A STUDY ON PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE AND AMP C BETA LACTAMASE PRODUCTION AMONG *Escherichia coli* AND *Klebsiella* ISOLATED IN URINARY TRACT INFECTION

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CHENNAI, TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY ON PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE AND AMP C BETA LACTAMASE PRODUCTION AMONG *Escherichia coli* AND *Klebsiella* ISOLATED IN URINARY TRACT INFECTION**" submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology is a record of original research work done by Dr. V. P. Sarasu at the Department of Microbiology, Thanjavur Medical College, Thanjavur during the period from August 2009 to July 2010 under my guidance and supervision and the conclusions reached in this study are her own.

Dean	Signature of the Guide
Thanjavur Medical College	Professor & Head, Department of Microbiology

DECLARATION

I, Dr. V. P. Sarasu solemnly declare that the dissertation entitled "**A STUDY ON PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE AND AMP C BETA LACTAMASE PRODUCTION AMONG *Escherichia coli* AND *Klebsiella* ISOLATED IN URINARY TRACT INFECTION**" submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of regulations required for the award of M.D. Degree in Microbiology, was done by me at the Department of Microbiology, Thanjavur Medical College, Thanjavur during August 2009 to July 2010. I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

Place:

Date: (V. P. SARASU)

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LIST OF ABBREVIATIONS

ATCC- American Type Culture Collection

CFA - Colonising Factor

CFU – Colony Forming Unit

CLSI- Clinical and Laboratory Standards Institute

CNF - Cytotoxic Necrotising Factor

CONS – Coagulase Negative Staphylococcus

DM – Diabetes Mellitus

EDTA- Ethylene diamine tetra acetic acid

ESBL – Extended Spectrum β- lactamase

GIT - Gastero Intestinal Tract

GNB - Gram Negative Bacteria

GPC - Gram Positive Cocci

MHA – Muller Hinton Agar

MIC – Minimum Inhibitory Concentration

MSSA- Methicillin Sensitive Staphylococcus aureus

MSU – Mid Stream Urine

QC – Quality Control

SHV - Sulph Hydryl Variable plasmid

TEM – Temoniere plasmid

UTI – Urinary tract infection

CONTENTS

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the community and the hospital settings affecting approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global economy[1, 2]. Incidence of UTI in India is 50,000/ million persons per year and accounts for 1-2 % of patients in primary care[3]. About 50% of all women experiences atleast 1 episode of UTI during their life time.Recurrent infection occurring in 20 – 30 % of the females results in high morbidity and mortality[4].

The incidence of UTI is greatly influenced by age, sex, anatomical and functional abnormalities of the urinary tract. Infected urine in pregnant women stimulates an immunological and inflammatory response in the unborn leading to many complications such as premature babies and IUGR as well as Hypertension and renal failure ending fatally from cradle to grave[5].

More than 90% of acute UTI in community is acquired infection caused by *Escherichia coli* and 10 – 20 % by CONS especially *Staphylococcus saphrophyticus* which is the second most common cause in young sexually active women and 5% or less by other Enterobacteriaceae and Enterococci. In complicated cases of UTI resulting from anatomical obstruction and catheterization, the most common causative agents are *E. coli* followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* spp[6, 7].

In general, the urinary tract infections are treated mostly with broad spectrum Cephalosporins, Flouroquinolones and Aminoglycosides. The Cephalosporins which includes, Cephelexin, Cefotaxime, Ceftazidime etc., are cell wall inhibitors and are

used commonly for treating infections caused by Gram negative organism. The Fluroquinolones consisting of Norfloxacin, Ciprofloxacin, Oflaxicin, Sparfloxicin, Levofloxicin etc., are antibiotics which act by inhibiting the activity of DNA gyrase and Topoisomerase the enzymes that are essential for bacterial DNA replication. The aminoglycosides antibiotics include Gentamicin, Kanamicin and Amikacin etc., and they act by inhibiting bacterial protein synthesis[8].

The *E. coli* would have developed resistance to antimicrobial agents and the phenomenon is increasing both in outpatients and hospitalized patients[7, 9]. Among members of the Enterobacteriaceae family, resistance to β- lactams has been reported to be associated with ESBL and Amp C β- lactamase [10]. ESBL producing organisms hydrolyze oxyimino β- lactams like Cefotaxime, Ceftriaxone, Ceftazidime and Monobactams but have no effect on Cephamicins, Carbapenems and related compounds [11]. Amp C β- lactamases are clinically important because they confer rsistance to narrow -, expanded -, and broad spectrum Cepahalosporins, β- lactam- β- lactamase inhibitor combinations and Aztreonam[12].

ESBL producing *E. coli* in patients with UTI has been observed by several workers; its prevalence was variously reported from 28 to 67.5%[13-17, 7]. The prevalence of ESBL producing *Klebsiella* has been reported to be more than 55%[18].

Production of β- lactamase is frequently plasmid encoded and bears clinical significance. Plasmids responsible for ESBL and Amp C β- lactamase production frequently carry genes encoding resistance to other drugs also and therefore antibiotic options in the treatment of β- lactamases producing organisms are extremely limited[19].

The major concern in the occurrence of ESBL and Amp C β- lactamases is the spread of ESBL and Amp C β-lactamases positive bacteria within hospitals, which may lead to outbreaks or endemic situation[20 - 23]. In addition, as therapeutic choices are much limited, it is equally difficult to treat the infections caused by ESBL and Amp C β-lactamase positive organisms[19]. Many clinical laboratories currently test *E. coli* and *Klebsiella* spp. for production of ESBL's but do not attempt to detect plasmid mediated Amp C β- lactamases[12]. It is necessary to investigate the prevalence of ESBL positive and Amp C β- lactamase strains in hospitals, so as to guide the clinician for better therapy.

The prevalence of antimicrobial resistance pattern may vary between geographical areas. However, the publications available on the susceptibility pattern of bacterial isolates in UTI and ESBL prevalence in South East zone of Tamil Nadu are scanty. Hence, the present study is under taken to find out the frequency of the uropathogens, their susceptibility pattern and ESBL production in order to facilitate effective management of UTI.

OBJECTIVES

1. To find out the bacteriological profile of Urinary Tract Infections.
2. To identify the prevalence of *Escherichia coli* and *Klebsiella* among the isolated organisms.
3. To see the sensitivity pattern for the pathogens those are isolated.
4. To screen ESBL production among the isolated *E. coli* and *Klebsiella.*
5. To detect Amp C β- lactamase production among the isolated *E. coli* and *Klebsiella*.
6. To compare the sensitivity pattern among the isolated ESBL producing and non ESBL producing *E. coli* and *Klebsiella.*
7. To provide guidelines to the clinician regarding the treatment of UTI caused by ESBL and Amp C β- lactamase producing *E. coli* and *Klebsiella*.

REVIEW OF LITERATURE

Historical Review

Individual cases of UTI were recorded in antiquity as early as 1412. The first case of UTI was recorded by John Arden in Britain. Later in 1863, Pasteur has recognized urine as a good culture media for bacteria and Roberts (1881) [24] related the presence of bacteria in the urine to symptoms, but very little progress was made in exploring the relationship until quantitative assessments of the number of bacteria in the urine of patients with urinary tract infection were carried out by many authors[25, 26, 27]. In 1995, Quantitative bacterial counting over 10^5 bacteria per ml was regarded as true (or) significant bacteriuria by Kass concept [28].

Classification of UTI [29]

I. Lower UTI

a. **Urethritis**: Infection of the urethra which present as dysurea and frequency.
b. **Cystitis:** Infection of the bladder with features of dysurea, frequency, urgency, supra pubic tenderness, etc.
c. **Acute urethral syndrome:** young sexually active women with dysurea, frequency and urgency but yield organisms less than 10^5 cfu/ml.
d. **Prostatitis:** Infection of the prostate.

II. Upper UTI

a. Pyelonephritis: Inflammation of the kidney, parenchyma, calices and Pelvis caused by bacterial infection.

b. Ureteritis: Rare, usually due to tuberculosis.

Types of UTI [29]

1. **Uncomplicated UTI** refers to infection in a structurally and neurologically normal urinary tract.
2. **Complicated urinary tract infection** refers to infection in a urinary tract with functional (or) structural abnormalities.
3. **Relapse**: Recurrence of bacteriuria with the same infecting micro organism that was present before therapy was started due to persistence of the organism in the urinary tract.
4. **Reinfection**: Recurrence of bacteriuria with a micro organism different from the original infecting bacterium.
5. **Asymptomatic bacteriuria:** Isolation of significant count of bacteria from a person without signs and symptoms of UTI.

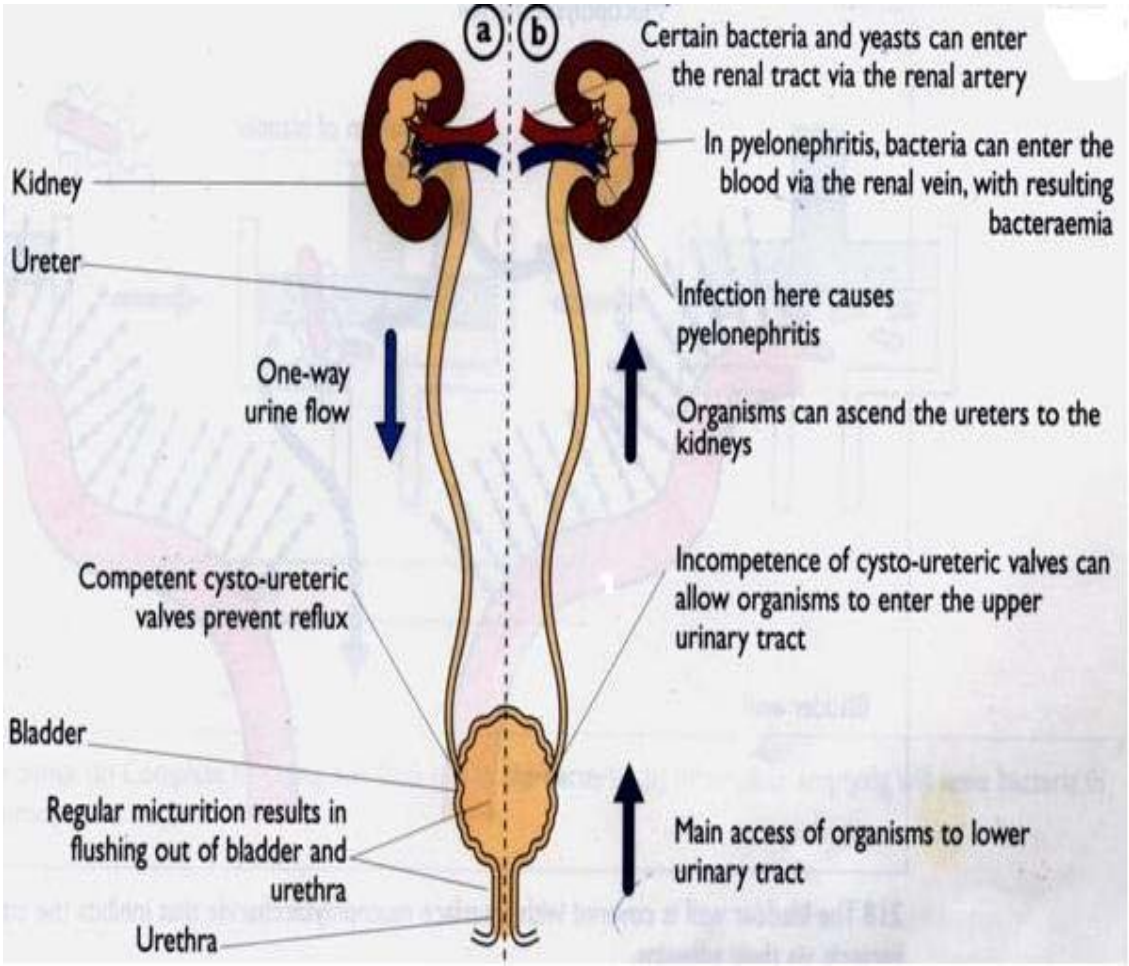
Epidemiology of Urinary Tract Infection[30]

Acute community acquired UTI's are very common. In female, 1-3 per cent School girls affected by UTI and then increases markedly with the onset of sexual activity and it is most common among women between 20 – 50 years of age. In the male population, acute symptomatic UTI's occur in first year of life often in association with urologic abnormalities; thereafter UTI's are unusual in male patients under the age of 50.

Pathogenesis of Urinary Tract Infection:

A thorough knowledge on the normal anatomy of urinary tract is essential to understand the pathogenesis of UTI.

Normal Anatomy **Route of Transmission of infection**



Source [31]

Route of infection

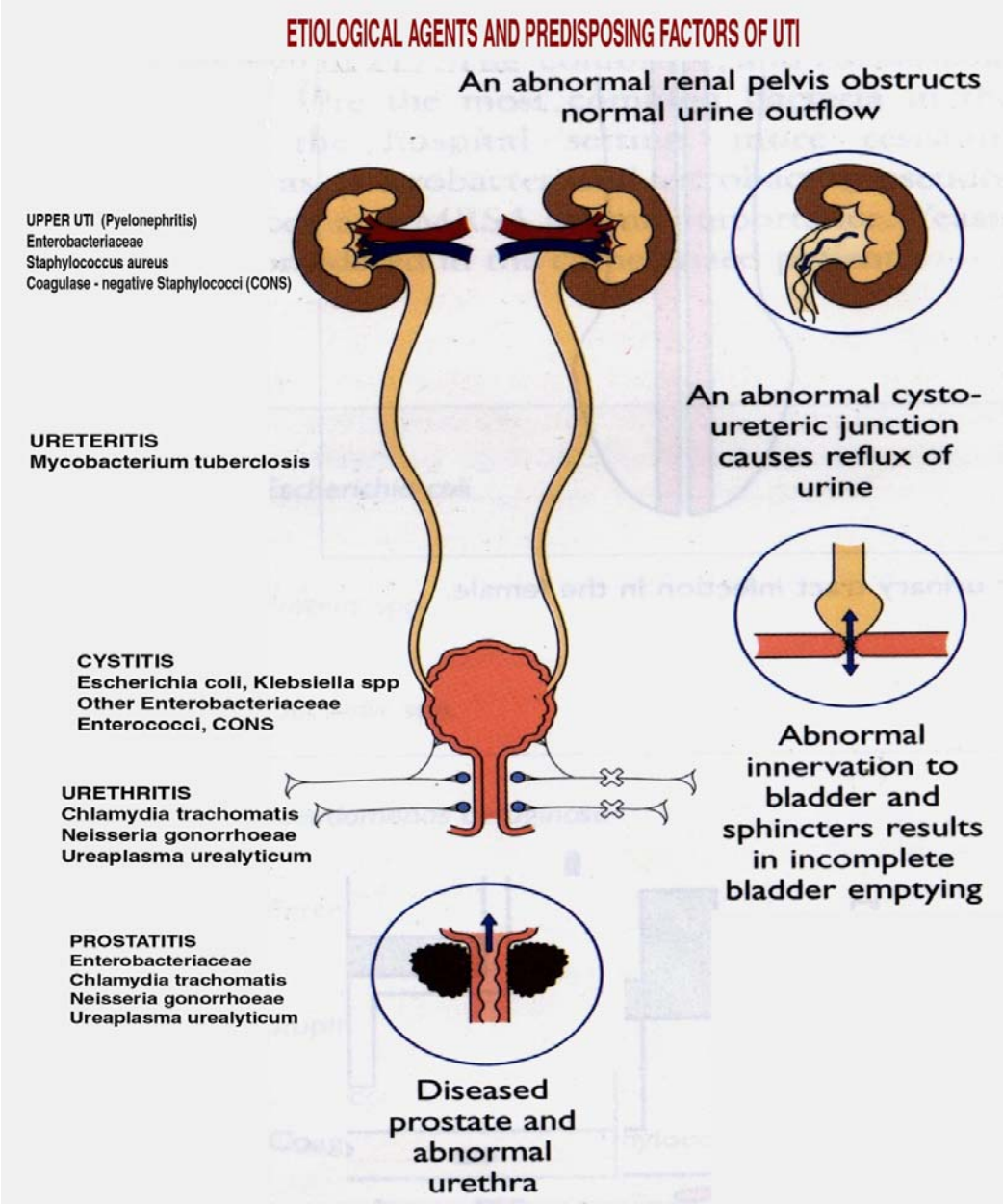
Urine is normally a sterile fluid. Bacteria can invade and cause UTI via two major routes. Ascending route is the most common route of infection in females.

Catheterization and cystoscopy also can cause UTI in both sexes by ascending route. Enteric Gram Negative Bacteria (GNB) and other organisms that originate in Gastro Intestinal Tract (GIT) must be able to colonise the vaginal cavity and periurethral area. Once they gain access to the bladder, multiply and pass up to the ureter and kidneys.[32, 33,34] 5 % of UTI is due to haematogenous spread as a result of bacteraemia e.g. *Staphylococcus aureus* and *Salmonella typhi.*

Host defence against UTI [34, 29]

1. Urine itself is inhibitory to anaerobic bacteria and the low pH, high osmolarity, high organic acid content and constant flushing action of urine inhibit the bacterial colonization.
2. Valve like membranes at the junction of bladder and ureter that prevents back flow of urine.
3. Immune system – Lipo Poly Saccharides of bacteria activates the host cell and releases cytokines such as TNF- α and IFN - γ, activation of complement system.
4. Tamm-Horsfall Protein (or) uromucoid serves as anti adherence factor by binding to *E.coli* expressing type I fimbriae.
5. Defensins – group of small antimicrobial peptides produced by macrophages, neutrophils and cells in the urinary tract and attached to the bacterial cell, eventually kill the bacteria.

Predisposing factors [35, 29]



Source [31, 36]

The following are the factors that favours development of UTI [29, 35]

- Any abnormality of the urinary tract that obstructs or slows the flow of urine e.g. Tumour, stricture and in men enlarged prostate can obstruct the urine flow and make infection difficult to treat.

- UTI occur in small percentage of infants due to congenital abnormality that required surgery.
- People on Immuno suppressive state – Diabetes Mellitus.
- UTI is more common in females because of the shorter urethra that opens in to the moist introitus which is colonised by bacteria. For many women, sexual intercourse seems to precipitate UTI. Women who are using diaphragm and (or) spermicides as a contraceptive are more likely to develop UTI than with other forms of contraception.
- Pregnant women (2-8%) are more susceptible to UTI's attributed to impairment of urine flow partly due to hormonal changes (decreased ureteral tone, decreased ureteral peristalsis and temporary incontinent of vesico ureteric valve) and partly due to pressure on the urinary tract[37, 38] 20 – 30 per cent of women with asymptomatic bacteriuria progress to pyelonephritis.
- Post menopausal women due to oestrogen deficiency and with prolapsed uterus.
- Patients with neurogenic bladder e.g., spinal cord injury, tabes dorsalis, multiple sclerosis and diabetes mellitus (or) bladder diverticulum.

