa'a lama hincaalle keessatti qorannoon irratti geggeeffamuu eegala.

Sababa Qorannoo Kana Hirmaachuun Saaxilamummaa / raakkoo mudachuu danda'u

Yeroo odeeffannoo fi saamuuda kennuun hirmaataan qisaasessu irraan kan hafe haalli qorannoo kanaa haala kamiinuu hirmaataa miidhaa kamiifuu kan hin saaxilleedha.

Faayidaa qorannoo kana irraa hirmaataan kallattii fi al-kallattiin argatu

Akka dhuunfaatti hirmaataan kan qabame jarmiiwwan qorichoota "ESBL waliin walbaruun isaanii qorannoo kana keessatti beekameen yoo ta'e, haakiimni isaanii hubannoo ga'aa argatee yaalii fooyya'aa akka kennuufiif jecha haakiimicha qofaaf firiin qorannoo ni beeksifama.

Iccitiii

Iccitiin dhuunfaa hirmaataa sababa qorannoo kanaatiin beekame iccitummaan isaa haala kamiinuu akka hin saaxilamne ni eegama.

Kaffaltiii

Hirmaataan qorannoo kana keessatti hirmaachuu isaatiif kaffaltiin addaa kaffalamu tokko illee hin jiru.

Mirkanaa'uu Seeraqabeessummaa Qorannoo kanaa

Piroojeektiin Qorannoo Kun Dippaartimantii dhimma qulqullina itiksii riisarchii fi garee sakatta'iinsa Itiksii yuuniivarsiitii Jimmaa, Inistitiyuutii fayyaa, Faakaaltii Saayinsii Fyyaa, Kutaa barnoota saayinsii Laaboraatoorii Medicaalaa irraa kan argate dha.

Madda odeeffannoo

Dhimma qorannoo kanaan walqabatu irratti gaaffii ykn akka ifa isiniif ta'u wanti barbaaddan yoo jiraate karaa teessoowwan armaan gadii fayyadamuu dandeessu

yuuniivarsiitii Jimmaa, Inistitiyuutii fayyaa, Faakaaltii Saayinsii Fyyaa, Kutaa barnoota saayinsii Laaboraatoorii Medicaalaa

Tel: +251-4-111-1457/60 **Fax:** +251-4-111-1450 E-mail: P.o.Box: 337, Jimma, Ethiopia

The address of investigator:

Tesfaye Sutuma Mobile: +251-941-52-04-32 E-mail: sutumanegeri@gmail.com

Annex Two:Concent Form and Data Collection Tools

Jimma UniversityInstitute of HealthCollege of Medical Laboratory ScienceDepartment of Medical Microbiology

Serialno_____ Cardno _____ Name of studyparticipant: _____

Ihavebeenrequestedtoparticipateinthisstudy,whichis plannedtodetermine the Prevalence of Extended Sspectrum Betalactamae producing Enterobacteriaceae and associated risk factors among DM patients with urinary tract infection at Jimma SGGH, South West Ethiopia, 2021.

I have been informed about this study which involves collecting of Urine specimen. During collection of the specimen I have been told that there is no harming procedure or phenomena and I have also read the information sheet or it has been read to me.

I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care access.

It is therefore with full understanding of the situations that I agreed to give the informed consent voluntarily to the researcher to use my urine specimen taken for the investigation. Moreover, I have had the opportunity to ask questions about the project and I have received clarification to my satisfaction. I was also told that results would bereported timely to there questing physicians for the appropriate treatment and management of the UTI.

IagreethatIamcontributingtothetreatmentofmyfellowsbyparticipatinginthisproject. Ihave asked some questions and clarification has been given to me. I have given my consent freely to participate in the study, and I approve my agreement with mysignature.

Participants' sign: _____ Date _____PrincipalInvestigator'ssign: _____ Date_____

At what time the interview started_____

I: Questions on Socio-demographic and clinical characteristics of the respondents

