STUDY OF PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITIES OF BACTERIA AND FUNGI ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN PATTUKKOTTAI, TAMIL NADU, INDIA

Thesis submitted to
BHARATHIDASAN UNIVERSITY
In partial fulfillment of the requirement
For the award of degree of
DOCTOR OF PHILOSOPHY IN ZOOLOGY

Submitted By
C. MANIKANDAN M. Sc., M. Phil., B.Ed.,

Under the Guidance of
Dr. A. AMSATH, M. Sc., M. Phil., Ph. D.

P. G. AND RESEARCH DEPARTMENT OF ZOOLOGY
KHADIR MOHIDEEN COLLEGE
ADIRAMPATTINAM- 614 701

DECEMBER-2013

Dr. A. AMSATH, M. Sc., M. Phil., Ph. D.,
Associate Professor and Research Advisor
P.G. and Research Department of Zoology,
Khadir Mohideen College,
Adirampattinam – 614 701.
Tamil Nadu, India

Date:……

CERTIFICATE

This is to certify that the thesis entitled, "**Study of prevalence and antimicrobial susceptibilities of bacteria and fungi isolated from patients with urinary tract infections in Pattukkottai, Tamil Nadu, India**" is a record of research work done by the candidate **Mr. C. Manikandan,** during the year 2011-2013 submitted to Bharathidasan University, Tiruchirappalli in partial fulfillment of the requirements for the award of Degree of **Doctor of Philosophy in Zoology** under my supervision and that the thesis has not been submitted earlier for the award of any Degree anywhere.

DECLARATION

I hereby declare that the thesis entitled "**Study of prevalence and antimicrobial susceptibilities of bacteria and fungi isolated from patients with urinary tract infections in Pattukkottai, Tamil Nadu, India**" is a record of research work done by me in the Post Graduate and Research Department of Zoology, Khadir Mohideen College, Adirampattinam under the supervision of **Dr. A. AMSATH,** during the year 2011-2013 submitted to Bharathidasan University, Tiruchirappalli in partial fulfillment of the requirements for the award of Degree of **Doctor of Philosophy in Zoology** and that the thesis has not been submitted earlier for the award of any Degree anywhere.

Station: Adirampattinam.

Date : (C.MANIKANDAN)

ACKNOWLEDGEMENTS

I give thanks to almighty GOD for his providence, grace and mercy upon my life, and for His divine enablement without Him nothing would have been possible.

In the first place I would like to record my gratitude to Dr. A. AMSATH, M.Sc., M.Phil., Ph.D., Associate Professor of Zoology, Khadir Mohideen College, Adirampattinam for suggesting the problem, supervision, advice, guidance and encouragement from the very early stage of this research as well as giving me extraordinary experiences throughout the work. His truly scientist intuition has made him as a constant oasis of ideas and passions in science, which exceptionally inspire and enrich my growth as a student, a researcher and a scientist want to be. I am indebted to him more than he knows.

My heartiest thanks to honorable Prof. Dr. A. JALAL, M.Com., M.Phil., Ph.D., Principal, Khadir Mohideen College, Adirampattinam for his kind support for completing this Research in a successful manner. I would to express my thanks to honorable Prof. Dr. A. UDUMAN MOHIDEEN, M.Sc., M.Phil., Ph.D., Vice Principal, Khadir Mohideen College, Adirampattinam for his encouragement during the study period.

Words are insufficient to express my deep sense of gratitude and heartfelt thanks to Honorable Prof. Dr. P. KUMARASAMY, M.Sc., M.Phil., Ph.D., HOD and Associate Professor of Zoology,

Khadir Mohideen College, Adirampattinam for having permitted me to pursue my research work and for having given me the opportunity and necessary facilities for my research programme in the college of excellence.

I wish to express my special thanks to Prof. Dr. S. RAVEENDRAN, M.Sc., M.Phil., B.Ed., BLIS., Ph.D., Associate Professor and Dr. O. SATHIK, M.Sc., M.Phil., Ph.D., Assistant Professor, Doctoral committee members, Department of zoology, Khadir Mohideen College, for their immense help for completing my task.

I sincerely express my heart full thanks to Dr. P. PRABHAHARAN, M.Sc., M.Phil., Ph.D., Assistant Professor, Department of Biotechnology, Srimad Andavan Arts and Science College, Thiruchirapalli for his assistance to publish the research work.