Virulence factors for *E. coli* [29, 39]

Fimbria, which binds on urothelium persist within the urinary tract. Three types of fimbriae are Type 'S' fimbriae (S FA-1), Type 'P' fimbriae and Type 'Dr' fimbriae.

Other factors are Siderophores, Toxins – Haemolysin subdivided in α - lysine which lysis the RBC and β Lysine which lysis the RBC's, lymphocytes and inhibit the phagocytosis, Cytotoxic necrotising factor – CNF 1,2, Uropathogenic strain specific

proteins (USP), Protectins, TIR Domain containing proteins (tcp C), Intimin, Colonising factor – CFA I, II, III.

Virulence factors for Klebsiella

Four factors of virulence in *Klebsiella spp*. are Capsular antigen, Type I and Type III pili, serum resistance LPS and siderophore.

Treatment

UTI is commonly treated with Cephalosporins, Fluroquinolones and Amino glycosides. Among these third generation Cephalosporins are most widely prescribed because of its broad spectrum of activity, low toxicity, ease of administration and its favourable pharmacokinetic profile[29]. Because of their extensive use they also developed resistance to many organisms especially ESBL producing organism[26].

Extended Spectrum β Lactamase (ESBL)

Antibiotic era started with discovery of penicillin by Alexander Flemming in 1928 [40]. Use of Penicillin started in 1941. Emergence of penicillin resistance is identified in S*taphylococcus aureus* due to plasmid encoded β-lactamase. First plasmid mediated β-lactamase in gram negative organisms- TEM-1 was described in early 1960's. It was first isolated in *Escherichia coli* from a patient Temoniera in Greece and the gene responsible for it was named after him. It spread to other genera soon. Another common plasmid mediated β-lactamase found in *Klebsiella pneumoniae* and *Escherichia coli* are SHV-1 (Sulph Hydryl in Variable). Over the last 20 years many new β - lactam antibiotics have been developed which were

resistant to hydrolytic action of β - lactamases but, because of indiscriminate use, these antibiotics also became resistant.

To overcome it, around 1980, 3rd generation cephalosporins are also called broad spectrum Cephalosporins were introduced. Because of their extensive use, they also became resistant.

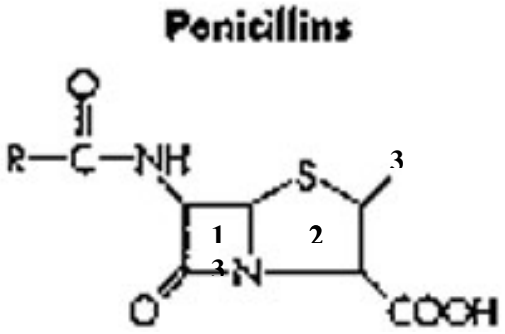
In Germany during 1983, isolates of *Klebsiella pneumoniae* and other Enterobacteriaceae were found to produce a plasmid-determined β-lactamase that hydrolyzed Cefotaxime, as well as other newer 3rd generation of Cephalosporins. This new β-lactamase, called SHV-2 was derived from a mutation in the well-known SHV-1β-lactamase commonly found in *Klebsiella* [37] and as they lead to resistance of extended spectrum cephalosporin they are called extended spectrum β-lactamases[29].

β- Lactam antibiotics: [40]

They are antibiotics having a β - lactam ring. It comprises of Penicillins, Cephalosporins, Monobactams, Carbapenems. All the β-lactam antibiotics have β-lactam ring in common which is made of 3 carbon atoms and one nitrogen atom. The other rings vary in respect of each group of antibiotics.

1. Penicillins:

The structure of Penicillin is



1. β-lactam ring
2. Thiazolidone ring
3. site where β-lactamase will act

Types of Penicillin

I. Natural Penicillin e.g., Penicillin G

II. Semi synthetic Penicillin

1. Acid resistant penicillin e.g., Penicillin V
2. Penicillinase resistant Penicillin e.g., Methicillin, Oxacillin,Cloxacillin
3. Extended spectrum Penicillin.
    a. Amino Penicillin e.g., Ampicillin, Amoxycillin
    b. Carboxy penicillin e.g., Carbenicillin
    c. Ureido Penicillin e.g., Piperacillin, Mezlocillin

2. Cephalosporins:

Have β- lactam ring attached to six member sulfur containing dihyro thiazine ring. By addition of different side chains to dihyro thiazine ring a large number of semi-synthetic compounds have been produced. They are divided into four generations

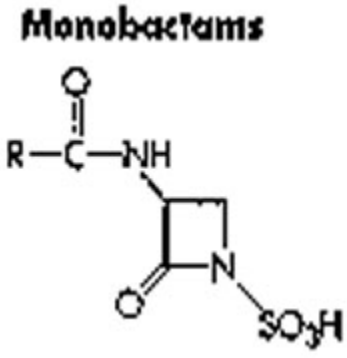
1st Generation Cephalosporins are active against streptococcus, Methicillin Sensitive *Straphylococcus aureus (MSSA)* and Gram Negative Bacteria (GNB) eg., Cephalothin, Cephalexin, Cefadroxil, Cefazolin.

2nd Generation cephalosporin are having greater stability against β- lactamase inactivation and broader spectrum of activity to Gram Positive Cocci [GPC], GNB and anaerobes. Eg, Cefuroxime, Cefuroxime Axetil, Cefaclor, Cefoxitin.

3rd Generation cephalosporin are having high degree of invitro potency to β lactamase stability, broad spectrum of activity against common GNB, anaerobes, good activity against *streptococcus* and less activity against *Staphylococcus e.g.,* Cefotaxim, Ceftazidime, Cefixim, Cefoperazone ceftriaxone.

4th Generation cephalosporin are greater activity against GPC, Enterobacteriaceae and Pseudomonas. e.g., Cefepime, Cefpirome.

3. Monobactams:



Monobactams have a monocyclic β - lactam ring and are resistant to β - lactamase. They are active against gram negative bacteria but not against gram positive bacteria. e.g., Aztreonam.

4. Carbapenems:

These drugs are structurally related to the β-lactam antibiotics. They are extremely potent and have very broad spectrum of activity. e.g., Imipenem [39]

Mechanism of Action of β-lactam antibiotics [40]

The β-lactam antibiotics act by inhibiting cell wall synthesis of bacteria. Bacteria synthesise UDP – N – Acetyl muramic acid pentapeptide and UDP – N – Acetyl glucosamine. Once Peptidoglycan residues are linked together and UDP is split off. Final step is cleavage of the terminal D-alanine of the peptide chains by Tran's peptidases and cross linking between peptide chains of the neighbouring strands is formed. β- lactam antibiotics inhibits trans peptidases so that cross linking is not formed. [40]

Mechanism of bacterial resistance to β-lactam antibiotics [40]

1. Enzymatic inhibition: It is a plasmid mediated e.g., β-lactamase
2. Membrane impermeability in both chromosome and plasmid mediated
3. Alteration of target protein e.g., Penicillin binding protein.
4. Enhanced efflux of the drug from the periplasmic space.

The β- Lactamase

This is a heterogeneous group of Penicillin recognizing proteins. They are members of a super family of active site serine proteases. They act by cleaving an amide bond of beta- lactam ring to form an acyl-enzyme complex. Any β-lactam antibiotic may be inactivated by these enzymes. There are about > 170 enzymes of this kind [41, 42].

Classification of beta- lactamases[43]

Early classification scheme was developed by **Richmon** and **Sykes** based on substrate profile and the location of genes encoding the β-lactamases. Modern scheme based on molecular structure was proposed by **Ambler**. Class A, C, and D are serine β-lactamases, where as class B enzymes are metallo β-lactamase that requires zinc for activity.

Recent classification of β-lactamases is by **Bush-Jacoby – Medeiros** scheme according to substrate profile and inhibition by clavulanic acid. [41]

Table 1: Bush–Jacoby–Medeiros Functional classification scheme for β- lactamases (with Correlation to the Ambler Molecular Classification Scheme) [44, 43]

Group	Enzyme Type	Location	Inhibition by Clavulanate	Amblers Molecular Class	No. of Enzymes	Example
1	Cephalosporinase	Plasmid Chromosome	No	C	57	Resistance to all β – Lactams except Carbapenems
2a	Penicillinase	Plasmid Chromosome	Yes	A	20	*Staphylococcus aureus, S. Epidermidis; Enterococcus spp.;* Penicillins
2b	Broad spectrum	Plasmid Chromosome	Yes	A	16	SHV-1 TEM-1,penicillins and cephalosporins
2be	Extended spectrum	Plasmid Chromosome	Yes	A	81	*Klebsiella oxytoca*, K1, TEM-3 , SHV-2, Penicillins and Cephalosporins
2br	Inhibitor resistant	Plasmid	Diminished	A	13	TEM-30, (IRT-2)
2c	Carbenicillinase	Plasmid Chromosome	Yes	A	15	AER-1 , PSE-1 , CARB-3, Cabenicillin
2d	Cloxacillinase	Plasmid Chromosome	Yes	D or A	21	*Streptomyces cacaoi* OXA-1,Cloxacilllin/Oxacilllin
2e	Cephalosporinase	Plasmid Chromosome	Yes	A	19	*Proteus vulgaris, FEC-1, Cephalosporins*
2f	Carbapenemase	Chromosome	Yes	A	3	IMI-1 , NMC-A, Carbapenems
3	Metalloenzyme	Plasmid Chromosome	No	B	15	*Stenotrophomonas maltophila, L1, All* β- lactams except monobactams
4	Penicillinase	Plasmid Chromosome	No		7	*Burkholderia cepacia*

The broad spectrum, plasmid mediated β - lactamases of Gram negative bacilli such as TEM-1 and SHV-1 produced by Class A were stable for many years.

From 1980, a series of enzymatic variants appeared that had a broadened spectrum of activity against the newly developed β-lactam antibiotics. These ESBLs were first found in Europe most commonly in *Klebsiella* species, less commonly in *E. coli.* The number of enzymes continues to increase.

The new enzymes are located on TEM-1 and SHV-1 plasmids. But they might have been derived originally from a chromosomal enzyme. Many of the new β-lactamases differ from each other only in single amino acid substitution but the changes have profound implications for clinical management of infectious diseases.

Detection of β - lactamases[45]

Detection of β-lactamases was done by various biochemical tests for the enzymes. This was by measuring Penicilloic acids which was produced when β-lactamases hydrolyse benzyl Penicillins. The acid production was detected by measuring the change in pH of an indicator dye (**acidometric method**), by exploiting the ability of Penicilloic acid to reduce iodine and reverse the formation of the blue colour when iodine complexes with starch (**Iodometric method**) and **Chromogenic Cephalosporin method** by using Nitrocephin. Nitrocephin was normally yellow but when the β-lactam ring was hydrolysed it turns red.

β -lactamase inhibitors [43]

These compounds structurally resemble β-lactam antibiotics. They can bind to β-lactam antibiotics either reversibly or irreversibly protecting the antibiotics from destruction. They serve as **suicide bombers** utilizing all available enzymes. These compounds have weak antibacterial activity but are potent inhibitors of many plasmid-encoded and some chromosome encoded β-lactamases. Three important β-lactamase inhibitors are Clavulanic acid, Sulbactam and Tazobactam.

Clavulanic acid show only low level of antibacterial action but when combined with β-lactam antibiotics, inhibition of bacteria is enhanced which are otherwise resistant to β-lactam antibiotics was noted. Sulbactam has broader spectrum of inhibition but less potent. Tazobactam is as potent as Clavulanic acid.

Extended spectrum of β-lactamase

Enzymes capable of hydrolyzing major β-lactam antibiotics including third generation Cephalosporins are called as extended spectrum beta- lactamases.

Characteristics of ESBLs: [43]

They are mostly class- A Cephalosporinases carried on plasmids.

They are more common in *Klebsiella species* followed by *Escherichia coli* described first in Germany and France.

1) All enzymes active against Cephalothin.
2) Imipenem and Cefoxitin not hydrolysed.
3) Comparative activity against Cefotaxime and Ceftazidine varies with enzymes.
4) Some enzymes active against Aztreonam.
5) Inhibition of activity by β-lactamase inhibitors can be demonstrated.

Table 2: Characteristics of the most common plasmid mediated "Wide Spectrum" β- lactamases[43]

Parameter	Extended Spectrum β-lactamase	Amp C -lactamases
Year first reported	1983	1988
Bacterial species affected	*Escherichia coli, Klebsiella pneumonia, Enterobacter spp., Salmonella spp., Proteus spp., Citrobacter spp., Morganella morganii, Serratia marcescens, Shigella dysenteriae, Pseudomonas aeruginosa, Burkholderia cepacia, Capnocytophaga ochracea* and others	*E. coli, K. pneumonia, Salmonella spp, Citrobacter freundii, Enterobacter aerogenes, Proteus mirabilis*
Inhibition by inhibitors of β-lactamases (Clavulanate)	Yes	No
Location of enzyme	Plasmid	Plasmid (derived from inducible chromosomal enzyme)
Expression	Constitutive	Constitutive (one expression)
Indicator antibiotics	Aztreonam, Ceftazidime, Ceftriaxone,Cefotaxime, Cefpodoxime	Possibly Cefoxticin or Cefotetan
Detection mechanism (screening)	Indicator antibiotic with and without β-lactamases inhibitor	Problematic : Use *K. pnemoniae* (no chromosomal Amp C enzyme) as an " institutional screen"
Associated problems	Presence of an ESBL may be masked by coincident Amp C enzyme	Difficult to differentiate plasmid from chromosomal enzyme
Inoculum effect	Yes	Yes
Efficacy of apparently susceptible β-lactamase in the presence of enzyme	No	Probably no
Therapy with β-lactam / β-lactamase inhibitor combinations	Controversial	No

Major risk factors for ESBL production

Risk factors are prolonged stay in ICU, long term use of antibiotics, nursing home residency, severe illness, high rate use of Ceftazidime and other Third Generation Cephalosporins and use of lifelines and catheters.

Detection methods of ESBL [43, 45]

There are several methods are available to detect the ESBL. Among the various methods some of them are discussed below.

a**.** Double-disk approximation test of Tarlier

b. Three Dimensional Test

c. Inhibitor Potentiated Disc Diffusion Test

d. MIC Reduction test

e. E- test

f. Phenotypic Confirmation Test

g. Molecular detection methods

a. Double-disk approximation test of Tarlier

Organism is swabbed onto a Muller – Hinton agar plate. An antibiotic disk containing one of the Oxyimino β-lactam antibiotics placed 30mm (centre to centre) from the Amoxicillin –Clavulanic acid disk. Enhancement of zone of inhibition of the Oxyimino β-lactam caused by synergy of Clavulanate present in Amoxy-clav disk indicates a positive result [43, 45].

b. Three Dimensional test

Advantage is simultaneous determination of antibiotic susceptibility and β- lactamase substrate profile. 2 types of inoculum are prepared.

a. Inoculum-1: contains $10^9 – 10^{10}$ CFU/ml of active ESBL producers.

b. Inoculum-2: Contains 0.5 Mc Farland Std. (150 million organisms/ml)

Plate is inoculated as for disc diffusion procedure with inoculam - 2. A circular slit was cut on the agar 4mm inside the position at which the antibiotic discs were placed and 10^9-10^{10} inoculum was poured into it. Distortion or discontinuity in the circular inhibition zone is interpreted as positive for ESBL production.

c. Inhibitor Potentiated Disc Diffusion test

Cephalosporin discs are placed on MHA plates, with Clavulanate and without Clavulanate. More than 10mm increase in the zone of inhibition of the Clavulanate containing MHA plate indicates ESBL production.

d. MIC Reduction test

An eight fold reduction in the MIC of 3rd generation Cephalosporins in the presence of Clavulanic acid indicates production of ESBL.

e. E test:

E test ESBL strips have 2 gradients, on one end Ceftazidime and on the opposite end Ceftazidime plus Clavulanic acid. MIC is the point of intersection of the inhibition ellipse with the E-test strip edge. Ratio of Ceftazidime MIC and Ceftazidime Clavulanic acid MIC $\geq$ 8 indicates presence of ESBLs.

g. Phenotypic Confirmation Test

Antibiotic susceptibility testing done on Muller Hinton Agar with 0.5 Mc Farland's standard of the organism[36]. Lawn culture of the organism was made and 3rd generation cephalosporin, Ceftazidime (30µg) disc was tested alone and along with their combination for 10mg of Clavulanic acid. Organisms with 5mm increase in zone of inhibition for Ceftazidime / Clavulanic acid (30µg/10µg) are confirmed as ESBLs. (NCCLS recommends MIC $\geq$ 2µg/ml for Cefotaxime, Ceftazidime, Astreonam, Ceftriaxone (or) Cefpodoxime as potential ESBL producers) [43 53].

Two indicators of ESBLs are

1. 4 fold reduction in MIC when 3rd Generation Cephalosporins are used with Clavulanic acid.
2. 5mm increase in diameter of inhibition zone when using disc diffusion method with 3rd generation Cephalosporin and Clavulanic acid combined disc.

h. Molecular detection methods:

Tests already described only presumptively identify the presence of ESBL. Earlier, determination of iso-electric point was sufficient for studying ESBL, But nowadays since there are >90 TEM type and >25 SHV type of β-lactamase and many of them have same iso- electric point, it has become impossible to detect the individual ESBLs. So detection of β-lactamases using DNA probes that were specific for SHV was used but they were labour intensive. The easiest and most reliable molecular method used to detect ESBLs is PCR with oligonucleotide primers that are specific for a β-lactamase gene Oligonucleotide primers can be chosen from sequence

available in Gene Bank. Primers are usually chosen to anneal to regions where various point mutation are known to occur.