No.	Variables	Response categories
1	Sex of the respondent	1. Male 2. Female
2	Age of the respondent	1.≤18rs 3. 25-34yrs 5. 45-54 yrs 7.>64yrs 2. 18-24yrs 4. 35-44yrs 6. 55-64 yrs
3	Respondent's Marital status	1. Single2. Married 3. Separated 4. Divorced5. Widowed
4	Respondent's occupation	1. House wife 4. Student 7. Farmer 2. Gov't Employee 5. Merchant 3. Non- Gov't E 6. Daily laborer
5	Respondent's education status	Not able to read and write 3. Secondary education Primary education (1-8) 4. Above secondary
6	Respondents Residence	1. Urban 2. Rural
7	Respondents Fasting blood glucose	1. ≤126mg/dl2. >126mg/dl
8	Urine glucose level	_____ (Grading)
9	Duration of DM	_____(in years)
10	History of UTI in previous time	1. Yes 2. No
11	Is the respondent hospitalized in last 3 months	1.Yes 2. No
12	Does the respondent used antibiotics in last 3months	1.Yes 2.No
13	repeated outpatient visits at hospital in the last 30 days,	1.Yes 2. No
14	Currently do you have burning sensation when you are urinating?	1. Yes 2. No
	Currently do you passing frequent small amount of urine?	1.Yes 2. No
	Currently is your urine color changed to red, bright pink or coca loloured?	1.Yes 2. No

At what time the questions are finished __Interviewer name _____ Sign. ___Thank the respondent**.**

B, Data collection tool and concent form Amharic version

የመረጃመሰብሰብያ ና ለተሳትፎ ፍቃደኝነት መጠየቅያ ቅፀበአማርኛመለያ (ID):_____

በጅማ ዩኒቨርሲቲ የጤና እንስቲቱት ሜድካልላብራቶሪ ሣይንስትምህርት ክፍልና ማይክሮባዮሎጂ ድፓርትመንት

ተ.ቁ_____ካርድቁጥር _____ የስልጠናዉተሳታፊስም: _____

‘በጅማ በሸነንግቤጀነራል ሆስፒታል (SGGH) መደበኛ መደሃኒቶች ጋር የተላመዱ ህዋሳት ልየታ ፡ ስርጭት፡ና፡ ተጋላጭነት (Determination of ESBLs prevalence, and associated risk factors among Enterobacteriaceae isolates from DM-UTI patients) ጅማ ደቡብ ምዕራብ ኢትዮጵያ’ በምል ርእስ የሚካሄደዉ ጥናት ለይ እንድሳተፍ ፍቃደኝነቴን ተጠይቄዋለዉ

ስለዚህ ጥናት እንድዉም የናሙና (ሽንት) አወሳሰድምንም አይነት ጉዳት እነደማያስከትል ተረድቸዋለሁኝ ።የስምምነት ቅፁንም አንብቤ ተስማምቸዋለሁኝ። በተጨማሪም በመጠይቆቹ ዉስጥ ሚሰጣቸዉ መረጃዎች በምሽጥራዊ እንደሚያዙም ጭምር ተረድቸዋለዉ።

በሌላም በኩል የተጤየኩትን መረጃ ያለመስጠት፡የተመቸኝ ሰአት የማቆም፡የመተባበርምሆኔ ያለመተባበር ሙሉ መብት እንዳለኝ ና በዚህ ምክንት ምንም አይነት ጉዳት እንማያስከትልብን ተረድቸዋለዉ።

በዚዉ መሰረት በራሴ ፍላጎት ጥናቱ ላይ ለመሳተፍና ለጥናቱ የሚወሰደዉ የሽንት ናሙና ለጥናቱ ፈቅጀዋለዉ።

ጉዳዩን በተመለከቴ ዬትኛንም መረጃ መጠየቅ . እንደምችል ና የኔጥናቱ ዉስጥ መሳተፍ የሌሎችን የወደፊት ህክምናን የተሸሌ ለማድረግ እንደሚጠቅም ስለተረዳዉ በነፃነት ጥናቱ ላይ ለመሳተፍ እንደተስማማዉ በፊረማዬ አረጋግጣለዉ።

ጥናቱ ተሳታፊ ፊርማ: _____ ቀን _____ጥናቱን የምያካሀደዉ ሰዉ ፊርማ: _____ ቀን_____

Witness_____ Date_____ ቃለመጠይቁ የተጀመረበት ሰአት _____

I: Questions on Socio-demographic and clinical characteristics of the respondents