Words are inadequate in offering my thanks to Dr. A. MOHAMED HATHA, M.Sc., M.Phil., Ph.D., HOD of Environmental sciences, Cochin University of Science and Technology, Cochin, who has spent their valuable time to help me do molecular work.

I express my sincere thanks to Dr. R. SUBHASHKUMAR, M.Sc., M.Phil., Ph.D., project convener, Department of Microbiology, Kongu Nadu Arts and Science College, Coimabatore and Dr. T. THAUMANAVAN, M.Sc., M.Phil., Ph.D., Department

of Microbiology, Assistant Professor of Microbiology, SNR College, Coimbatore, for their friendly guidance and help

It is my privilege to thank Dr. P. BASKARAN MS., Medical officer, Government Hospital, Pattukkottai, Dr. P. SRINIVASAN MBBS, D.Ortho., Devasena Hospital, Dr. M. GUNASEKARAN MD., Thiravidan Hospital, Pattukkottai, Dr. M. AYYASAMY MBBS., DCH, Dr. S. SURIYAMOORTHY MBBS., DCH., and Dr. T. NEELAVATHI MBBS., DGO., Janaki Hospital, Dr. ADR. ANBARASAN MS., and Dr. A. MAHESHWARI MBBS. DGO., GRAM Hospital, Dr. T. DEVI MD., DGO., Sangeetha hospital, Dr. R. IGBAL SHERIFF MBBS.,DNB., Dr. A. MAHALINGAM MD., Dr., C. CHINNADURAI MS., Elangovan Hospital, Dr. C. TAMILSELVAN MD., DCH., Dr. R.RAJAGOPAL MS., (URO), Ln.U.POTHIYAPPAN B.Sc., DMLT, Jayam Clinical Laboratory, Pattukkottai for their prompt help in sample collection and cooperation at various phases of the experimental work.

My sincere thanks will also extend to all members of Zoology Department, Khadir Mohideen College for exchanging knowledge and experiences and assisting me during studies.

Words fail me to express my appreciation to my wife M. MAHALAKSHMI, B.Sc., B.Ed., MCA., M.Phil., for her dedication, love and persistent confidence in me, has taken the load off my shoulder. I owe her for being unselfishly let her intelligence, passions, and ambitions collide with mine. I express my lovable thanks to my elder son M. SHANMUGAPRAKASH, my younger

son M. POKUTTEZHINI for their cooperation during my study Period.

I would like to thank the Laboratory Technologists C. SUGANYA B.Sc., DMLT, B. DHANALAKSHMI B.Sc., DMLT and R. SATHYA B.Sc., DMLT of Gangasaras Diagnostic and Research Centre, Pattukkottai for the facilities provided to carry out the work and their consistent support and help in this study.

I express my heartfelt thanks to my friends for their help and wishes for the successful completion of this project.

I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Last, but not the least, I am very much grateful to all patients without whom is study would not have been completed.

C. Manikandan

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
1	INTRODUCTION	1
	Objectives of the study	20
2	REVIEW OF LITERATURE	22
3	MATERIALS AND METHODS	67
3.1	Study of prevalence of urinary tract infections (UTIs)	67
3.2	Isolation and identification of bacteria causing UTIs.	69
3.3	Antibacterial susceptibility testing	86
3.4	Isolation and Characterisation of the pathogenic *Candida* species	90
3.5	Antifungal susceptibility testing	91
3.6	Plasmid analysis- molecular characterisation of ESBL producing *E.coli*	93
4	RESULTS	95
4.1.	Study of the prevalence of UTIs	95
	4.1.1. Study population	95
	4.1.2. Distribution of uropathogens according to age groups and gender	95

4.2.	Isolation and identification of pathogenic bacteria from UTIs	97
	4.2.1. Sources of isolated strains with percentage	97
	4.2.2. Correlation between pyuria, Gram's stain and culture in UTIs	98
	4.2.3. Uropathogens identified on Chromagar media according to pigment reactions	101
	4.2.4. Distribution of isolates among positive urine specimens	106
4.3.	Antibacterial susceptibility pattern of bacterial isolates from the UTIs	107
	4.3.1. Characterisation and susceptibility pattern of *E.coli*	107
	4.3.2. Characterisation and susceptibility pattern of *Klebsiella pneumoniae*	113
	4.3.3. Characterisation and susceptibility pattern of *Pseudomonas* spp.	115
	4.3.4. Characterisation and susceptibility pattern of *Proteus* spp.	117
	4.3.5. Characterisation and susceptibility pattern of *Enterobacter* spp.	119
	4.3.6. Characterisation of Gram Positive Bacteria	121
	4.3.7. Antibiotic susceptibility pattern of CoNS	121
	4.3.8. Antibiotic susceptibility pattern of *Enterococcus* spp.	123
	4.3.9. Antibiotic susceptibility pattern of *Staphylococcus saprophyticus*	124
	4.3.10. Antibiotic susceptibility pattern of *Staphylococcus aureus*	125