Detection of Amp C β-lactamase

a. Disc Antagonism test

The organisms that exhibited resistance to 3rd generation cephalosporins and cefoxitens were swabbed onto a Muller - Hinton Agar Plate and Cefoxiten (30 µg) and Ceftaxidime (30 µg) discs are placed at a distance of 20mm from centre to centre and incubated overnight at 37° C. Amp C β-lactamases inducibility was recognised by blunting of the Ceftazidime zone adjacent to Cefoxiten disc[12].

b. Amp C disc test (Black *et al*., 2005) [46]

The test is based on the use of Tris – EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. Amp C discs (i.e., filter paper disks containing Tris-EDTA) were prepared in house by applying 20 µl of 1:1 mixture of saline and 100 X Tris – EDTA to sterile filter paper discs allowing the discs to dry and storing them at 2- 8 °C. The surface of a MHA plate was inoculated with a lawn of Cefaxitin- susceptible *E. coli* ATCC 25922 according to the standard disc diffusion method. Immediately prior to use, Amp C discs were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disc. A 30 µg Cefoxiten disc was placed on the inoculated surface of the MHA. The inoculated Amp C disc was then placed almost touching the antibiotic disc with the inoculated side of the disc is in contact with the agar surface. The plate was then inverted and incubated overnight at 35 °C in ambient air.

After incubation, plates were examined for either a distortion, indicating no significant inactivation of Cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of Cefoxitin (negative result).

c. Modified three dimensional test

Fresh overnight growth from MHA is transferred to a pre weighed sterile micro centrifuge tube. The tube is weighed again to determine the weight of bacterial mass to obtain 10- 15mg of bacterial wet weight. The botanical mass is suspended in peptone water and pelleted by centrifugation at 3000 rpm for 15 minutes. Crude enzyme extract is prepared by repeated freeze thawing of bacterial pellet (approximately 10 cycles). Lawn culture of *E. coli* A7CC 25922 is prepared on MHA plates and Cefoxiten 30µg disc is placed on the plates. Linear slits (3cm) are made using sterile surgical blade, 3mm away from Cefoxiten disc. All the other end of the slit, a small circular well is made and the extracted enzyme is loaded. A total of 30 – 40 ml of extract is loaded in the well at 10 µl increment. The plates are kept upright for 5 – 10 m until the liquid dries and incubated at 37°C for 24 hrs. Enhanced growth of the surface organism at the point where the slit inserted the zone of inhibition of Cefoxiten is considered a positive three dimensional test and interpreted as evidence of Amp C β-lactamase.

Medical significance of detection of ESBL

Patients having infections caused by ESBL – producing organisms are at increased risk of treatment failure with expanded spectrum β-lactam antibiotics. So it is recommended that if an organism is confirmed to produce ESBL it is considered as resistant to all 3rd Generation Cephalosporins.

Many ESBL isolates will not be phenotypically resistant; even through their MIC is so high. ESBL producing strains have been established in many hospitals producing epidemic diseases especially in Intensive Care Units [43] failure to control outbreaks has resulted in new mutant types in some institution.

Treatment

Carbapenems are most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBLs due to the Trans 6 – hydroxy ethyl group. Alternatively, Fluoroquinolones and amino glycosides may be used if they show in vitro activity[47]. Although clinical data for their use are absent, a β- lactam and β-lactamase inhibitor combination such as Amoxicillin-Clavulanate or Piperacillin Tazobactam may also be a further option to consider[48]. All these agents should be used with caution, as their susceptibility varies among ESBL producers. Cephamycin, such as Cefoxitin and Cefotetan, although active in vitro are not recommended for treating such infections, because of the relative ease with which these strains decrease the expression of outer membrane proteins, rendering them resistant[47]. In urinary tract infection combination with Clavulanic acid can be used[41].

Prevention and control measures

Proper infection control practices and barrier methods are essential to prevent spreading and outbreaks of ESBL producing bacteria. Other practices that reduce the occurrence of ESBL's are optimization of local clinical microbiological laboratories, the rational use of antimicrobial drugs in the community, hospital and veterinary settings. Support of antimicrobial surveillance programmes at local and national levels[49].

MATERIALS AND METHODS

Place of study : Thanjavur Medical College Hospital, Thanjavur.

Study period : One year between August 2009 and July 2010

Collaborating departments : Medicine, Surgery, Paediatrics, Nephrology, Urology, Obstetrics, Gynaecology and STD

Design of study : Observational study

Ethical clearance : Prior approval obtained from Ethical Committee

Informed consent : Obtained from each patient

Inclusion criteria : 1. Fresh case of UTI

2. No H/O antibiotic intake
3. No H/O instrumentation
4. No H/O immune compromised state
5. Non pregnant women
6. No H/O recent delivery
7. No H/O liver or renal dysfunction

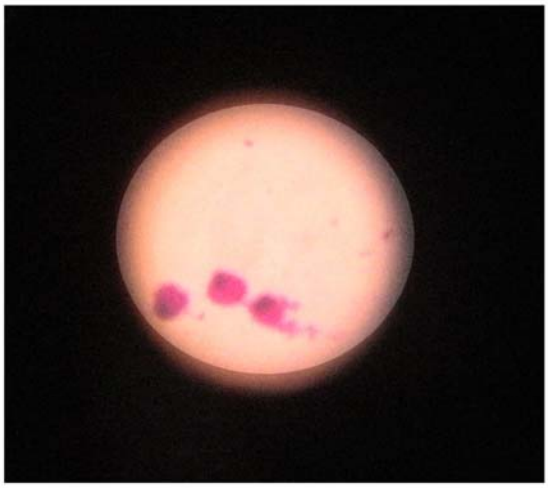
The patients of all age groups belonging to both the sex with fever, dysurea, frequency, urgency, lower abdominal pain / flank pain and supra pubic tenderness that are suggestive of upper and lower Urinary tract infections were considered and included in the study.

Exclusion criteria:

Those with Diabetes mellitus and associated co-morbid conditions, promiscuous individual, immuno compromised status, repeated catheterization, instrumentation and on antimicrobial therapy were excluded.

Plate 1. Urine specimen in wide mouth universal container

Plate 2. Direct Gramstain showing pus cells and Gram negative bacilli



Specimen collection and transport

Urine samples were collected in 50ml wide mouth sterile container as per CLSI guidelines for urine Group16-A2[34, 50].

Midstream urine collection[34]

- Female patients were asked to clean the area around the urethral opening with soap and water, and instructed to rinse well and collect the urine with the labia held apart.
- Male patients were asked to wash periurethral region and by retracting the foreskin.
- Patients were asked to void few ml of urine initially then collect about 20ml during midstream. Immediately after collection, the samples were labelled and transported to the laboratory and processed within two hours.

Specimen processing

Macroscopy[34, 50]

Urine specimen was examined macroscopically for the presence of colour and turbidity.

Wet mount

One drop of uncentrifuged urine was kept over a microscope slide then a cover slip was placed over it and it was examined under low power microscope for the presence of pus cells. A count of more than eight pus cells/mm^3 is suggestive of pyuria[34].

Plate 3. Semi quantitative culture in blood agar plate

Plate 4. Semi quantitative culture in Mac Conkey agar plate

Gram staining

A drop of well mixed uncentrifuged urine was placed over the microscope slide, thin smear was made air dried, heat fixed and gram staining was done and examined under oil immersion. Presence of ≥1 to 5 bacteria / OIF was taken as significant bacteriuria accounts for $>10^5$ CFU/ml, presence of pus cells taken as definite indication of UTI[34].

Culture[34, 51]

Uncentrifuged urine was mixed well by gently rotating the container by keeping it over the table. Using a calibrated loop (0.001). Each sample was inoculated in the following media 1. Nutrient agar,

2. Mac Conkey agar
3. Blood agar

Calibrated loop was flamed, after cooling it was inserted vertically into the urine to allow urine to adhere to the loop. And the culture plate was inoculated by keeping this loop in the centre of the plate and the inoculums was spread in a line on either side. Then without flaming, loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.

Plates were incubated for 24 hrs at 35-37ºC. Colonies were counted on each plate with the help of hand lens. The number of colonies was multiplied by 1000 to determine the number of microorganism per ml in the original specimen.

Plate 5. Gram Negative Bacilli

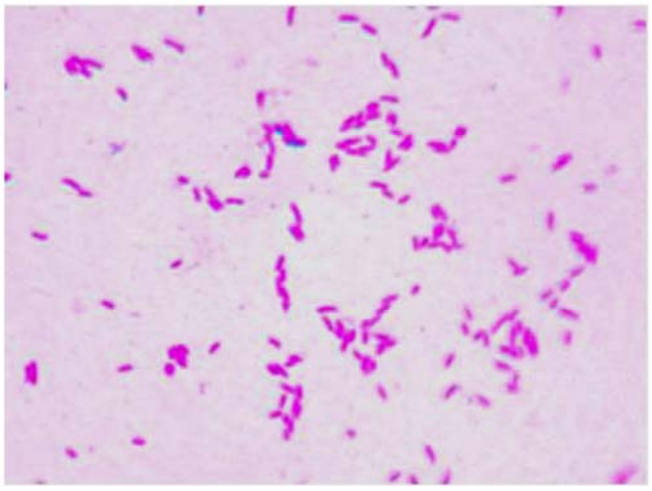


Plate 6. Gram Positive Cocci

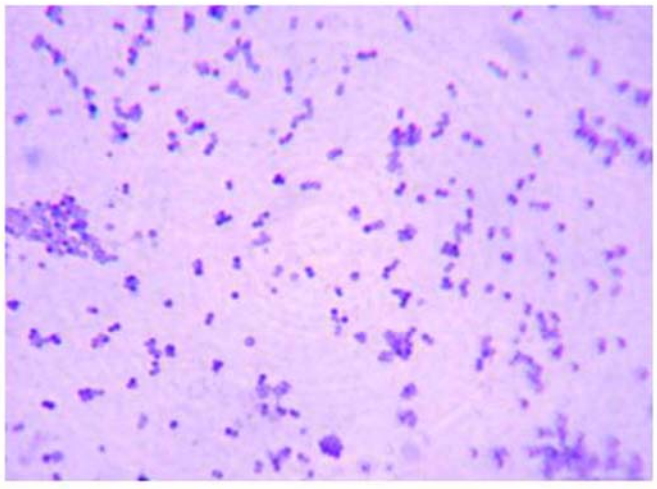


Plate 7. *Escherichia coli* in Mac Conkey agar showing lactose fermenting colonies

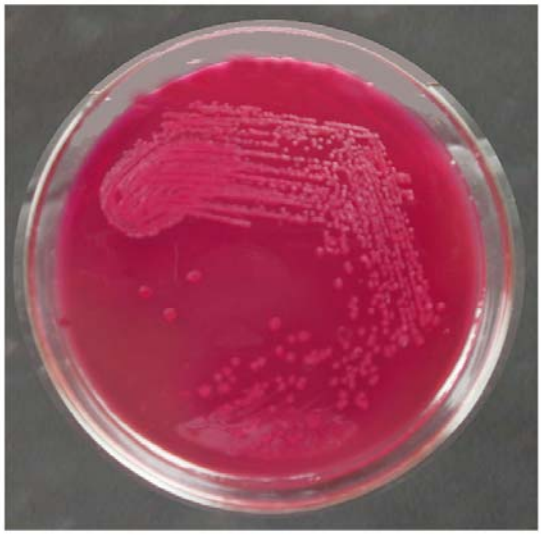


Plate 8. *Escherichia coli* - Biochemical reactions

Plate 9. *Klebsiella pneumoniae* in Mac Conkey agar showing lactose fermenting colonies

Plate 10. *Klebsiella pneumoniae* - Biochemical reactions

Plate 11. ***Proteus mirabilis in Blood agar showing swarming***

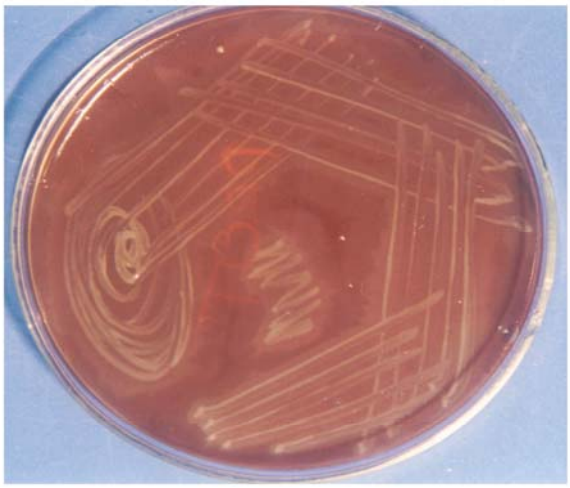


Plate 12. ***Proteus mirabilis - Biochemical reaction***

Plate 13. *Pseudomonas aeruginosa* in Mac Conkey agar showing non lactose fermenting colonies

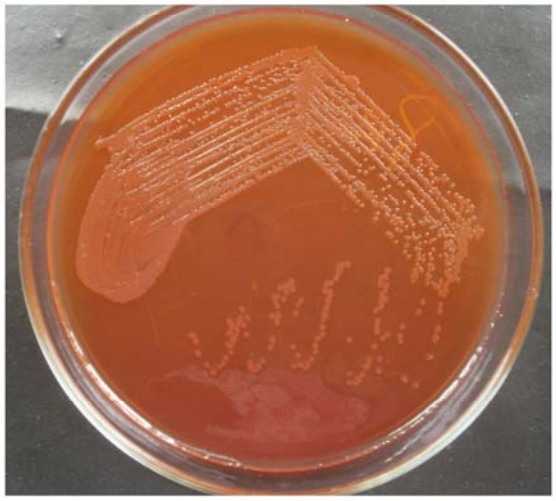


Plate 14. *Pseudomonas aeruginosa* - Biochemical reactions

Interpretation of culture[34, 51]

A single type of colony was counted and interpreted as follows,

Significant bacteriuria - More than 1, 00,000 CFU /ml.

Probably significant' - 10,000 -'.1, 00,000 CFU/ml – (Culture repeated)

Insignificant - < 10,000 CFU/ml

However, a pure culture of *Staphylococcus aureus* was considered to be significant regardless of the number of organism[34].

The isolated colony was identified by adopting the procedures of Gram staining, motility and routine biochemical reactions[51, 52].

The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs (Himedia, Mumbai).

Antimicrobial sensitivity testing[53]

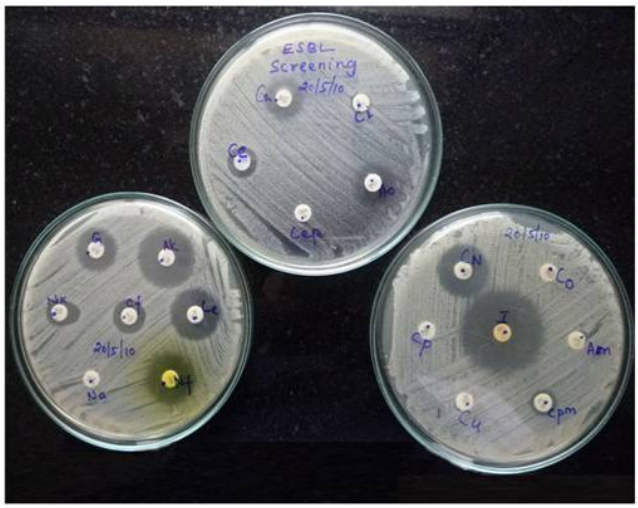
Storage of antimicrobial discs[53]

The antimicrobial disc container was refrigerated at 4 - 8° C while the β-lactum antibiotics were stored in the freezer compartment. Some labile agents like Imipenem and Clavulanic acid retained greater stability when stored frozen until the day of use.

Disc container was taken out from refrigerator one or two hours before use and brought to room temperature. Once a cartridge of discs has been removed from its sealed package, after the use it was replaced in a tightly sealed dry container.

Plate 15. Mc Farland turbidity standard

Plate 16. Antibiogram



Preparation of turbidity standard[36]

McFarland standards prepared by adding specific volumes of 1% Suphuric acid and 1.175 % barium chloride to obtain a barium sulphate solution with a specific optical density. The most commonly used is the McFarland 0.5 standard, which contains 99.5ml of 1% sulphuric acid and 0.5 ml of 1.175 % barium chloride. This solution is dispersed into tubes comparable to those used for inoculums preparation, which are sealed tightly and stored in the dark at Room temp. The McFarland 0.5 standard provides a turbidity comparable to that of a bacterial suspension containing approximately 1.5 X 10^{8} CFU/ml.

Preparation of Inoculum

In order to prepare the inoculum**,** about 3-5 representative colonies were picked up and inoculated in 4-5 ml of peptone water and incubated at 37°C for 2 – 6 hrs to attain 0.5 McFarland's standard which corresponds to 150 million organisms/ml. If it was more turbid, then some more quantity of peptone water was added and adjusted to 0.5 McFarland's standard by comparing against a card with white background and contrasting black lines.

Inoculation of MHA plates

Within 15 minutes of adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped and rotated several times. During this process, the swab was pressed firmly on the inside wall of the tube above the fluid level to remove excess of broth from the swab. Then the dried surface of Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times by rotating the plates at an angle of approximately 60°c to ensure an even distribution of inoculums and finally, the rim of

the agar were swabbed. The plate was closed and left for 3-5 minutes to allow any excess surface moisture to be absorbed before applying drug impregnated discs.

Application of discs to inoculated agar plates

The predetermined battery of antimicrobial discs of Gentamicin, Amikacin, Ampicillin, Cotrimaxazole, Nitrofurantoin, Nalidixic acid, Norfloxacin, Ciprofloxacin, Levofloxacin, Cephelexin, Cefuroxime, Cefoxitin, Ceftazidime, Ceftriaxone, Cefotaxim,Cefpodaxime, Cefipime, Aztreonam, Imipenam, and Amoxyclav were tested for all the isolates.

Along with the above drugs Azithromycin and Vancomycin were tested for gram positive cocci. Piperacillin-Tazobactum and Cefeperazone-Sulbactum were used only for *E. coli* and *klebsiella.* The entire disc were placed on agar plates and pressed down to ensure complete contact with the agar surface. Discs were distributed evenly so that they were not closer than 25 mm from centre to centre of the disc and incubated at 37° C for 16 – 18 hrs.

Reading and interpretation of results

After 16-18 hrs of incubation, each plate was examined for satisfactory streaking with uniformly circular zones of inhibition and confluent lawn of growth.

The diameter of the zones of complete inhibition including the diameter of the discs was measured. The zones were measured to the nearest millimetre using a ruler that was held on the back by inverting Petri plate. The Petri plate was held a few inches above a black, non reflecting background and illuminated with reflected light. The zone margin showing no obvious visible growth that could be detected with unaided eyes was considered as a zone of inhibition. The sizes of the zones of inhibition were interpreted by referring to the CLSI standards and reported as 'susceptible', 'intermediate' or 'resistant' to the drugs that were tested.