No.	Variables	Response categories
01	የምርምሩ ተሳታፊ ጾታ	1. ወንድ 2. ሴት
02	የምርምሩ ተሳታፊ እድሜ (በአመት)	1≤183. 25-34 5. 45-54 yrs 2. 18-24 4. 35-44yrs 6. 55-64 yrs 7.>64
03	የምርምሩ ተሳታፊ የጋብቻ ዉኔታ	1. ያላገባ/ች 2. ያገባ/ች3. የተለያዩ 4. የተፋቱ 5. የሞተባት/ቸበት
04	የምርምሩ ተሳታፊ የስራ ዉኔታ	1. የቤት እመቤት 2. የመንግስት ሰራተኛ 3. የግል ሰራተኛ 4. ተማሪ5. ነጋዴ 6. የቀን ሰራተኛ 7.ሌሎች (ይለዩ)
05	የምርምሩ ተሳታፊ የትምህርት ደረጃ	ı. መፃፍ ና ማንበብ የማይችል3. 2ኛ ደረጃ 2. አንደኛ ደረጃ 4. ከ2ኛ ደረጃ በላይ

06	የምርምሩ ተሳታፊ የኑሮ ቦታ	ነ. ከተማ 2. ገጠር
07	የምርምሩ ተሳታፊ የደም ዉስጥ ስካር መጠን	1. ≤126mg/dl2. >126mg/dl
08	የምርምሩ ተሳታፊ የሽንት ዉስጥ ስካር መጠን	_____ (Grading)
09	የምርምሩ ተሳታፊ የስካር ጋር ቆይታ	_____(በ አመት)
10	የምርምሩ ተሳታፊ በ3 ወር ዉስጥ UTI በሽታ	1. አዎ 2. አይደለም
11	የምርምሩ ተሳታፊ በለፉት 3 ወር ዉስጥ ሆስፒታል መተኛት	1. አዎ 2. አይደለም
12	የምርምሩ ተሳታፊ በለፉት 3ወራት ዉስጥ ጸረ ሕዋሳት መዉሰድ	1. አዎ 2. አይደለም
13	የምርምሩ ተሳታፊ በለፉት በደም ስር የሚሰጡ antibiotics መዉሰድ	1. አዎ 2. አይደለም
14	የምርምሩ ተሳታፊ በለፉት ግዜያት ሰዉሰራሽ የሽንት ቱቦ መጠቀም	1. አዎ 2. አይደለም
15	የስልጠናዉ ተሳታፊ በለፉት 30 ቀን ዉስጥ ሆስፒታል ተመላልሶ መታከም	1.አዎ 2. አይደለም
16	የስልጠናዉ ተሳታፊ በለፉት ገዜያት ከባድ ቀዶ-ጥገና ብሊቱ ላይ መደረግ	1. አዎ 2.አይደለም
	ሽንት ስሸኑ ያቃጥሎታል ወይ	1. አዎ 2 አይደለም
	ቶሎቶሎ የመሸናት ና የሽንት መጠን ማነስ ይታይቦታል ወይ	1. አዎ 2.አይደለም
	ከቅረብ ግዜ ወዲ የሽንትሕ መልክ ተቀይሮ ያዉቃል ወይ	1. አዎ 2.አይደለም

ቃለመጠይቁ ያለቀበት ሰአት _____ቃለመጠይቁን የካሀደዉ ሰዉ_____ፊረማ. _____

ተሳታፊየችን ከልብ እናመሰግናለን

C, Data collection tool and consent form Afan Oromo Version

Yuuniivarsiittii Jimmaatti, Inistitiyuutii fayyaa, Faakaaltii Saayinsii Fayyaa, Kutaa barnootaa Saayinsii Mediikaal Laaboraatoorii

Waraqaa Sassaabbii Odeeffannoo (Data) fi Eeyyamamummaan hirmaataa fudhachuuf tajaajilu

Tartiiba Lakk._____Lakk. Kaardii _____ Maqaa hirmaataa:_____

Akka qorannoo kana keessatti hirmaattanuuf Affeeramtaniittu. Qorannoo kana keessatti hirmaachuuf ykn hirmaachuu dhiisuu, murteessuu keessaniin dura . Yeroo xiqqoo fudhaatoo Odeeffannoowwan armaan gadii suuta hubannoon dubbisaa. Odeeffannoo dabalataa argachuu yoo barbaaddan ykn waan ifa isiniif hinta'in yoo qanaattan gaafachuu ni dandeessu. .

Yeroo odeeffannoo fi saamuuda kennuun hirmaataan qisaasessu irraan kan hafe haalli qorannoo kanaa haala kamiinuu hirmaataa miidhaa kamiifuu kan hin saaxilleedha.

Iccitiin dhuunfaa hirmaataa sababa qorannoo kanaatiin beekame iccitummaan isaa haala kamiinuu akka hin saaxilamne ni eegama.