		4.3.11. Antibiotic resistance patterns of all Bacterial isolates from UTIs	127
		4.3.12.Aminoglycoside resistance in bacterial isolates from UTIs	127
		4.3.13.Beta-lactam group of antibiotics resistance in bacterial isolates from UTIs	129
		4.3.14.Fluoroquinolone resistance in bacterial isolates from UTIs	130
		4.3.15.Two stand-alone antibiotics resistance in bacteria from UTIs	130
	4.4	Isolation and characterisation of the pathogenic *Candida* species	131
	4.5	Antifungal susceptibility pattern of *Candida* species causing UTIs	134
	4.6	Plasmid analysis-molecular characterisation of ESBL producing *E.coli*	137
		4.6.1. ESBL Positive *E.coli*	137
		4.6.2. PCR and RFLP analysis	138
5	DISCUSSION		140
6	SUMMARY AND CONCLUSION		158
	REFERENCES		162
	APPENDIX 1: LIST OF PAPER PUBLICATIONS		
	APPENDIX 2: LIST OF PAPER PRESENTATIONS		

LIST OF TABLES

Table No	CAPTION	Page No
1.	Pathogens in complicated and uncomplicated UTIs	27
2.	Antimicrobial drugs and their sensitivity profiles	89
3.	Result of urine culture among study population	95
4.	Distribution of urine pathogens according to age groups and gender.	96
5.	The sources of isolated strains from UTIs	97
6.	Correlation between pyuria, Gram's stain and culture in UTIs	99
7.	Urine isolates presumptively identified on Chromagar media	101
8.	Identification of uropathogens by Gram's staining, SBA and MA agar.	103
9.	Biochemical identification of bacteria isolated from UTIs	105
10.	Frequency of bacterial isolates from urine samples	106
11.	Antibiotic susceptibility pattern of *E. coli*	109
12.	Antibiotic susceptibility pattern of *K.pneumoniae*	114
13.	Antibiotic susceptibility pattern of *Pseudomonas* spp.,	116
14.	Antibiotic susceptibility pattern of *Proteus* spp.,	118

15.	Antibiotic susceptibility pattern of *Enterobacter* spp.,	120
16.	Antibiotic susceptibility pattern of CoNS	122
17.	Antibiotic susceptibility pattern of *Enterococcus* spp.,	123
18.	Antibiotic susceptibility pattern of *Staphylococcus saprophyticus*	125
19.	Antibiotic susceptibility pattern of *Staphylococcus aureus*	126
20.	Antibiotic resistance pattern of bacteria isolated from urine samples	128
21.	Characteristics of *Candida* species on Chromagar *Candida* media	131
22.	Distribution of isolated *Candida* species	132
23.	Identification of isolated *Candida* species by Direct wet mount and Germ tube methods	133
24.	Antifungal susceptibility pattern of *Candida* species causing UTIs	135

LIST OF FIGURES

Figure No	CAPTION	Page No
1.	Route of urinary tract infections	24
2.	Predisposing factors for complicated UTIs	25
3.	Distribution of symptomatic urinary tract infection and prevalence of asymptomatic bacteriuria by age and sex	30
4.	Urinary system of women and men	37
5.	The sources of isolated strains from UTIs	98
6.	Isolation of bacteria on Nutrient agar and isolation of *Candida* species on Sabouraud dextrose agar	99
7.	Gram's staining, Direct microscopy, Germ tube test	100
8.	Identification of bacterial species on Chromagar media	102
9.	Identification of bacteria on MacConkey and Blood agar	102
10.	Identification of bacteria through biochemical testing and selective media	104
11.	Antibiotic resistance of *E. coli* strains from patients with UTIs	109
12.	Antibiotic **s**usceptibility pattern of bacteria isolated from UTIs	110
13.	Antibiotic **s**usceptibility pattern of bacteria isolated from UTIs	111
14.	Antibiotic susceptibility pattern of *Proteus* spp., and MDR *E.coli* isolated from UTIs	112