Table 3: ZONE SIZE INTERPRETATIVE CHART IN ACCORDING TO CLSI[53]

Sl. No.	Antimicrobial agent	Symbol	Drug concentration (μg)	Zone size in mm		
				Resistant	Intermediate	Sensitive
A. Aminoglycosides						
1.	Gentamicin	G	10	< 12	13-14	> 15
2.	Amikacin	AK	30	<14	15-16	>17
B. Penicillin						
1.	Ampicillin	A	10	<13	14-16	>17
C. Sulphonamides						
1.	Cotrimoxazole	CO	1.25/23.75	<10	11-15	>16
D. Urinary antiseptics						
1.	Nitrofurantoin	NF	300	<14	15-16	>17
E. Quinolones						
1.	Nalidixic acid	NA	30	<13	14-13	>19
2.	Norfloxacin	NX	10	<12	13-16	>17
3.	Ciprofloxacin	CF	5	<15	16-20	>21
4.	Levofloxacin	LE	5	<13	14-16	>17
F. Cephalosporins						
1.	Cephelexin	CP	30	<14	15-17	>18
2.	Cefuroxine	CU	30	<14	15-17	>18
3.	Cefoxiten	CN	30	<14	15-17	>18
4.	Ceftazidime	CA	30	<14	15-17	>18
5.	Ceftriaxone	CI	30	<13	14-20	>21
6.	Cefotaxime	CE	30	<14	15-22	>23
7.	Cefpodoxime	CEP	10	<17	18-20	>21
8.	Cefipime	CPM	30	<14	15-17	>18
G. Monobactams						
18.	Aztreonam	AO	30	<15	16-21	>22
H. Carbapenems						
19.	Imipenam	I	10	<13	14-15	>16
I. β lactam - β lactamase inhibitor						
	Amoxyclav	AC	20/10	<13	14 – 17	>18
	Piperacillin-Tazobactum	PT	100/10	17	18 – 20	21
	Cefeperazone-Sulbactum	CFS	75 / 10	15	16 20	21
J. Macrolids						
21.	Azithromycin	AT	15	<13	14-17	>18
K. Glycopeptide						
22.	Vancomycin	V	30	<14	----	>15

Plate 17. ESBL screening

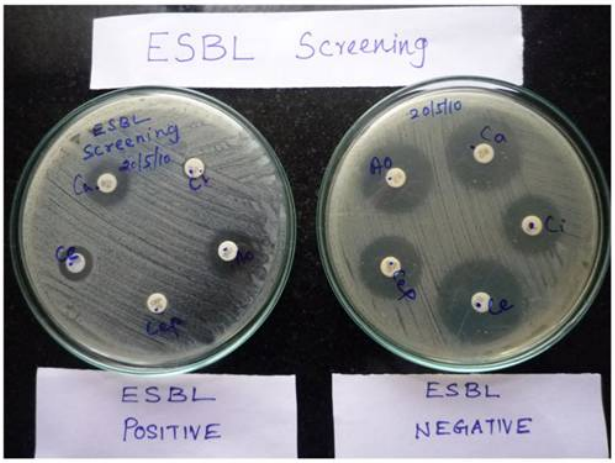


Plate 18. Double disc synergy test

Control strains used with each batch

i. *Escherichia coli* ATCC 25922

ii. *Pseudomonas aeruginosa* ATCC 27853

iii. *Staphylococcus aureus* ATCC 25923

Screening for ESBL production[53]

1. Modified Kirby Bauer disc diffusion method

Isolates showing inhibition zones ≤22 mm for Ceftazidme, ≤ 27 mm for Cephotaxime, ≤25 mm for Ceftriaxone, ≤22 mm for Cefpodoxime and ≤27 mm for Aztreonam were identified as potential ESBL producers and they were tested further.

2. Double disc synergy test[53]

To demonstrate a synergistic action between a 3rd generation Cephalosporin and Clavulanic acid, isolates were grown and adjusted to 0.5 McFarland's standard and lawn culture of it was made on MHA plate.

Discs of 3rd generation Cephalosporin, Cefotaxime (30µg) and Ceftazidime (30µg) were placed 20 mm apart from an amoxicillin (20µg) and Clavulanic acid (10µg) combined disc (Augmentin) centre to centre and incubated at 37°C for 16 – 18 hrs. If inhibition zone around the 3rd generation Cephalosporins showed a clear extension towards Augmentin disc then the organisms were said to be ESBL producing.

Phenotypic confirmation test

1. **Inhibitor potentiation disc diffusion test (NCCLS confirmatory test)**[53]

ESBL production was confirmed by Ceftazidime (30 µg) and Ceftazidime plus Clavulanic acid (30 /10 µg) placed on inoculated MHA plates and incubated. Organism

Plate 19. Double disc potentiation

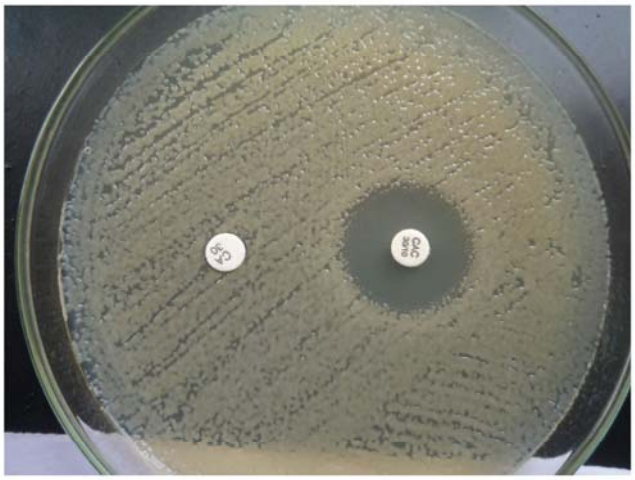


Plate 20. E test

was considered as ESBL producer if there was ≥ 5mm increase in diameter of Ceftazidime/ Clavulanate disc than that of Ceftazidime disc alone.

2. **E-test for ESBL** [43,49]

Combination of disc diffusion and Minimum Inhibitory Concentration (MIC) were studied using the E-test strips. The E-test strip contains Ceftazidime gradient at one end and Ceftazidime plus Clavulanate gradient on the opposite end. MHA were inoculated as for disc diffusion and the strips were placed on the inoculated lawn and incubated. MIC was the point of intersection of the inhibition ellipse with the E-test strip edge. Ratio of ceftazidime MIC and Ceftazidime Clavulanic acid MIC ≥ 8 indicated the presence of ESBL.

Quality Control (QC) used for ESBL production:

E. coli A7CC 25922 - Negative control

Klebsiella pnemoniae ATCC 700603 – Positive control

Tests for Amp C β lactamase production

a. Disc Antagonism test[12]

The organisms that exhibited resistance to 3rd generation cephalosporins and cefoxitins were swabbed onto a Muller - Hinton Agar Plate and Cefoxitin (30 µg) and Ceftaxidime (30 µg) discs are placed at a distance of 20mm from centre to centre and incubated overnight at 37° C. Amp C β-lactamases inducibility was recognized by blunting of the Ceftazidime zone adjacent to Cefoxitin disc[12, 54].

Plate 21. Disc antagonism test

Plate 22. Amp C Disc test

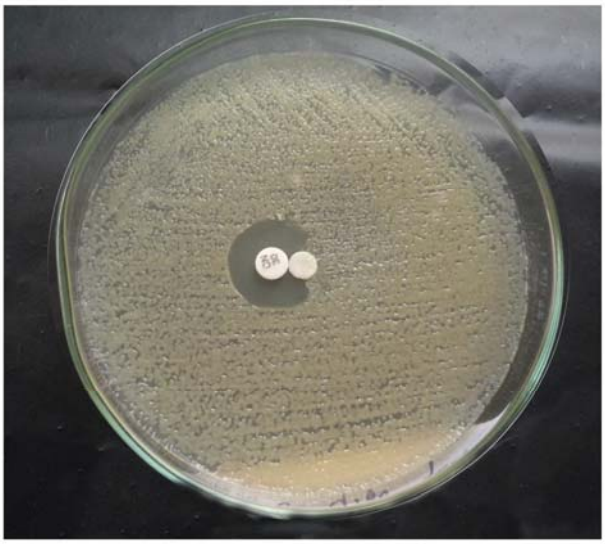


Plate 23. Antibiotic of choice for ESBL producers

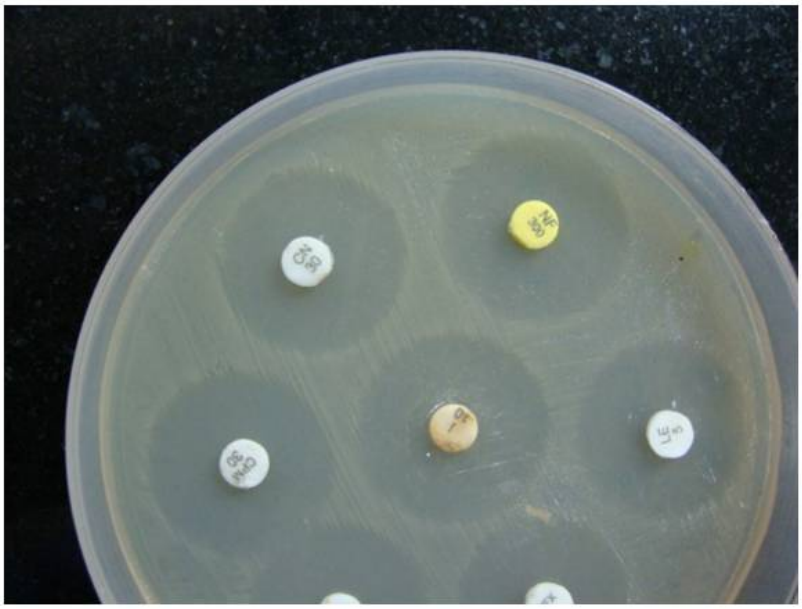
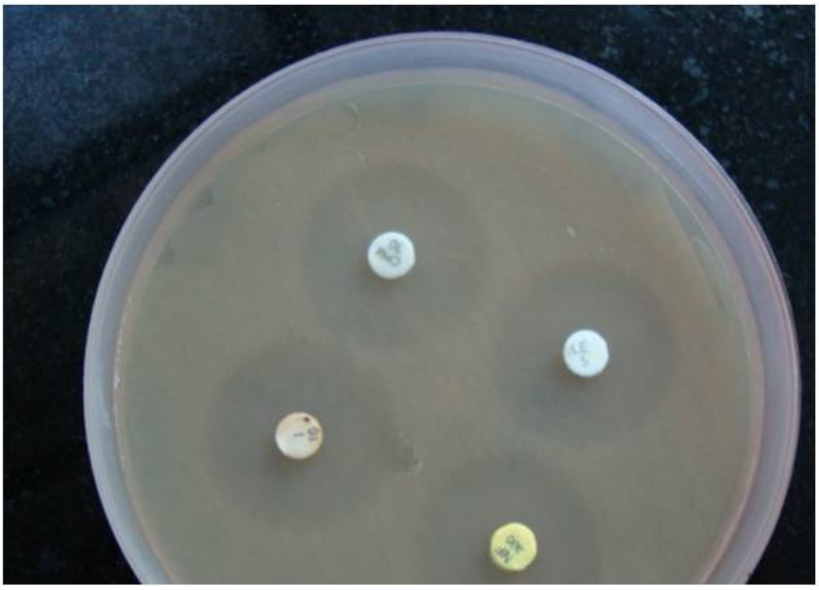


Plate 24. Antibiotic of choice for Amp C β Lactamase producers



b. Amp C disc test (Black *et al*., 2005)[46]

The test is based on the use of Tris – EDTA to permeabilize bacterial cell and release β-lactamases into the external environment. Amp C discs (i.e., filter paper disks containing Tris-EDTA) were prepared in house by applying 20 µl of 1:1 mixture of saline and 100 X Tris – EDTA to sterile filter paper discs allowing the discs to dry and storing them at 2- 8 °C. The surface of a MHA plate was inoculated with a lawn of cefoxitin- susceptible *E. coli* ATCC 25922 according to the standard disc diffusion method. Immediately prior to use, Amp C discs were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disc.

A 30 µg Cefoxitin disc was placed on the inoculated surface of the MHA. The inoculated Amp C disc was then placed almost touching the antibiotic disc with the inoculated disc face in contact with the Agar surface. The plate was then inverted and incubated overnight at 35 °C in ambient air. After incubation, plates were examined for either a distortion, indicating no significant inactivation of Cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of Cefoxitin (negative result).

STATISTICS: Simple descriptive statistics was used to analyse the data.

RESULTS

The results of the present investigation on bacteriological profile, antimicrobial susceptibility pattern, ESBL and Amp C β- lactamase producing strains status with reference to *Escherichia coli* and *Klebsiella spp* in urinary tract infections are presented below.

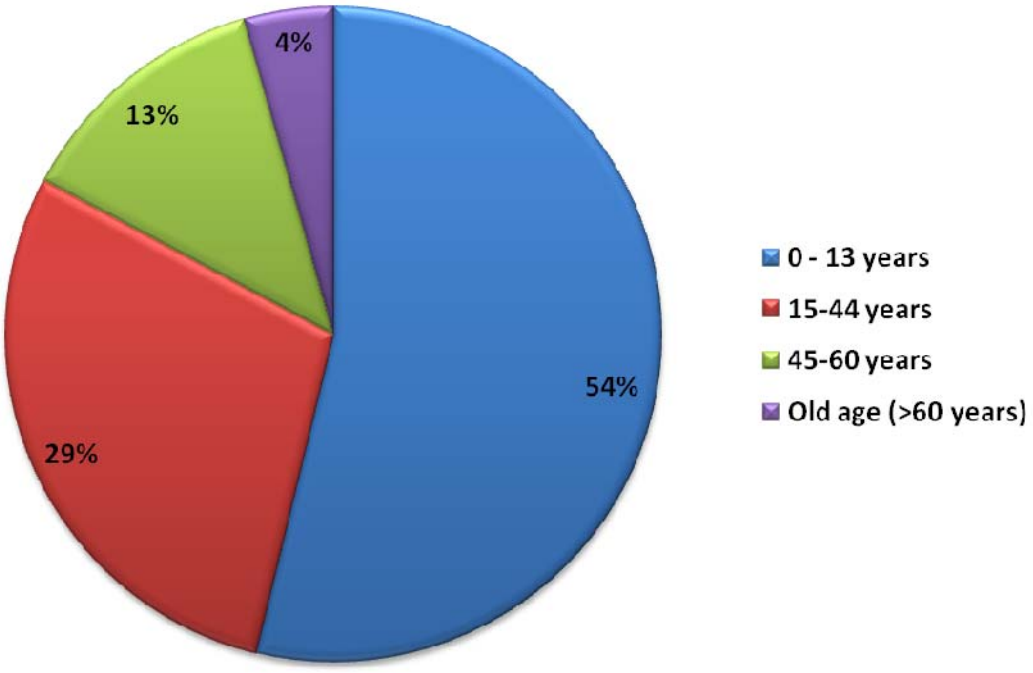
Table 4: Age and gender wise collection of samples (n = 500)

Age Group	Male	%	Female	%	Total	%
0 - 13 years	136	27.2	133	26.6	269	53.8
15-44 years	58	11.6	87	17.4	145	29.0
45-60 years	37	7.4	27	5.4	64	12.8
Old age (>60 years)	14	2.8	8	1.6	22	4.4
Total	245	49.0	255	51.0	500	100

Collection of samples based on age and gender

The data on the age and gender wise distribution of patients included in the study are presented in Table 4. Among the samples collected from 500 patients both Inpatient and Outpatients, 49% were male, 51% were female, mean age was 39 years with the range from new born to 78 years. The observations showed 53.8% (27.2% of male and female 26.6%) of the patients were less than 14 years of age. 29 % (11.6% + 17.4%) of patients were in reproductive age group, 12.8% (7.4% + 5.4%) of patients from middle age group, 4.4% (2.8% + 1.6%) of patients were in older age groups more than 60 years.