Akka waliigalaatti qorannoo kana irratti hirmaachuu fi dhiisuun fedhii hirmaataa (dhibamaa) ti .Hirmaachuu dhiisuun addabbii kan hin qabnee fi waan sila argachuu dandeessanu kamiinuu akka dhabdanu isin gochuu hin danda'u. Hirmaatichi erga hirmaachuu jalqabee booda odeeffannoo kennuu hin barbaanne kennuu dhaabuu, atooma gochuu dhiisuu fi saamuuda qorannoof b barbaachisu kennuu diduuf illee mirga guutuu qaba.

Haaluma kanaan qorannoo kana keessatti hirmaachuun koo yaalii namootaa gara fuulduaa keessatti bu'aa tokko buusanii darbuu ta'uu isaatti waanan amaneef qorannoo kana keessatti hirmaadhee odeeffannoo fi saamuuda qorannoo kanaaf oolu kennuu fi saamuuda kana qoannoo kanaaf dhimma asirratti ibsameef akka itti fayyadamau eeyyamuu mallattoo koo armaan gadiitiin nan mirkaneessa.

Mallattoo hirmaataa: _____ Guyyaa _____Mallattoo qoratichaa: _____ Date_____

Sa'aatii gaaffii fi deebiin itti eegale _____

I: Questions on Socio-demographic and clinical characteristics of the respondents

No.	Vaariyeebilii Qorannoo	Qoqqoodda deebiiwwan Eegamanii
01	Saada Hirmaataa	1. Dhiira 2. Dhalaa
02	UmriiHirmaataa	1. Waggaa ≤18 2. Waggaa 18-24 3. Waggaa 25-34 4. Waggaa 35-44 5. Waggaa 45-54 6. Waggaa 55-64 7. Waggaa >64
03	Haala fuudhaa fi heerumaaa Hirmaataa	1. Kan hinfuune/hin eerumne 2. Kan fuudhe/eerummte 3. Kan gargar ba'e/baate 4. Kan hike/hiikte 5. Kan irraa du'e/ jalaa duute
04	Haala hojii Hirmaataa	1. Haadha warraa 2. Hojjetaa moot. 3. Hojjetaa miti-mootummaa 4. Barataa/ttuu5. Daldalaa/ltuu 6. Hojjetaalttu guyyaa7. Kanbiraa
05	Sadarkaa Barumsaa Hirmaataa	1. Barumsa kan hinqabna 2. Barumsa Sad. 1^{ffaa} (1-8) 3. Barumsa sadarkaa 2^{ffaa} (9-12) 4. Barumsa sadarkaa 2^{ffaa} ol (12+)
06	Bakka jireenya hirmaataa qorannoo	1. Baadiyyaa 2. Magaalaa
07	Hanga shukkaara dhiiga keessaa Hirm. Qorannoo (mg/dl)	1. ≤126mg/dl 2. >126mg/dl
08	Hanga shukkaara fincaan keessaa Hirm. Qorannoo	1. Xiqqoo **2**. 1+**3**. 2+**4**. 3+**5**. 4+
09	Turtii dhukkubsataa dhibee sukkaaraa waliin	_____(waggaan)
10	Seenaa Dhukkuba ujummoo fincaanii ji'oota 3 darban	1. Eeyyee 2. Lakkii
11	Seenaa fayyadama ujummoo fincaanii nam-tolfe (catether) fincaanii ji'oota sadan darban keessa	1. Eeyyee 2. Lakkii
12	Seenaa fayyadama "Antibiotic" ji'oota 3 darban keessa	1. Eeyyee 2. Lakkii
13	dawaan karee hidda dhiigaa fudhattu jiraa	1. Eeyyee 2. Lakkii
14	Boolii bishaaniif Ujummoo namtolfe fayyadamtee beektaa	1. Eeyyee 2. Lakkii
15	Guyyoottan 30 darban keessa deddeebiin dawaa fudhattee?	1. Eeyyee 2. Lakkii
16	Duraan ujummoo fincaanii kee irratti baqaqsanii hodhuu guddaa geggeessitanii beektuu	1. Eeyyee 2. Lakkii
17	Yeroo fincooftan fincaan isin gubuun ni jiraa?	1. Eeyyee 2. Lakkii
18	Fincaan ammaa fi amma isin qabuu fi hammi fincaanii xiqqaachuun ni mul'ataa?	1. Eeyyee 2. Lakkii
19	fincaan keessan irratti halluun jijjiiramuun ni mul'ataa?	1. Eeyyee 2. Lakkii

Sa'aatii gaaffii fi deebiin itti dhume _____

Maqaa nama gaaffii fi deebii geggeessee _____mallattoo. _____

Hirmaataan keenya baay'ee galatoomi.