15.	Antibiotic resistance of *K.pneumoniae* from patients with UTIs	114
16.	Antibiotic resistance of *Pseudomonas* strains from patients with UTIs	116
17.	Antibiotic resistance of *Proteus* strains from patients with UTIs	118
18.	Antibiotic resistance of *Enterobacter* strains from patients with UTIs	119
19.	Antibiotic resistance of CoNS strains from patients with UTIs	122
20.	Antibiotic resistance of *Enterococcus* strains from patients with UTIs	124
21.	Antibiotic resistance of *S. saprophyticus* strains from patients with UTIs	124
22.	Antibiotic resistance of *S. aureus* strains from patients with UTIs	126
23.	Antibiotic resistant of all bacterial strains isolated from UTIs	127
24.	Characteristics of *Candida* species on Chromagar *Candida* media	132
25.	Antifungal susceptibility pattern of *Candida* species	136
26.	Antifungal susceptibility of *Candida* species isolated from UTIs	136
27.	Double disc synergy test	137
28.	Molecular Characteristics of ESBL producing MDR *E.coli*	139

ABBREVIATIONS

µg	: Micro gram
µl	: Micro litre
A	: Acid production
AATF	: Antimicrobial availability task force
ACB	: Antibody coated bacteria
AK	: Amikacin
AM	: Ampicillin
AX	: Amoxicillin
BPH	: Benign prostatic hyperplasia
Candida spp.,	: *Candida* species
CAUTI	: Catheter associated urinary tract infection
CAZ	: Ceftazidime
CDC	: Centers for Disease control
CFU	: Colony forming units
CIP	: Ciprofloxacin
CL	: Cephalexin
CLSI	: Clinical Laboratory Standards Institute
CoNS	: Coagulase negative *Staphylococcus*
COT	: Cotrimoxazole

CT	: Ceftriaxone
CTX	: Cefotaxime
DDST	: Double disc synergy test
E.coli	: *Escherichia coli*
ESBLs	: Extended-spectrum β-lactamases
ExPEC	: Extra-intestinal pathogenic *E.coli*
G	: Gas production
GEN	: Gentamicin
GNB	: Gram Negative Bacteria
GPC	: Gram Positive Cocci
HCW	: Health care workers
HIV	: Human immunodeficiency virus
HPF	: High power field
ICU	: Intensive care unit
IMViC	: Indole, Methyl red, Voges-Prokauer and citrate
IP	: In- patient
IPM	: Imipenem
K.pneumoniae	: *Klebsiella pneumoniae*
Kbp	: Kilo base pairs
M	: Marker
MA	: MacConkey Agar

MDR	: Muti-drug resistance
Mg	: Milligram
MH Agar	: Muller-Hinton Agar
Ml	: Milliliter
MICs	: Minimal inhibitory concentrations
mm	: Millimeter
MSU	: Mid Stream Urine
NA	: Nalidixic acid
NCCLS	: National Committee for Clinical Laboratory standards
NIT	: Nitrofurantoin
NX	: Norfloxacin
OF	: Ofloxacin
OP	: Out -patient
P.aeruginosa	: *Pseudomonas aeruginosa*
P.vulgaris	: *Proteus vulgaris*
PCDDT	: Phenotypic confirmatory disc diffusion test
PCR	: Polymerase Chain Reaction
PFGE	: Pulsed-field gel electrophoresis
RFLP	: Restriction fragment length polymorphism
rpm	: Revolution per minute

S. aureus	: *Staphylococcus aureus*
S. epidermidis	: *Staphylococcus epidermidis*
S.saprophyticus	: *Staphylococcus saprophyticus*
SDA	: Sabouraud Dextrose Agar
SHV	: Sulfhydryl –variable-active site
Spp.,	: Species
TB	: Tobramycin
TEM	: Temnoniera
TST	: Triple Sugar Iron
U	: Unit
UPEC	: Uropathogenic *Escherichia coli*
UTIs	: Urinary Tract Infections
UV	: Ultra violate
WBCs	: White blood corpuscles
Wt	: Weight
WHO	: World Health Organistion
β	: Beta

1. INTRODUCTION

1.1. General information

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits (Ronald, 2002; Kolawale *et al.,* 2009; Upadhyay *et al.,* 2013). Urinary tract infections (UTIs) are more common among women than men, although the prevalence in elderly men and women is similar. Most of the research on UTI has focused on young, sexually active women who are at high risk for developing an infection (Harrington and Hooton, 2000). The predominant UTI risk factors in young women are sexual intercourse and the use of spermicidal contraceptives. Worldwide, about 150 million people are diagnosed with UTI each year (Gupta, 2001; Amin *et al.,* 2011). Urinary tract infections (UTIs) is one of the common infectious diseases, and nearly 10% of people will experience a UTI during their life-time (Foxman *et al.,* 2003). UTI is the leading cause of morbidity and health care expenditures in persons of all the ages (Prabhu and Selvaraj, 2012).