Age wise distribution of patients (n= 500)



Age and Gender wise distribution of patients (n=500)

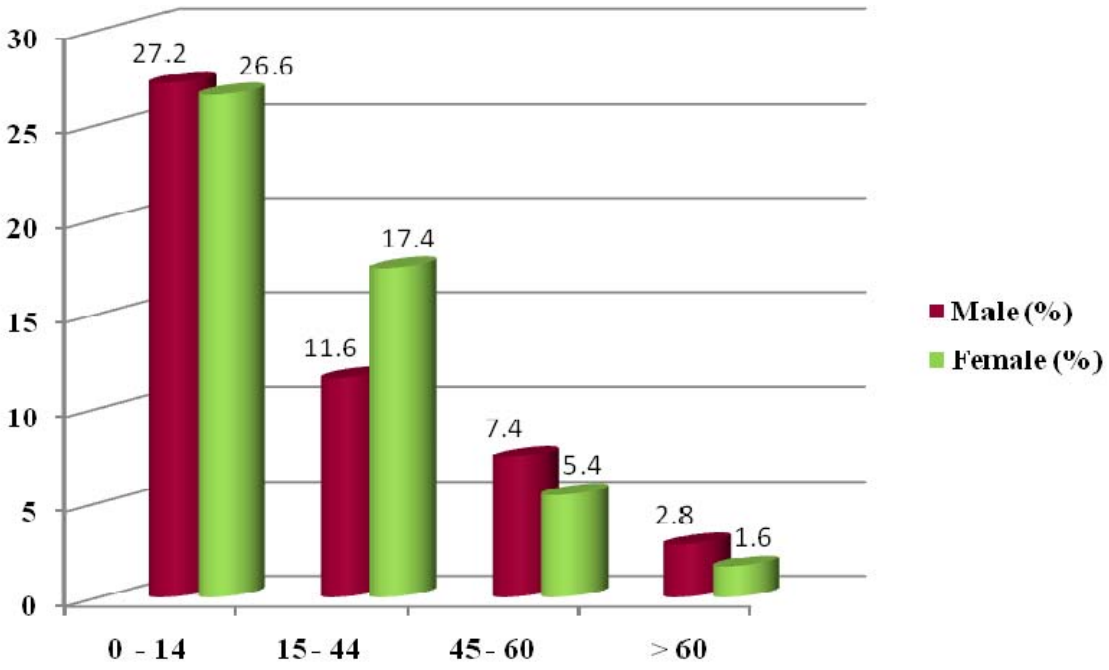


Table 5: Age and gender wise prevalence of UTI

Age Group	Male	%	Female	%	Total	%
0-13 years	26	17.3	36	24.0	62	41.3
15-44 years	19	12.7	31	20.7	50	33.4
45-60 years	15	10.0	8	5.3	23	15.3
Old age (>60 years)	9	6.0	6	4.0	15	10.0
Total	69	46.0	81	54.0	150	100

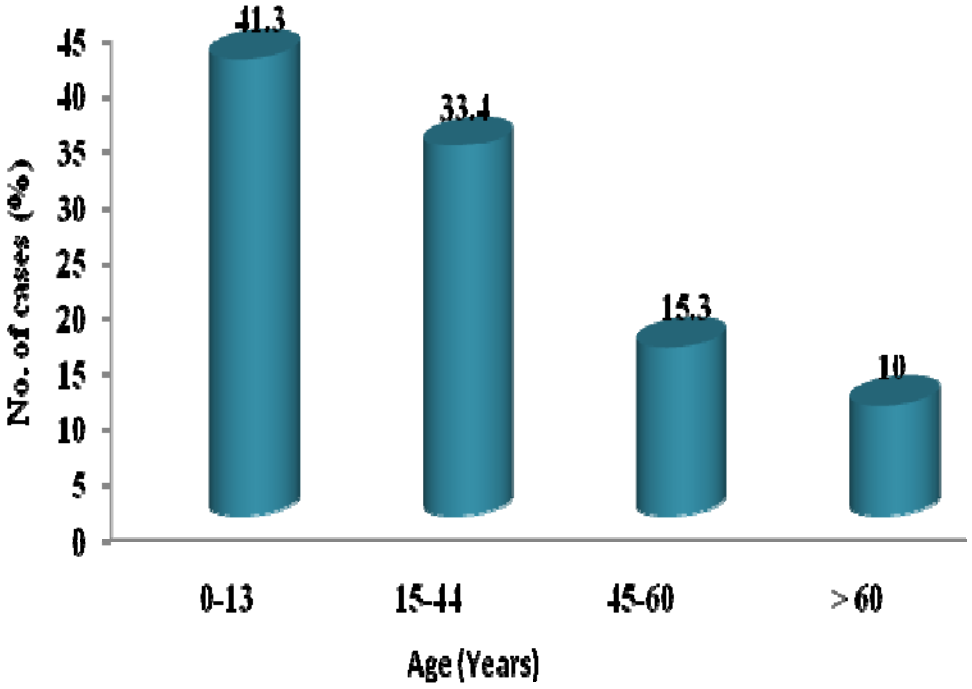
Age and gender wise prevalence of UTI

Among the 500 samples tested only 30% showed the significant bacteriuria. In 150 bacterial isolates 54% were from female patients and 46 % from male patients with a male to female ratio of 1:1.2 (Table 5). Large numbers of isolates were found in paediatric age group 41% (17.3 % + 24 %) followed by 33.4% (12.7% + 20.7%) were from reproductive age group and 15.3% (10 % + 5.3%) from middle age and the rest 10% (6% + 4%) were from old age group.

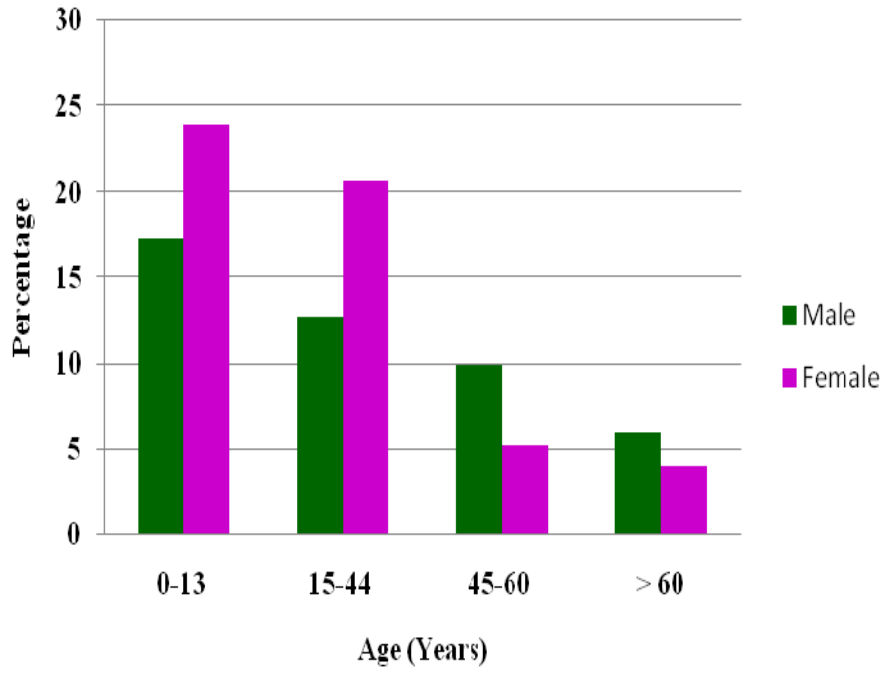
Table 6: Department wise prevalence of UTI (n= 150)

Department	IP		OP		Total (n= 150)	
	Number	%	Number	%	Number	%
Paediatrics	52	34.6	5	3.4	57	38
Medicine	35	23.3	5	3.4	40	26.6
Surgery	21	14.0	2	1.4	23	15.3
Obstetrics & gynaecology	13	8.6	2	1.4	15	10
Urology	7	4.6	4	2.6	11	7.3
Nephrology	2	1.3	-	-	2	1.4
STD	-	-	2	1.4	2	1.4
Total	**130**	**86.4**	**20**	**13.6**	**150**	**100**

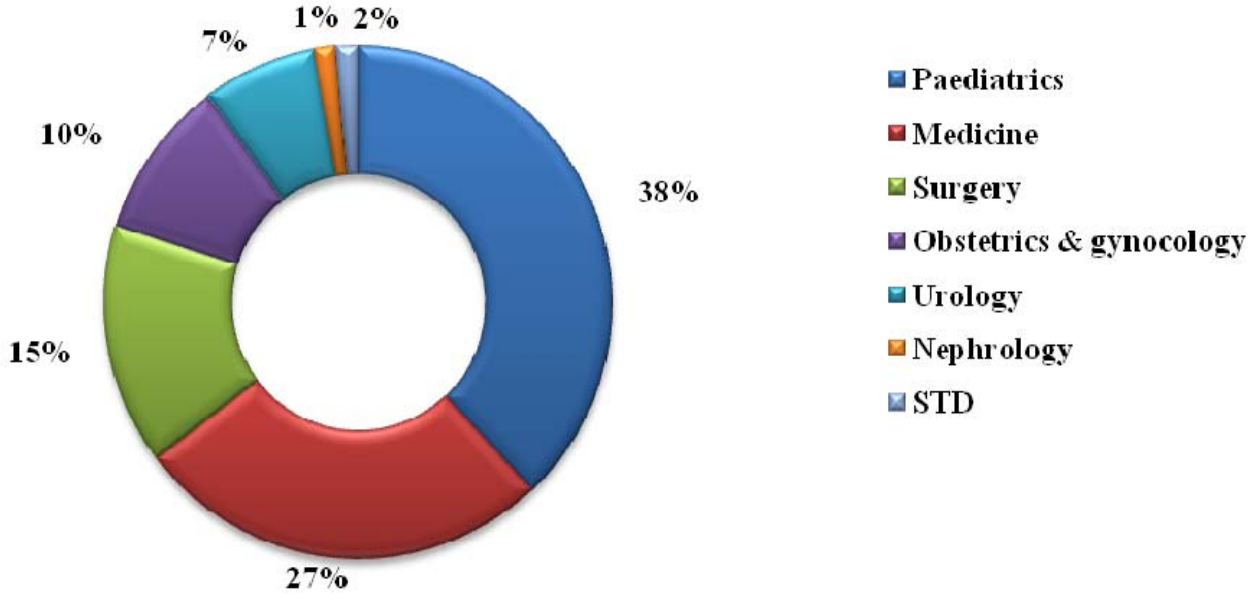
Age wise prevalence of UTI (n=150)



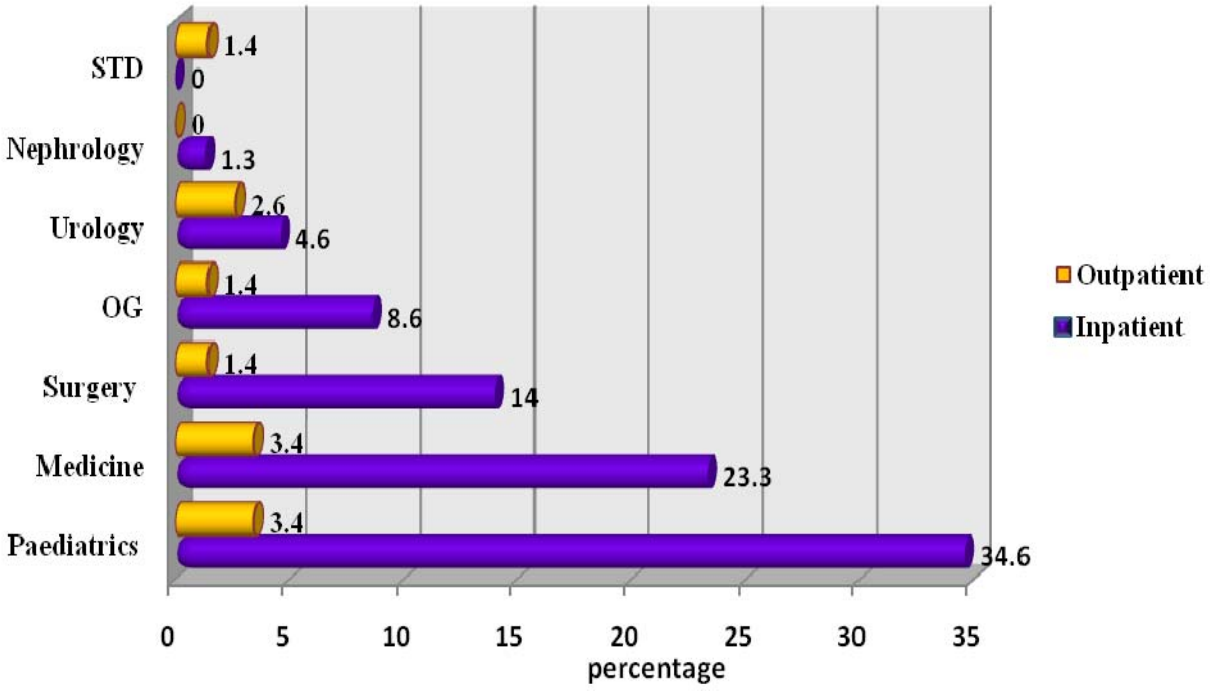
Age and sex wise prevalence of UTI (n=150)



Department wise prevalence of UTI (n=150)



Inpatient and outpatient wise distribution of UTI (n=150)

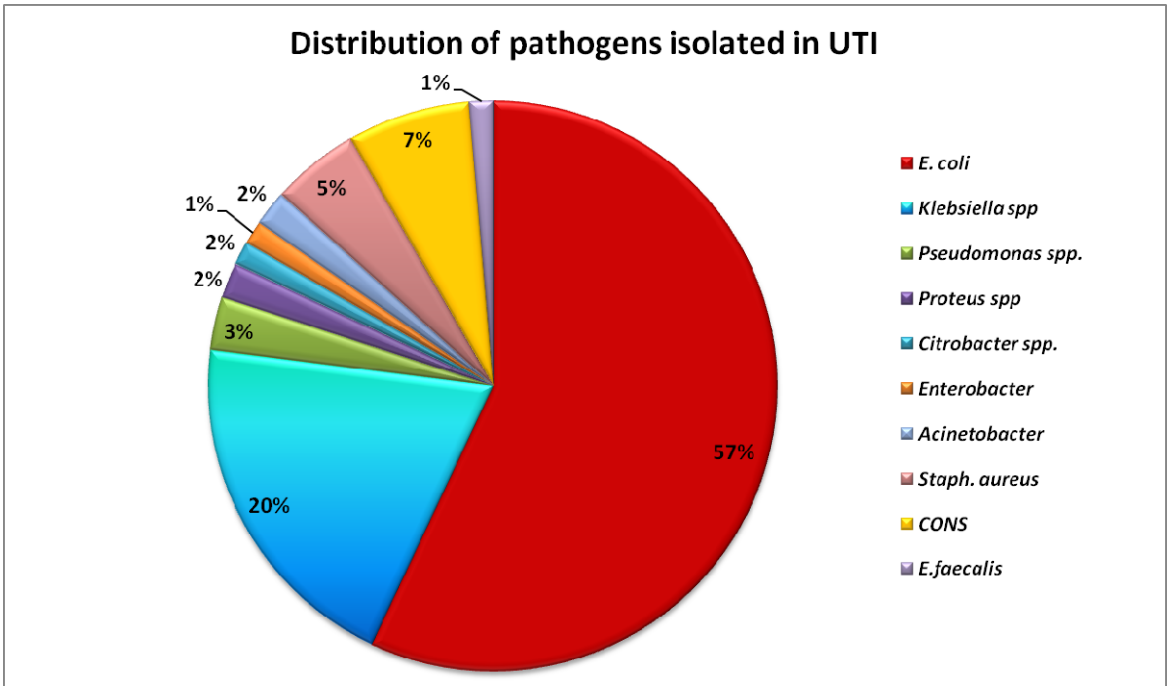


Department wise prevalence of UTI

The data on department wise prevalence of UTI cases is presented in Table.6 and among the 150 patients showing UTI, 130 patients i.e., 86.4% were In-patients and the remaining 20 patients (13.6%) were Out-patients. The department wise distribution of patients revealed that, the prevalence of UTI was maximum in the patients admitted in the department of Paediatrics which registered the highest number of 57 UTI cases (38%) followed by departments of Medicine (26.6%) and Surgery (15.3%). Of the 15 UTI cases in the department of Obstetrics and gynaecology, 13 were from in-patients and only two were from out-patients. Among all the departments, the departments of Nephrology and STD recorded the least cases of UTI (1.4% each).

Table 7: Distribution of pathogens isolated in UTI

Sl.No	Organism	Frequency (n= 150)	Percentage
1.	*Escherichia coli*	85	57
2.	*Klebsiella pneumonia*	21	14
3.	*Klebsiella oxytoca*	9	6
4.	*Pseudomonas aeruginosa*	4	3
5.	*Proteus spp*	3	2
6.	*Citrobacter koseri*	2	1.33
7.	*Enterobacter*	2	1.33
8.	*Acinetobacter*	3	2
9.	*Staphylococcus aureus*	8	5
10.	CONS	11	7
11.	*Enterococcus faecalis*	2	1.33
	Total	**150**	**100**


Distribution of pathogens isolated in UTI
1%
7%
5%
2%
1%
2%
2%
3%
20%
57%
E. coli
Klebsiella spp
Pseudomonas spp.
Proteus spp
Citrobacter spp.
Enterobacter
Acinetobacter
Staph. aureus
CONS
E.faecalis

Prevalence of urinary pathogens

Isolation of the 500 samples that were collected during the study period indicated the presence of 150 pathogens and the details are presented in Table 7. Among the 150 pathogens isolated from the samples, the Gram Negative Bacilli (GNB) with 129 isolates (86.0%) was the major cause for UTI while only 21 isolates were Gram positive cocci (GPC). Among the 129 GNB, the *Escherichia coli* and *Klebsiella spp* alone constituted 77 % of total isolates with 85 isolates of *E. coli* and 30 isolates of *Klebsiella* spp. The remaining GNB isolates includes, 4 isolates of *Pseudomonas auregenosa*, 3 isolates from *Proteus spp* and Acinetobacter. *Citrobacter koseri* and Enterobacter accounted 2 each. Among the 21 isolates of GPC, 11 were coagulase negative *Staphylococcus,* 8 was *Staphylococcus aureus* and 2 isolates were *Enterococcus faecalis.* Out of 11 CONS 6 were *Staphylococcus epidermidis* and 5 isolates were *Staphylococcus saprophyticus.*

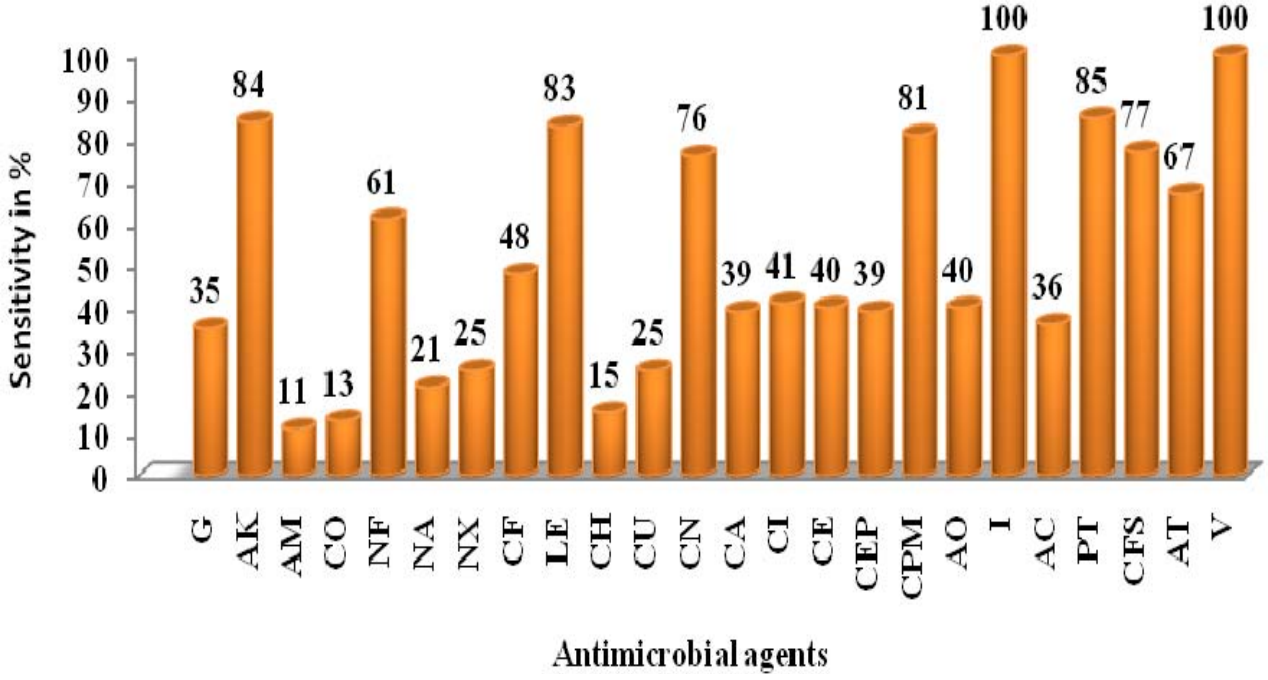
Antimicrobial susceptibility pattern

A total of 150 urine samples were collected and processed for culture and sensitivity assay. The antibiogram revealed that, all the isolated bacteria such as *E.coli, Klebsiella spp, Pseudomonas aurogenosa*, Citrobacter, Enterobacter, Acinetobacter, had a maximum sensitivity pattern to Imipenum (100%) and *Staphylococcus aureus,* CONS and *Enterococcus faecalis* had a maximum sensitivity to Vancomycin(100%) followed by Amikacin (84%), Levofloxaicin (83%), Cefepime (81%), Cefoxitin (76%), Nitrofurantoin (61%) and Ciprofloxacin (48%). Lower sensitivity pattern observed in Ampicillin (11%),