Annex Three: Laboratory Procedures And Analysis

A. Step- by- step instruction for Midstream urine sample collection

Step 1, ParticipantswereWash and driedtheir hands thoroughly.

Step 2, she/he were inistructed to removed the lid on the container and set it aside without touching the inner surface of the containers and lids.

Step 3, They were cleaning their genital area prior to voiding .

Step 4, All participants were instructed to pass a small amount of urine into the toilet.

Step 5, Midway through urination fill the urine to half full (minimum of 10 Millilitre.

Step 6, She/he Finish voiding in the toilet.

Step 7, She/hehave been replacing the lid and tighten firmly.

Step 8, Participantshave been Washing and drying their hands thoroughly after collection.

Step 9, The sample containerswere labelled with the patient's first name and last name, MRN, date and time of collection.

Step 10, For sanitary reason the container were enclosed in plastic biohazard bag.

Note ; Due to high probability of contaminations at vulva female study participants were provided with especial inistructions. Forinistance they were told to hold appart or spread labia majora (outer folds) and swipe each two sides of labia minora (inner folds) with sterile separate towelettes using a single downward stroke. With the third towelette, cleanse meatus (center area) with a single downward stroke.Then void first few(20-25) milliliters of urine into the toilet and in between collect ½ to 2/3 full of provided urine cup & pass the rest urine into the toilet again(62).

B. Step-by-Step Procedure of the Calibrated Loop/Surface Streak Method

Sterilized inoculating loop was used for urine sample innoculation. This was achieved by putting the loop into the flame until it is red hot and allowed it to cool.

The urine cap was tip over to re-mix the urine sample.

The capswere removed and end of a sterile 1-µL inoculating loop was dipped into the urine and removed vertically making sure that there is no urine up the loop.

The loop wasTipped and the inoculum was spread over the surface of first quadrant (approximately 1/4 of the plate) of standard MacConkey/nutrient agar plate prepared according to the instructions of the manufacturing company.

The loop was flamed and cool for the next use.

4. The plate was turn 90° and the loop will lightly sweep 1-2 times through the inoculated area, then streak into the next quadrant without overlapping the previous streaks.

5. The loop was flamed cool for the next use again

6. The plate was again turn 90° and the loop was lightly sweep 1-2 times, and streaked into the next quadrant as in step 4.

7. The loop was flamed cool for the next use

8. #6 was repeated by streaking the remainder of the plate.

9. The plate was Inverted and incubated at 37°C for 24 hr.

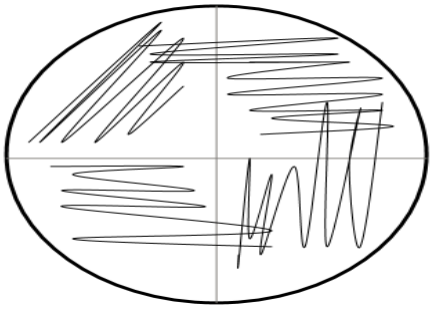

Figure 6 Urine culture using the calibrated loop/surface streak method **Source**; Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens inLow-Resource Settings, ondon W12 0HS, UK, Oct. 2020.)

B. Procedure of Gram Staining

Clean, grease free slide were prepared.

1. On the clean slide smear of suspension were prepared with a loopful of sample.
2. The smear were air dried and heat fixed.
3. Crystal Violet was poured& kept for about 30 seconds to 1 minute & rinsed with water.
4. The smears were next flood with gram's iodine for 1 minute and washed again with water.
5. Smears were washed with 95% alcohol or acetone for about 10-20 seconds & rinsed with water.
6. Finally safranin stain was added and kept for about 1 minute and wash with water.
7. The stained sample on the slideswere air or blot dried and Observed under Microscope.

C. Biochemical identifications of bacterial isolates from urine sample

Positive urine culture is usually followed by a variety of biochemical identification tests to determine the species/genus of the implicated bacterium

Basic (Level-1) identification

Results of the basic biochemical tests were available in one day after receiving the sample. At this level, we assigned the unknown pathogen into a bacterial group (Enterobacterales, Pseudomonas-like glucose-non-fermenter Gram-negative rods, Acinetobacter-like glucose-non-fermenter Gram-negative rods, etc). This will be sufficient to select a suitable panel of antimicrobial agents for the AST.