Table 8: Antimicrobial susceptibility pattern (number and percentage)

Sl. No		Escherichia coli (n=85)	Klebsiella spp (n= 30)	Pseudomonas aeruginosa (n=4)	Proteus spp(n=3)	Citrobacter spp(n=2)	Enterobacter (n=2)	Acinitobacter (n=3)	Staphylococcus aureus(n=8)	CONS(n=11)	Enterococcus faecalis(n=2)	Total n =150
1.	Gentamycin (**G**)	32**(38)**	**10(33)**	3**(75)**	1**(33)**	0	0	1**(33)**	2 **(25)**	4 **(36)**	0	53 **(35)**
2.	Amikacin (**AK**)	74**(87)**	26**(87)**	3**(75)**	2 **(66)**	**2(100)**	1**(50)**	2 **(66)**	6 **(75)**	8 **(72)**	0	126 **(84)**
3.	Ampicillin **(A)**	**14(16)**	2**(7)**	0	0	0	0	0	0	0	0	16 **(11)**
4.	Co-trimoxazole(**CO**)	**15(18)**	5**(17)**	0	0	0	0	0	0	0	0	**20 (13)**
5.	Nitrofurantoin(**NF**)	**66(78)**	19 **(63)**	1**(25)**	0	1**(50)**	1**(50)**	2 **(66)**	1**(13)**	1**(9)**	0	92 **(61)**
6.	Nalidixic acid (**NA**)	20 **(24)**	9 **(30)**	0	0	0	0	0	2 **(25)**	1**(9)**	0	32 **(21)**
7.	Norfloxacin (**NX**)	19 **(22)**	9 **(30)**	2 **(50)**	0	1**(50)**	1**(50)**	2**(66)**	1**(13)**	2**(18)**	0	37 **(25)**
8.	Ciprofloxacin (**CF**)	41 **(48)**	14 **(47)**	2 **(50)**	1**(33)**	1**(50)**	2**(100)**	2**(66)**	3**(38)**	6**(54)**	0	**72 (48)**
9.	Levofloxacin (**LE**)	73 **(86)**	26 **(87)**	3 **(75)**	2 **(66)**	2 **(100)**	2 **(100)**	2 **(66)**	5 **(63)**	9 **(82)**	0	124 **(83)**
10.	Cephalexin (**CH**)	15 **(18)**	3 **(10)**	0	0	0	0	0	2 **(25)**	2 **(18)**	0	22 **(15)**
11.	Cefuroxime (**CU**)	19 **(22)**	6 **(20)**	0	0	0	0	0	5 **(63)**	7 **(64)**	0	37 **(25)**
12.	Cefoxitin (**CN**)	69 **(81)**	21**(70)**	4**(100)**	2**(66)**	2**(100)**	2**(100)**	3**(100)**	4**(50)**	5**(45)**	1**(50)**	114 **(76)**
13.	Ceftazidime (**CA**)	33 **(39)**	11 **(37)**	3 **(75)**	3 **(100)**	1 **(50)**	0	1**(33)**	3 **(38)**	3 **(27)**	0	58 **(39)**
14.	Ceftriaxone (**CI**)	34 **(40)**	12 **(40)**	2 **(50)**	3 **(100)**	1 **(50)**	0	2 **(66)**	3 **(38)**	5 **(45)**	0	62 **(41)**
15.	Cefotaxime (**CE**)	37 **(44)**	12 **(40)**	1**(25)**	2 **(66)**	0	0	2 **(66)**	2 **(25)**	5 **(45)**	0	61 **(40)**
16.	Cefpodaxime (**CEP**)	35 **(41)**	11 **(37)**	2 **(50)**	3 **(100)**	0	0	1**(33)**	2 **(25)**	4 **(36)**	0	58 **(39)**
17.	Cefipime (**CPM**)	79 **(93)**	26 **(87)**	3 **(75)**	3 **(100)**	1**(50)**	1**(50)**	1**(33)**	3**(38)**	4**(36)**	1**(50)**	122 **(81)**
18.	Aztreonam (**AO**)	41 **(48)**	11 **(37)**	2 **(50)**	3**(100)**	2**(100)**	0	2**(66)**	n=129			61 **(40)**
19.	Imipenam (**I**)	85**(100)**	30**(100)**	4**(100)**	2**(66)**	2**(100)**	2**(100)**	3**(100)**				129**(100)**
20.	Amoxyclav (**AC**)	33 (**39**)	**13(43)**	0	0	0	0	0	4**(50)**	5**(45)**	1**(50)**	56 **(37)**
21.	Piperacillin-Tazobactum (**PT**)	**74(87)**	24**(80)**	n = 115								98 (**85**)
22.	Cefeperazone-Sulbactum (**CFS**)	**65(76)**	23(**77**)									89 (**77**)
23.	Azithromycin(**A**)	-	-	**n = 21**					5**(63)**	**8(73)**	1**(50)**	14 **(67)**
24.	Vancomycin (**V**)								8(**100**)	11(**100**)	2(**100**)	21 (**100**)

Antimicrobial susceptibility pattern of isolated pathogens



Co-trimoxazole (13%), Nalidixic acid (21%), Cephelexin (15%), Cefurioxime (25%), Norfloxacin (25%) and Gentamicin (35%).

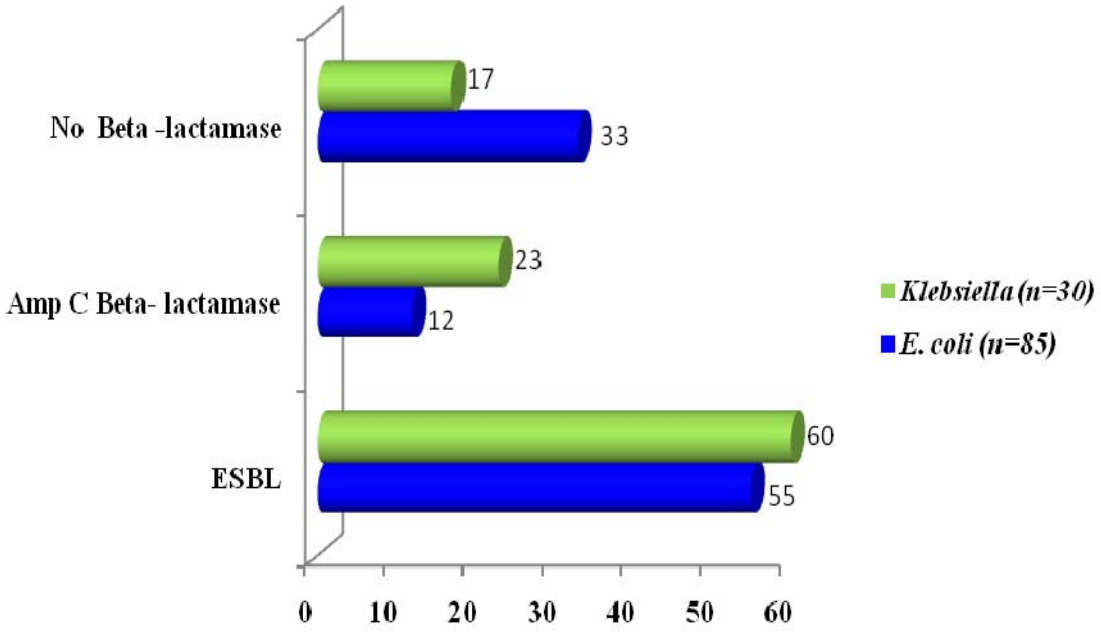
Of the 85 isolates of *E. coli,* 57 showed resistance to anyone of the third generation Cephalosporins (Ceftazidime, Cefotaxime, Ceftrioxone, Cefpodoxime and Aztreonam). Among the 30 isolates of *Klebsiella spp* 25 found resistance to the above drugs (Table.8). The above isolated *E.coli* and *Klebsiella spp* were screened for ESBL production by Double disc synergy test.

Table 9: Prevalence ESBL and Amp C β - lactamase

Organism	ESBL	Amp C β -lactamase	No β -lactamase	Total (%)
*E. coli (*n=85)	47(55%)	10 (12%)	28(33%)	**100**
Klebsiella spp.(n=30)	18(60%)	7 (23%)	5(17%)	**100**
Total (n = 115)	65(56%)	17(15%)	33(29%)	**100**

The NCCLS phenotypic confirmation test and E test revealed that 55% of *E. coli* and 60% of *Klebsiella* isolates were found to be ESBL producer. The rest of the isolates were screened for Amp C β- lactamase production by Disc antagonism test. 12% of *E. coli* and 23% of *Klebsiella* were confirmed Amp C β - lactamase producer by Amp C disc test (Table 9). Among isolated 115 *E. coli* and *Klebsiella* ESBL production was found in 56% and Amp C β- lactamase production was 15%.

Prevalence of ESBL and Amp C Beta- lactamase



In-patient and out-patient wise prevalence of ESBL and Amp C Beta- lactamase

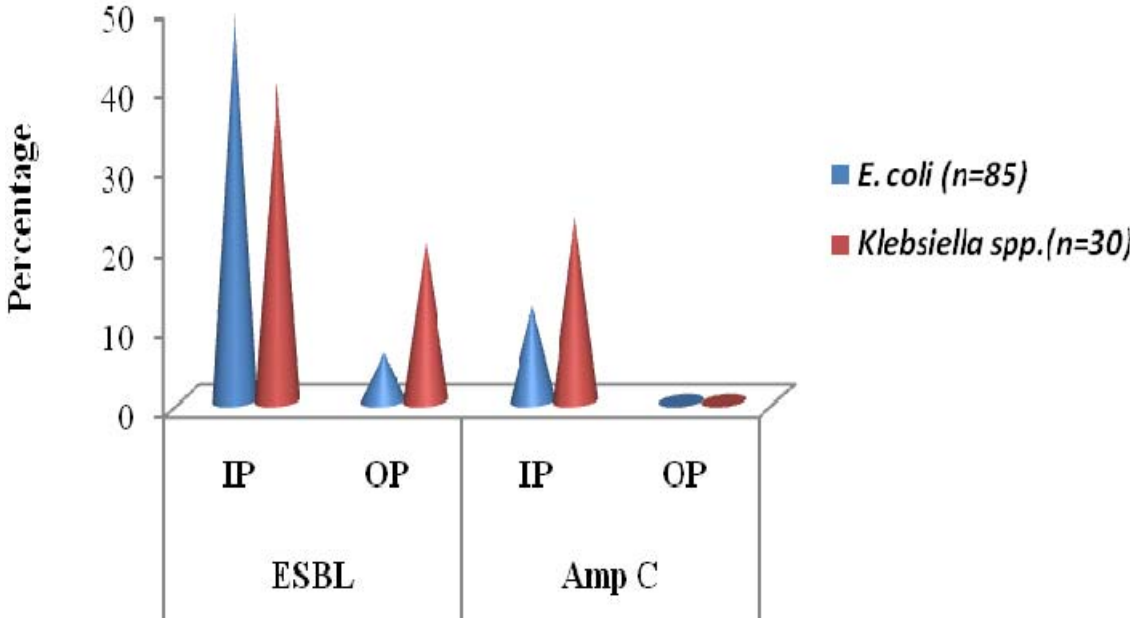


Table 10: Inpatient and Outpatient wise prevalence of ESBL and Amp C β -lactamase

Organism	ESBL		Amp C β -lactamase		No β -lactamase		Total (%)
	IP	OP	IP	OP	IP	OP	
*E. coli (*n=85)	42(49%)	5 (6 %)	10 (12%)	0	23(27%)	5(6%)	**100**
Klebsiella spp.(n=30)	12(40%)	6(20%)	7 (23%)	-	3(10%)	2(7%)	**100**
Total (n= 115)	54(47%)	11(9%)	17(15%)	-	26(23%)	7(6%)	**100**

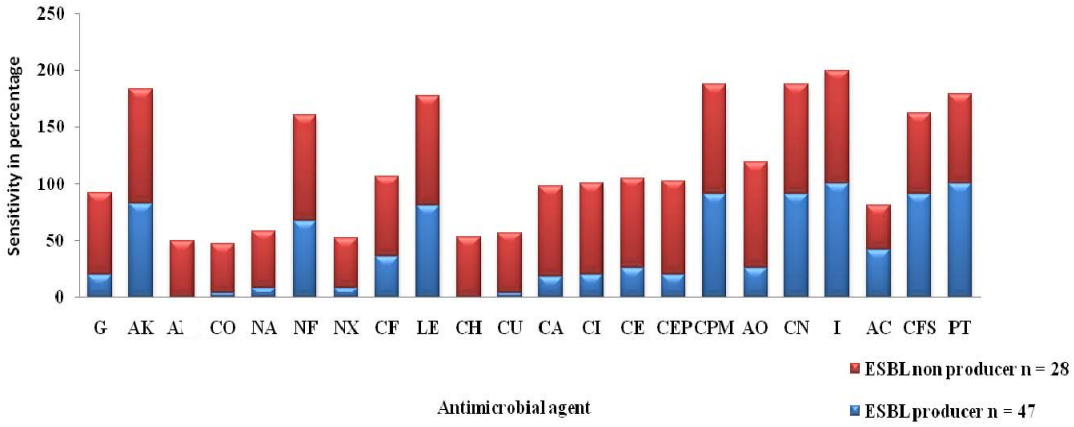
In the present study, among the in-patients, ESBL producing *E. coli* was found to be the most prevalent organism with 49% prevalence followed by *Klebsiella spp* (40%). Whereas, in outpatients *Klebsiella* (20%) was the most prevalent ESBL producing organism followed by *E. coli* (6%). Among the inpatients, the prevalence of Amp C β- lactamase producing *E. coli* and *Klebsiella* 12% and 23% respectively while, in case of outpatient, the Amp C β- lactamase production was nil.

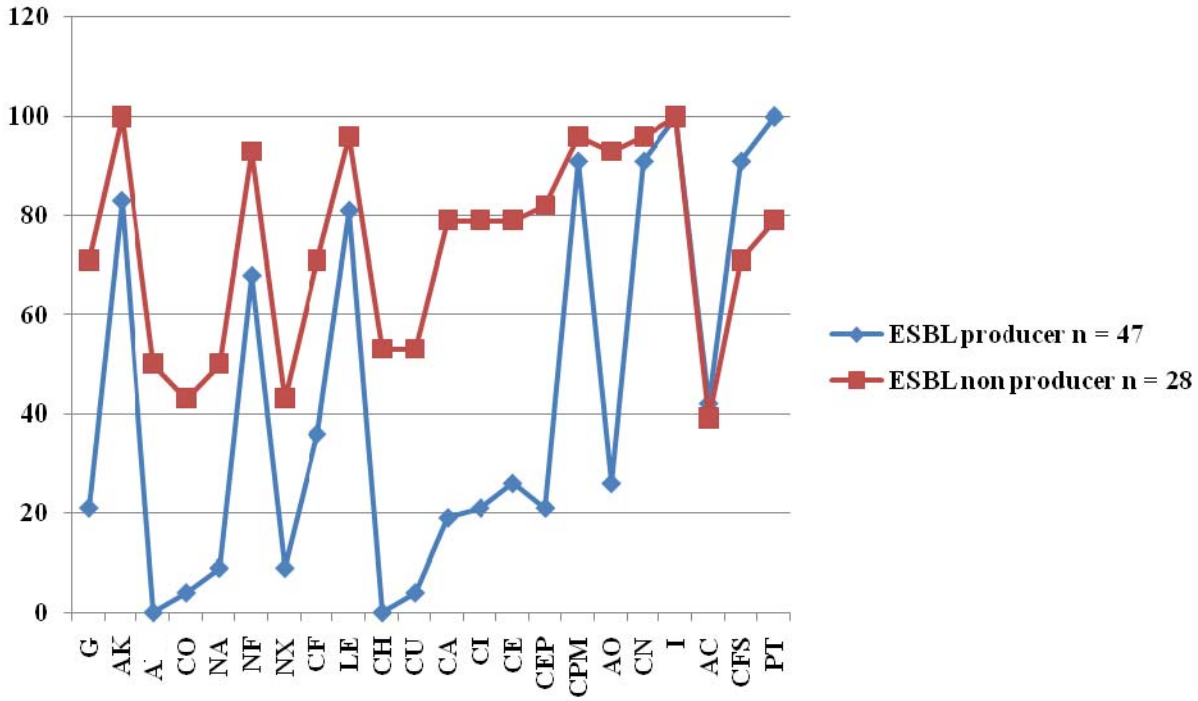
Table 11: Antimicrobial susceptibility pattern of ESBL producer and non ESBL producer among isolated *E. coli* and *Klebsiella spp.*

Organism		G	AK	A	CO	NF	NA	NX	CF	LE	CH	CU	CN	CA	CI	CE	CEP	CPM	AO	I	AC	CFS	PT
E. coli	ESBL producer n =47	10 **(21)**	39 **(83)**	0 **0**	2 **(4)**	32 **(68)**	4 **(9)**	4 **(9)**	17 **(36)**	38 **(81)**	0 **0**	2 **(4)**	43 **(91)**	9 **(19)**	**10** **(21)**	12 **(26)**	10 **(21)**	43 **(91)**	12 **(26)**	47 **(100)**	20 **(42)**	43 **(91)**	47 **(100)**
	ESBL non producer n = 28	20 **(71)**	28 **(100)**	14 **(50)**	12 **(43)**	26 **(93)**	14 **(50)**	12 **(43)**	20 **(71)**	27 **(96)**	15 **(53)**	15 **(53)**	27 **(96)**	22 **(79)**	**22** **(79)**	22 **(79)**	23 **(82)**	27 **(96)**	26 **(93)**	28 **(100)**	11 **(39)**	20 **(71)**	22 **(79)**
	Amp C β Lactamase producer n = 10	2 **(20)**	**7** **(70)**	**0** **(0)**	1 **(10)**	**8** **(80)**	**2** **(20)**	3 **(30)**	**4** **(40)**	**8** **(80)**	0 **(0)**	**2** **(20)**	**0** **(0)**	**2** **(20)**	2 (20)	**3** **(30)**	**2** **(20)**	**9** **(90)**	**3** **(30)**	**10** **(100)**	2 **(20)**	**2** **(20)**	5 **(50)**
Klebsiella spp	ESBL producer n = 18	3 **(17)**	15 **(83)**	0 **0**	2 **(11)**	10 **(56)**	4 **(22)**	4 **(22)**	9 **(50)**	16 **(89)**	0 **0**	3 **(17)**	**16** **(89)**	5 **(28)**	**6** **(33)**	6 **(33)**	5 **(28)**	16 **(89)**	6 **(33)**	18 **(100)**	9 **(50)**	16 **(89)**	17 **(94)**
	ESBL non producer N =5	4 **(80)**	5 **(100)**	2 **(40)**	3 **(60)**	4 **(80)**	3 **(60)**	3 **(60)**	**3** **(60)**	4 **(80)**	2 **(40)**	2 **(40)**	5 **(100)**	4 **(80)**	**4** **(80)**	4 **(80)**	4 **(80)**	5 **(100)**	4 **(80)**	5 **(100)**	2 **(40)**	4 **(80)**	3 **(60)**
	Amp C β Lactamase producer n = 7	3 **(43)**	6 **(86)**	0 **(0)**	0 **(00)**	5 **(71)**	2 **(29)**	2 **(29)**	2 **(29)**	6 **(86)**	1 **(14)**	1 **(14)**	0 **(0)**	2 **(29)**	**2** **(29)**	2 **(29)**	2 **(29)**	5 **(71)**	1 **(14)**	7 **(100)**	2 **(29)**	3 **(43)**	4 **(57)**

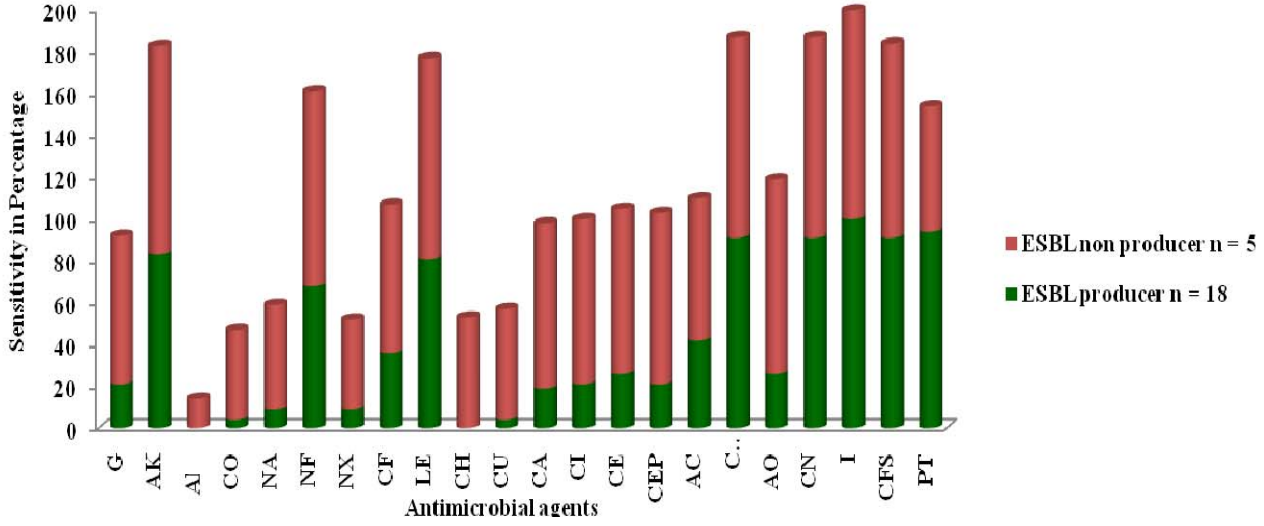
*. G-Gentamicin, AK-Amikacin, A-Ampicillin, CO-Co-trimoxazole, NF-Nitrofurantoin, NA-Nalidixic acid, NX-Norfloxacin, CF-Ciprofloxacin, LE-Levofloxacin, CH-Cephelexin, CU-Cefuroxime, CN-Cefoxitin, CA-Ceftazidime, CI-Ceftriaxone, CE-Cefotaxime, CEP-Cefpodaxime, CPM-Cefipime, AO-Aztreonam, I-Imipenam, AC- Amoxyclav, CFS-Cefeperazone-Sulbactum, PT- Piperacillin-Tazobactum

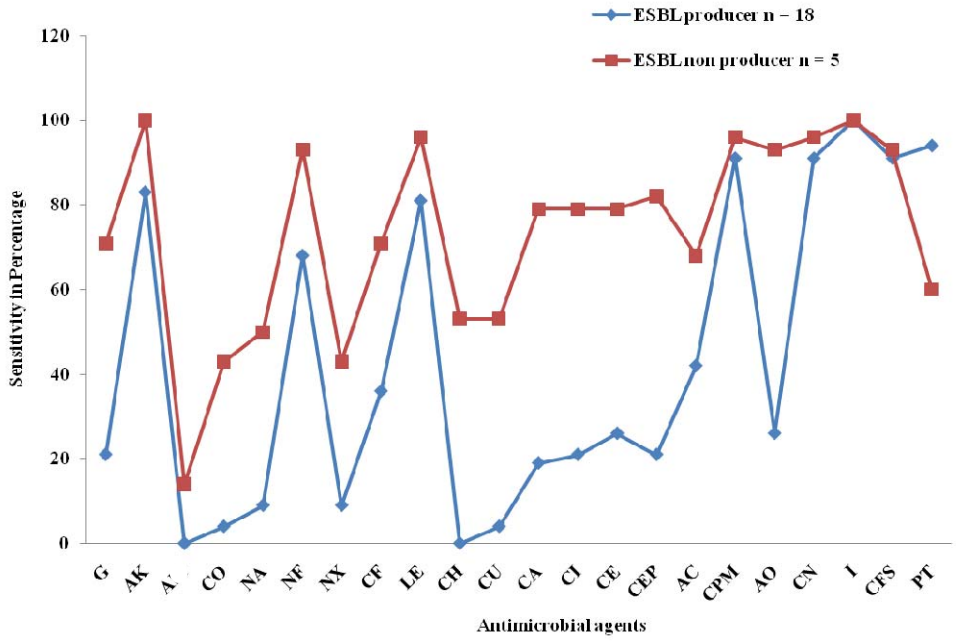
Comparing the sensitive pattern of ESBL producer Vs non ESBL producer (*E. coli*)





Comparing the sensitive pattern of ESBL producer Vs Non ESBL producer (*Klebsiella* spp)





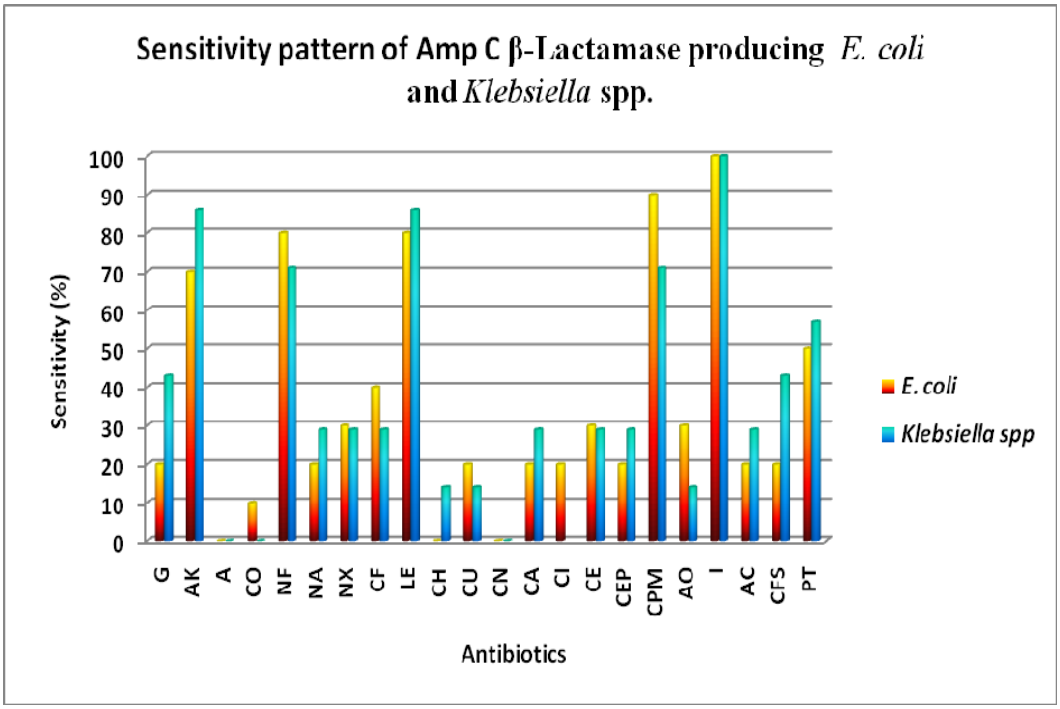
Comparison of the antimicrobial sensitivity pattern of ESBL producer and non ESBL producers

In the present study Antimicrobial resistance pattern in *E. coli, Klebsiella spp* of ESBL producer and non- producer, were compared and presented in the Table 11.

The ESBL producer shows multiple drug resistance than the non- ESBL producers. In case of *E. coli,* sensitivity of Gentamicin is reduced from (71% to 21%). Amikacin shows (17%) reduction in sensitivity. The co-resistance activity was found higher in Co- trimoxazole showing decreased sensitivity from 43% to 4%. Fluroquinolones also showed co-resistant pattern, sensitivity reduced in Nalidixic acid (50 % to 9 %), Norfloxacin (43% to 9%), Ciprofloxacin (71% to 36%) and Levofloxacin (96 % to 81%).

Whereas, in *Klebsiella spp* the highest resistance was found for Gentamicin, sensitivity diminished from (80% to 17%). Other drugs also showed reduction in sensitivity pattern in the following manner Co-trimoxozole (60% to 11%), Nalidixic acid (60 % to 22%), Norfloxacin (60% to 22%) and Ciprofloxacin (60% to 50%).

The antimicrobial susceptibility of ESBL *E. coli and Klebsiella spp* to Imipenem, Piperacillin-Tazobactum, Cefipime, Cefoxitin, Cefeparazone-Sulbactum, Amikacin, Levofloxacin and Nitrofurantoin were 100%/100%, 100%/94%, 91%/89%, 91%/89%, 91%/89%, 83/ 83%, 81/89% and 68/56% respectively.


Sensitivity pattern of Amp C β-Lactamase producing E. coli and Klebsiella spp.
Sensitivity (%)
100
90
80
70
60
50
40
30
20
10
0
G
AK
A
CO
NF
NA
NX
CF
LE
CH
CU
CN
CA
CI
CE
CEP
CPM
AO
I
AC
CFS
PT
Antibiotics
E. coli
Klebsiella spp

The antibiotic of choice for Amp C β- Lactamase producing *E.coli* and *Klebsiella spp* in UTI are Imipenam (100%/100%), Cefipime (90%/71%), Levofloxacin(80%/86%), Amikacin (70%/86%) and Nitrofurantoin (80%/71%).

The sensitivity of Non ESBL producing *E. coli* and *Klebsiella* for different antibiotics was as follows; Imipenam (100/%100%), Amikacin (100%/100%), Levofloxacin (96%/80%), Piperacillin-Tazobactum (79%/60%), Cefipime (96%/100%), Nitrofurantoin (93%/80%), Cefeperazone-Sulbactum (71%/80%), Cefoxiten (96%/100%) Aztreonam (93%/80%) and 3rd generation Cephalosporins like ceftazidine, ceftrioxone, cefotoxime (79%/80%) and cefpodoxime (82%/80%).

DISCUSSION

Urinary tract infections (UTI) are the most common bacterial infection among the humans. Perhaps one of the most important factors impacting the management of UTI over the past decade is emergence of anti microbial resistance among uropathogens.[55] Production of ESBLs and Amp C β-lactamases are the most common mechanism of anti microbial resistance in gram negative bacteria. A prospective study was under taken to know the occurrence of ESBL, Amp C β-lactamases producing strains and their anti microbial susceptibilities to newer agents to guide therapy for urinary tract infection.

During the neonatal period about one per cent of all babies have bacteria in bladder urine then up to three months UTI is more common in male babies after that UTI predominates in females of all age groups. Again after 60 years UTI incidence increases in males[36].

In the present study 500 samples were collected from MSU. Among the samples 150 (30%) showed significant growth of bacteria. According to Chua *et al*. (1988)[56 in] Philippines, the clean-catch midstream urine collection is primarily aimed at avoiding contamination of voided urine by urethral and perineal flora, which might confuse the interpretation of culture results. The normal urethral flora consists primarily of diphtheriods, streptococci and staphylococci[30]. In contrast, Morris *et al*. (1979)[57]concluded that in ambulant adult perineal cleansing before voided urine sample is taken does not influence the bacteriologic finding. In a study, Turner (1961)[58] on pregnant women, demonstrated that vulvar cleansing did not decrease the contamination rate of midstream voided specimen. It is also probable that in some instance, the use of disinfectant and antiseptics in the cleansing procedure might alter and decrease the true bacterial count.

Sex and Age wise prevalence of UTI

Significant bacteriuria showed in 30% of 500 tested samples. Out of 150 bacterial isolates 46% were males and females were 54% with the male to female ratio of 1:1.2. Similar finding (1:1.3) was shown by Baby padmini and Appala Raju (2004)[14] in Tamil Nadu. According to Foxman *et al.* (2003)[35], USA the ratio was 1:4.2. In every age group there was a higher incidence of UTI in adult female than male with an annual incidence of 12.6% in women as compared with 3% among men. Incidence of UTI is higher in females because of the shorter urethra that bacteria have less distance to travel to reach the bladder. In addition, the urethral meatus opens into the moist introitus which is colonized by bacteria have the potential to cause cystitis. Sexual intercourse, pregnancy and post menopausal state also favours occurrence of UTI in females[29].

In the present study, more number of UTI were found in paediatric age group of 41.3%, followed by 33.3% were from reproductive age,15.3% were in middle age and elderly accounts for 10%. Bacteriuria is common in association with UTI in male newborns. When infection occurs in pre-school boys, it is frequently associated with serious congenital abnormalities. The prevalence of significant bacteriuria in this age group is 4.5% for girls and 0.5% for boys. About one third of these patients had some referable urinary tract abnormality when bacteriuria was first detected. The presence of bacteriuria in childhood defines a population at higher risk for development of bacteriuria in adult. ESBL producing *E. coli* may be causative agent of UTI in children without any specific risk factor[59]. The similar findings showed in which the prevalence of bacteriuria in adult men is low (0.1% or less). In young men, a lack of circumcision may also increase the risk for UTI caused by uropathogenic strain of *E. coli* including the development of symptomatic urethritis[60].

Department wise prevalence of UTI

In the present study, the results on the department wise prevalence of UTI found to be highest in paediatric and followed by medicine. But, in the study by Ullal *et al*. (2009)[61] Pakistan, patients from gynaecology contributed maximum number of isolates (42.2%) followed by medicine department as in the case of the present study.

Table 12: Comparision of Common organisms causing UTI

Organisms	**Latin America**[49]	**India**[7]	**Tamilnadu**[78]	**Present study**
E. coli	60.4%	61%	30.2%	57%
Klebsiella	11.1%	22%	22%	20%
Pseudomonas	8.3%	4%	12.35%	3%
Acinetobacter	10%	3%	8.3%	2%
Proteus	4.6%	-	6.7%	2%
Enterobacter	14%	-	35%	1.3%
Citrobacter	7%	2%	2.5%	1.3%
CONS	-	7%	5%	7%
Enterococcus	2.3%	1%	9.5%	1.3%

In the present study, *E. coli* (57%) was the commonest organism isolated followed by *Klebsiella* (20%), *CONS* (7%) and the least isolated was *Citrobacter, Enterobacter, Enterococcus* each accounting 1.3%. The study conducted in India by Akram *et.al* (2007)[7] and in Latin America by Ana.C.Gales *et.al* (1998) [49] showed that the *E. coli* was the commonest organism isolated followed by *Klebsiella.* In this study 7% of CONS were isolated and the same was found in Akram *et.al* (2007) [7]. In the present study, only 1.3% of Enterococcus was isolated, but the study conducted by

Ana.C.Gales *et.al* (1998) [49] showed the highest isolation of about 14% and Ramesh *et.al* (2008) [78] Tamil Nadu showed 9.58% of isolation.

Table 13: Comparison of *E. coli* isolates among UTI in various part of the world

Sl.No.	Name of the Country/ State	*E. coli* (%)	Reference
A	**International**		Sana *et al*. (2005)[63]
1**.**	Israel	94	
1.	USA	90	
2.	Kuwait	90	
3.	England	75	
4.	Sweden	74	
5.	Italy	69	
6.	Japan	65	
B	**National**		
1.	Kashmir	90	Chatterjee *et al*. (2009)[65]
2.	Maharashtra	83.4	Chatterjee *et al*. (2009) [65]
3.	Tamil Nadu	49.3	Baby Padmini and Appalaraju (2004)[14]
4.	New Delhi	46	Mohanty *et al.* (2005)[64]
5.	**Present study**	**57**	

E. coli was the predominant pathogen isolated from patients with community acquired UTI[11, 62]. Among the 500 samples obtained, 150 pathogens were isolated and 129 out of them were Gram negative bacilli were the leading cause of UTI followed by Gram positive cocci with 21 isolates. A higher isolate rate was reported by 61 % reported by Akram *et al*., (2007)[7], but lower isolate rate of 43.5% reported by Sana *et al*., (2005)[63] in Kuwait and 46 % *E. coli* by Mohanty *et al.* (2005) [64] in New Delhi.