Procedure of Basic Identification

1. Colonies were examined & registered for their ability to grow on nutrient agar & MacConkey agar plates.
2. Colonies were examined and registered for their ability to ferment lactose on the standard MacConkey agar plate.
3. Gram-stained smear from an isolated colony were Performed and examined.
4. For Gram-negative rods, standard oxidase test was performed & the results were registered.

Table 6. Basic biochemical identification of common uropathogens (**Source**; Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens in Low-Resource Settings, ondon W12 0HS, UK, Oct. 2020)

Bacterium	Growth on	Gram Staining Bacterial morphology	Glucose	Oxidase	Catalas	PYR	Lancified
Enterobacterales	+	Red or pink rod-shaped	+	-	NA	NA	NA
Pseudomonas-like NGF Gm-ve rods	+	Red or pink rod-shaped	-	+	NA	NA	NA
Acinetobacter-like NGF Gm-ve rods	+	Red or pink rod-shaped	-	-	NA	NA	NA
Staphylococci	-	Clusters of purple/mauve sphere-shaped	NA	NA	+	NA	NA
Enterococci	-	Pairs or short chains of purple or mauve sphere-shaped	NA	NA	-	+	D
Streptococci	-	Chains of purple or mauve sphere-shaped	NA	NA	-	-	B/D

Advanced Level of identification

The advanced level was also also used to guide a correct read of the AST results, and provide data on the epidemiology of local uropathogens.

Procedure of Advanced Identification of Enterobacterales

1. Standard tests for Sugar fermentation, citrate, urease, hydrogen sulfide (H2S) production, indole,andmotilitywere performed and the results were registered .

2. Presence/absence of nitrites from the urine dipstick test was retrieved.

• The presence of nitrites indicates a positive nitrate reduction test.

Glucose Non-Fermenting Gram-Negative Rods

1. If oxidase positive, it was planned to be sub-cultured on Cetrimide agar. Incubated in air conditions at 35–37◦C for 18–24 h. Examined for the production of pigments. But none of our isolate wereoxidase positive gram negative rod.

2. Standard tests for indole, citrate, urease, H2S production & motility were Performed.

3. The presence/absence of nitrites from the urine dipstick test was Retrieved.

• The presence of nitirtes indicates a positive nitrate reduction test. However, the absence of nitrites could be due to a negative nitrate reduction test (as for Acinetobacter baumannii) or because nitrites have been further reduced to nitric oxide, nitrous oxide and/or nitrogen (as for Pseudomonas aeruginosa).

Table 7. Advanced biochemical identification of common uropathogens (**Source**; Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens inLow-Resource Settings, ondon W12 0HS, UK, Oct. 2020)

Enterobacterales (see also Supplementary Table S1)	Lac [1]	Ind [1]	Cit [1]	VP [1]	Ure [1]	Mot [1]	H2S [1]	LDC [1]	Nit [1]
Escherichia coli	+	+	–	–	–	+	–	+	+
Klebsiella pneumoniae	+	–	+	+	+	–	–	+	+
Klebsiella oxytoca	+	+	+	+	+	–	–	+	+
Enterobacter cloacae	+	–	+	+	V	+	–	–	+
Enterobacter aerogenes	+	–	+	+	–	+	–	+	+
Citrobacter freundii	V	–	+	–	V	+	(+)	–	+
Citrobacter koseri	V	+	+	–	V	+	–	–	+
Proteus mirabilis	–	–	V	V	+	+	+	–	+
Proteus vulgaris	–	+	(–)	–	+	+	+	–	+
Providencia stuartii	–	+	+	–	V	(+)	–	–	+
Morganella morganii	–	+	–	–	+	+	–	–	+
Serratia marcescens	–	–	+	+	(–)	+	–	+	+
Glucose-non-fermenting Gram-negative rods	**Oxi [1]**	**Lac [1]**	**Ind [1]**	**Cit [1]**	**VP [1]**	**Ure [1]**	**Mot [1]**	**H2S [1]**	**LDC [1]**	**Nit [1]**
Pseudomonas aeruginosa	+	-	-	V	-	(-)	+	-	-	V
Acinetobacter baumannii	-	-	-	+	-	-	-	-	-	-

Declaration

I the undersigned, declare that this MSc thesis is my original work, has not been presented for adegree in Jimma University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

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Internal Examinor: Mr. Zewdneh Sahlemariam: **Sign._____**

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