While seeing the current status of UTI, in worldwide studies have revealed a preponderance of *E .coli* in urinary isolates 65% in Japan, 69 % in Italy, 74 % in Sweden, 75 % in England up to 90 % in USA and as high as 94 % in Israel.[63]

Antibiotic susceptibility pattern

The most effective antibiotics against all isolates were Imipenam (100%) followed by Amikacin (84%), Levofloxacin (83%), Cefepime (81%), Cefoxitin (76%), Nitrofurantoin (61%) and Ciprofloxacin (48%). However, Akram *et. al*. (2007)[7] from India have reported 100% activity of Imipenem against *E. Coli* and similar findings were also reported by Ullal *et. al*. (2009) from Pakistan[61].

Both Ampicillin and Co-trimoxazole were highly resistant shows only 11% and 13% sensitivity. Studies from USA, Europe and most other countries have shown better susceptibility pattern for pathogens isolated from UTI against Co-trimoxazole[66, 67, 68, 69]. But, in this region of the world Co-trimoxazole has shown poor activity[15, 7]. A reason for this lack of sensitivity may be that in the past, Co-trimoxazole has been extensively used in this region. Among the 85 *E. coli*, 70 (82.4%) strains were resistant to Co-trimoxazole. Hence, Co-trimoxazole cannot be recommended as an empiric therapy for the treatment of UTI in India. Rest of the antibiotics were resistant to all the isolated pathogen by 50% and more.

Prevalence of ESBL production

The NCCLS phenotypic confirmation test and E test revealed that out of 85 isolates of *E. coli,* 47 (55%) were found to be ESBL producer, out of 30 isolates of *Klebsiella,* 18 (60%) were found to be ESBL producer.

Whereas, lower occurrence of ESBL producer in urinary isolates of *E. coli* and *Klebsiella* was found to be 40 and 41 % respectively as reported by Baby Padmini and Appalaraju, 2004[14] Coimbatore.

This was higher than the reported figures of *E. coli* and *Klebsiella* in USA (2.2/6.6%), Canada (2.7/ 6.2%) and India (24.7 /38.5%) by Sumeeta *et al*., (2002)[70] Much higher prevalence of ESBL producers (*E. coli* 60% and *Klebsiella* 44%) in urinary isolates of Gram negative bacilli was observed in Norway by Toftelan (2007)[71] and 70% of *E. coli* and 54.5% *Klebsiella* was observed in India (Purva *et al*., 2002)[72]. Study conducted at Mangalore by Beena Antony *et al*. (2010)[73] showed the prevalence of 70% and 75% of *E. coli* and *Klebsiella spp* respectively producing ESBL.

Table 14: ESBL production among *E. coli* and *Klebsiella* from various geographical areas

S.No	Geographical area	*E. Coli* (%)	*Klebsiella* (%)	Reference
1.	USA	2.2	6.6	Sumeeta *et al*., 2002[70]
2.	Canada	2.7	6.7	Sumeeta *et al*., 2002[70]
3.	Norway	60	44	Sumeeta *et al*., 2002[70]
4.	Europe	5.3	22.6	Calbo et al., 2006[74]
5.	Pakistan	51	40	Mumtaz, 2006[75]
6..	India	70	54.5	Singhal *et al*.,20[76]
7..	Manipal	35	41	Shoba *et al*.,2007[77]
8.	Mangalore,	70	75	Beena Antony *et al*., 2010[73]
8.	Nagpur	34	25.6	Tankiwale *et al*., 2004[15]
9.	Kerala	62.3	67.4	Sashikala *et al*., 2010[48]
10.	Tamil Nadu	41	40	Baby Padmini and Appalaraju, 2004[14]
11.	**Present study**	**55**	**60**	

Prevalence of Amp C β- lactamase

In the present study, the prevalence of Amp C β- lactamase producer was lesser among *E. coli* (12%) as compared to the 23% prevalence among *Klebsiella*. This is in accordance with the findings of Ramesh *et al* (2008)[78] from Tamil Nadu who had recorded lesser prevalence of Amp C β- lactamase in *E. coli* (9.9%) and more among *Klebsiella (*31.1%). In contrast, under Mangalore conditions Beena Antony *et al*. (2010)[37] recorded much higher production of Amp C β- lactamase among *E. coli* (15.4 %) than in *Klebsiella* (5.4 %). However, Singhal *et al*. (2005)[76] recorded almost equal production of Amp C β- lactamase both among *E. coli* (6.97%) and *Klebsiella (*6.18%).

Inpatient and outpatient wise prevalence of ESBL and Amp C β- lactamase

In the present study, among the in-patients, ESBL producing *E. coli* (49%) was found to be most prevalent organism followed by *Klebsiella spp* (40%). While in Outpatients *Klebsiella* (20%) was the most prevalent ESBL producing organism, followed by *E. coli* (6%). A similar finding was observed by Mumtaz (2006) in Pakistan[75]. According to Calbo *et al*. (2006) in Europe, prevalence of infection due to ESBL producing *E. coli* in UTIs increased from 0.4 % to 1.7 % in the span of 3 years. Community onset ESBL producing *E. coli* infection shifted from 50% too 79.5 % within the period of 3 years from 2000 to 2003[74]. According to Pena *et al*., 2006 from Barcelona, Spain, 68% of the hospitalized patients develop infection, yielding one or more clinical isolates of ESBLs producing *E. coli*. A significant increase was observed the incidence of ESBL producing *E. coli* colonization or infection during the study period[21].

Another study showed that among hospitalized patients, the most prevalent ESBL producer was *Klebsiella* followed by *E. coli.* A study by Luzzaro *et al*. (2006) in Italy and Lin *et al*. (2006) in Taiwan, showed that ESBL producing *E. coli* were 16.3 % from Outpatients[79,80].

Among the inpatients Amp C β- lactamase producing E. *coli* was 12% and *Klebsiella* was 23% but in case of out patient Amp C β- lactamase production was found to be nil. Singhal *et al*., 2005[76] also reported that in outpatients, out of 74% of *Klebsiella* and 62% of *E. coli* UTI isolates, the Amp C β- lactamase production was nil. It has been reported that at present in India Amp C β- lactamase harbouring isolates are largely restricted to the hospitalised patients only[76].

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates varies greatly worldwide and in geographical areas and are rapidly changing over time[81].

Co-resistance pattern of ESBL producers

The ESBL producer shows multiple drug resistance than the non- ESBL producers. In case of *E. coli,* sensitivity of Gentamicin is reduced from 71 % to 21 %. Whereas Amikacin shows 17% reduction in sensitivity. The co-resistance activity was found higher in Co- trimoxazole showing decreased sensitivity from and for *E. coli* (43 % to 4 %). Fluroquinolones also shows co- resistance pattern, Sensitivity reduced in Nalidixic acid (50% to 9%), Norfloxacin (43% to 9%), Ciprofloxacin (71 % to 36 %) and Levofloxacin (96 % to 81%). Whereas, in *Klebsiella spp* the highest resistance was found in Gentamicin, sensitivity diminished from (80% to17 %). Other drugs shown to be resistance are Co-trimoxazole (60% to 11%), Nalidixic acid (60% to 22%), Norfloxacin (60% to 22%) and Ciprofloxacin (60% to 50%).

Genes coding for resistance to β - Lactam antibiotics and Quinolones are located on the same plasmid and thus passed on together among different Enterobacteriaceae, in addition loss of outer membrane proteins (or) efflux pump over expression plays a major role in co-resistance[82].

Genes that encode resistance to Aminoglycosides and Co- trimoxazole are located on a wide range of genetic elements such as class 1, 2, and 3 integrons or transposable elements have been associated with different multidrug resistant ESBL plasmids from human and animal origin[74].

In contrast, at Mangalore higher resistance was observed in Co- trimoxazole (84 -100%), Gentamicin (40 – 93%), Ciprofloxacin (91 – 100%) by Beena Antony *et al*. (2010)[73]. In our study, the resistance to Fluoroquinolones varied from 9 – 41% for various organisms. In contrast, 27.6 to 90% of resistance was observed at Bangalore by Mahesh *et al*. (2010). Gordon *et al*. (2003)[83, 68] reported Quinolones as the most active against UTI pathogens in North America. Peterson *et al*. (2007) have reported a resistance rate of 5- 20% in their study in USA[84].

In our study, 32% of the *E. coli* and 33% *Klebsiella* isolates only sensitive to Gentamicin. The co-resistance was comparatively low for Amikacinf (17%). Similar findings were observed by Baby Padmini and Appalaraju (2004)[14].

Co-Resistance was high in Amp C β- Lactamase producers than ESBL producers. Among the Amp C β- Lactamase producers, the Aminoglycosides showed the highest resistance followed by Cotrimoxazole and fluroquinolones.

Sensitivity to β - Lactam and β - Lactamase inhibitor in ESBL producers

In the present study, the ESBL producing *E. coli* and *Klebsiella* showed 100% and 94% sensitivity to Piperacillin-Tazobactum respectively followed by 91% and 89%

sensitivity against Cefeperazone-Sulbactum. A similar finding was reported by Shasikala *et al*. (2010)[48] in Kerala that *E. coli* exhibited 96.80% and 93.3% sensitivity to Piperacillin-Tazobactum and Cefeperazone-Sulbactum.

Whereas in Mangalore, Shigu e*t al*. (2010)[85] showed 100% sensitivity of the ESBL producing *E. coli* and 98% and 88% sensitivity of the ESBL producing *Klebsiella* to Piperacillin-Tazobactum and Cefeperazone - sulbactum respectively.

Antibiotic of choice for ESBL producing *E. coli* and *Klebsiella spp*

The present study revealed that the most effective antibiotic against ESBL producing *E. coli*, and *Klebsiella spp*. in UTI are Imipenam (100/100%), Piperacillin-Tazobactum (100%/94%), Cefipime (91%/89%), Cefeperazone-sulbactum (91%/89%), Cefoxiten (91%/89%) Amikacin (83%/83%), Levofloxacin (81%/89%) and Nitrofurantoin (68/%56%). Baby Padmini and Appalaraju, (2004)[14] and Tankhiwale, (2004)[15] suggested Imipenam (100%), Nitrofurantoin (89%) and Amikacin (86%) is the drug of choice for ESBL producers which are similar to this study.

Study conducted in Kerala by (Shasikala *et al*., 2010) showed Piperacillin-Tazobactum (96.8%), Cefeperazone-sulbactum (92.2%), were sensitive to ESBL producers[48].

Antibiotic of choice for Amp C β- Lactamase producing *E. coli* and *Klebsiella spp*

The antibiotic of choice for Amp C β- Lactamase producing *E. coli* and *Klebsiella spp* in UTI are Imipenam (100/100%), Cefipime (90%/71%), Amikacin (70%/86%), Levofloxacin (80%/86%) and Nitrofurantoin (80%/71%). It is comparable with Neelam Taneja (2006) India[12] and Zanetti *et.al (*2003) [86] both of them suggests carbapenam are more effective than cefipime. Amp C β- Lactamases are clinically

important because they confer resistance to Extended spectrum Cephalosporins,Cephamycins,Monobactums β - Lactam and β - Lactamase inhibitor combinations[12].

Imipenam resistant organisms were isolated in different hospitals in India which was widely discussed as Super bug in press and parliament (Lancet, 2010)[87]. During the present study, no resistance was noticed to Imipenam. Imipenam should be used as the second line of drug to prevent further resistance and rest of the drugs can be used as the first line of choice. The routine susceptibility testing by clinical laboratories fail to detect ESBL positive strains and shows false invitro sensitivity to Cephalosporins. Screening for ESBL and Amp C β- Lactamase production as a routine procedure in clinical laboratories gives valuable information to the clinician in appropriate selection of antibiotics.

Limitation of the study

1. Single centred study
2. Follow up of cases not done
3. Due to technical constraints the studies were limited to phenotypic methods only.

Strength of the study

1. Strict criteria for case selection
2. Samples were collected and processed by single person
3. Processing was carried out within shortest time.
4. Quality control maintained

Suggestions

1. Avoiding indiscriminate use of antibiotic by ensuring that the indication for, and the dose and duration of treatment are appropriate,
2. Restricting the use of antimicrobial combinations to appropriate circumstances,
3. Constantly monitoring the resistance patterns in a hospital or community and change recommended antibiotics used for empirical treatment and limiting the newest group of antimicrobials as long as the current drugs are effective.
4. Maintenance of infection control in hospitals, such as the isolation of carriers, hand hygiene practices for ward staff prevent the spread of resistant bacteria.
5. Restricting the use of catheter, if needed it should be carried out with all strict aseptic precaution.
6. Conducting periodic educational programme on antimicrobial use for the practitioner in order to limit the use and to minimize the antibiotic resistance

SUMMARY AND CONCLUSION

The study was conducted at Thanjavur Medical College Hospital, Thanjavur over a period of one year from 1st August 2009 to 30th July 2010 with 500 patients suffering from UTI, which included 49 % of males and 51 % of females. Among 500 patients 150 (30%) of them had significant bacteriuria. UTI was higher in paediatric age groups 41.3% followed by reproductive age group (33.3%).

- In this study, 129 Gram Negative Bacilli and 21 Gram Positive Cocci were isolated among which *E. coli* (57%) was the commonest organism isolated followed by *Klebsiella spp* 20%, *Pseudomonas aeruginosa* 3%, Proteus 2%, Enterobacter and *Citrobacter Koseri* each accounting1.35 % and Acinetobacter 2%. Among the Gram Positive cocci, 7% were CONS, 5% were *Staphalococcus aureus* and 1.3% were *Enterococcus faecalis*.
- All the Gram negative bacilli were mostly sensitive to Imepenam (100%) and Gram positive cocci were sensitive to vancomycin (100%) followed by Amikacin (84%), Levofloxacin (83%), Cefipime (81%), Cefoxitin (76%), and Nitrofurantoin (61%).
- Prevalence of ESBL production was found in 55 % of the *E. coli* and 60 % of the *Klebsiella spp.*
- Prevalence of Amp C β lactamase production was 12 % in *E.coli* and 23% in *Klebsiella spp.*
- ESBL producing strains showed multiple drug resistance than the non ESBL production. The ESBL producing *E. coli and Klebsiella* showed highest resistance to Gentamicin, Co-trimoxazole, Nalidixic acid, Norfloxacin and

Ciprofloxacin. Low level of resistance was seen in Nitrofurantoin, Amikacin and Levofloxacin.

- The sensitivity pattern of ESBL *E. coli and Klebsiella spp* are Imipenem (100 %/100%), Piperacillin-Tazobactum (100%/94%), Cefipime (91%/89%), Cefoxitin (91%/89%), Cefeperazone-Sulbatum (91%/89%), Amikacin (83%/83%), Levofloxacin (81% / 89%) and Nitrofurantoin (68% / 56%).

- The antibiotic of choice for Amp C β Lactamase producing *E. coli* and *Klebsiella spp* in UTI are Imipenam (100%/100%), Cefipime (90%/71%), Amikacin (70%/86%), Levofloxacin (80%/86%) and Nitrofurantoin (80%/ 71%).

Even though all the isolates are 100% sensitive to Imepenam, it should be kept in reserve as the second line of drug. Other drugs which is most economic and orally effective like Nitrofurantoin and Levofloxacin can be given to outpatients. Amikacin, Cefipime and β lactamase Inhibitors Piperacillin-Tazobactum, Cefeperazone- Sulbatum can be given to inpatients.

Based on our study, we conclude that, there is a high prevalence rate of ESBL and Amp C β- Lactamase production seen among uropathogenic *E. coli* and *Klebsiella spp*. Based on the prevalence rate of the ESBL and Amp C β- Lactamase production in a health care facility, institutional antibiotic policy can be tailored to achieve superior therapeutic outcome and bring about a reduction in healthcare costs. It also eliminates misuse of conventional cephalosporin in a significant proportion of patients. Drug resistance pattern varied from place to place which may be related to the nature of the pathogen and preferred antimicrobial agents.

ANNEXURE

LIST OF TABLES

LIST OF PLATES

INFORMED CONSENT

I have been informed about the study of Urinary Tract Infection. I totally agree to participate in the study, as I realize the importance of the study. I also aware that I can withdraw from the study whenever like.

Date : Signature of the patient

Department :

PROFORMA

Name:	Serial No:
Age:	Lab No:
Sex:	OP/IP No:
Education:	D.O.A:
Occupation:	D.O.D:
Income:	Provisional Diagnosis:
Address:	

Chief complaints:

Fever

Dysurea

Frequency

Urgency

Lower abdominal/ flank pain

H/O Present illness:

Associated conditions- instrumentation/ surgery in urinary tract

Calculi

Diabetes mellitus

Chronic kidney and liver diseases

Benign Prostatic Hypertrophy

Pregnancy

Immuno compromised state

Treatment History: H/O anti biotic intake, duration

Past History: H/O Similar episode in the past

Instrumentation/ surgery in urinary tract

Family History:

Personal History:

General Examination: Stature, nourishment, anaemia, jaundice, cyanosis, clubbing, lymphadenopathy, pedal edema.

Vital signs: Temperature, pulse rate, respiratory rate, blood pressure.

Systemic examination: Abdomen

Inspection: shape of the abdomen

Position of the umbilicus

Movements of the abdominal wall

Skin and surface of the abdomen

Palpation : Mass

Tenderness (Suprapubic)

Rigidity

Organomegaly

Percussion : Any free fluid

Auscultation : Bowel sounds

Bruit

Examination of groin and genital region

P/V:

P/R:

Examination of other systems

CVS:

RS;

CNS:

Definitive Diagnosis

WORKSHEET

Specimen: Urine

Method of collection : MSU/Indwelling catheter/Cystoscope/Suprapubic aspiration

I. Macroscopic Examination: Color

Turbidity

II. Microscopic Examination: Wet mount

Gram staining

III. Culture : Nutrient agar

MacConkey agar

Blood agar

IV. Biochemical Reactions:

Gram staining :

Motility :

Catalase :

Oxidase :

Sugar fermentation tests :

IMViC :

Urease :

TSI :

LAO : Special Tests:

Coagulase :

Micro organism isolated :

V. Anti Microbial Susceptibility test:

VI. Screening for ESBL : 1. Antibiogram

2. Double disc synergy test

VII. Conformation of ESBL : 1. Double disc potentiation

2. E Test

Screening for Amp C β-lactamases: 1. Cefoxitin resistant strains

2. Double disc antagonism test

Confirmation for Amp C β-lactamases: Amp C disc test

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