UTI is one of the most common infectious diseases which have been extremely studied in the field of clinical practice (Payam *et al.,* 2010). It is the most common health care associated group of bacterial infections affecting humans in India (Suman *et al.,* 2013). UTI is among the most common bacterial infections in humans both in community and hospital settings which occur in all age groups and in both genders (Orret and Davis, 2006). UTI is the major cause of morbidity in both the hospital and community settings (Omigie *et al.,* 2009; Prakash and Saxena, 2013) and affecting both outpatients and inpatients (Suwangool, 2012).

Under normal circumstances, the urine is sterile until it reaches the distal urethra. Various defense mechanisms of body prevent the infection of urinary tract (Ramzan *et al.,* 2004). One of the most important defense mechanism is the flow of urine that washes bacteria out of the body. In men prostate gland produces secretions that prevent bacterial growth. The acidic pH (5.5) and low osmolarity of urine also discourage the bacterial growth (Acharya, 1992). Similarly there are a number of factors that increase the risk of developing urinary tract infection. Some of these are: sex, age, pregnancy, catheterisation, kidney stones, tumors, urethral structures, neurological diseases, congenital or acquired anomalies of bladder, vesica-ureteric reflux, suppressed immune system, Diabetes mellitus, enlarged prostate and ureteric and stresses (Ramzan *et al.,* 2004).

Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency, and occasionally suprapubic tenderness. Acute pyelonephritis (Lane and Takhar, 2011) describes the clinical syndrome characterised by flank pain or tenderness, or both, and fever, often associated with dysuria, urgency, and frequency (Mandell *et al.,* 2005). More than 95% of urinary tract infections are caused by a single bacterial species (Bush *et al.,* 2011).

1.2. Epidemiology

UTI spans all age groups from neonates to elder. It is much more common in boys during first three months, often in association with urologic abnormalities. During preschool years it is common in girls than boys. Presence of bacteriuria in childhood defines a population at higher risk for development of bacteriuria in adulthood (Sobel and Kaye, 2005; Najar *et al.,* 2009).

Once adulthood is reached, prevalence of asymptomatic bacteriuria increases in the female population (Richard *et al.*, 2006). Up to 40% to 50% of female population will experience asymptomatic UTI at some time during their life. The prevalence of bacteriuria in adult men is low, until later years, when it rises. In young men lack of circumcision increases the risk for UTI caused by *Escherichia coli* including the development of symptomatic urethritis (Spach *et al.*, 1992; Singh *et al.*, 2005).

Obstructive uropathy due to enlarged prostate and loss of bactericidal activity of prostatic secretions in men and poor emptying of bladder due to prolapse of uterus in women are the possible reasons (Nicolle, 2001). As a result of anatomic and hormonal changes that favour development of UTIs, the incidence of bacteriuria increases during pregnancy. UTIs are important complications of diabetes, renal disease, renal transplantation and structural abnormalities that interfere with urine flow (Forbes, 2007).

1.3. Sources of urinary tract infection spread

Urinary tract infection (UTI) is an infection of one or more structures in the urinary system. The urinary tract includes urethra, bladder, ureters, prostate and kidneys. So UTI encompasses a broad range of clinical entities namely;

- Urethritis
- Acute urethral syndrome
- Prostatitis
- Cystitis
- Pyelonephritis
- Asymptomatic bacteriuria

Clinical presentation of UTI varies from asymptomatic infection to full blown pyelonephritis. Some symptoms may be non-specific and frequently symptoms overlap in patients with lower UTIs and upper UTIs. Symptoms of bladder infection (lower UTI) relate to inflammation-frequency, urgency and dysuria. The upper UTI which involves infection of ureters and kidneys may be associated with loin pain, fever, rigors, and vomiting (Forbes, 2007).

1.3.1. Urethritis

Urethritis is inflammation of the urethra. It is usually an ascending infection in men. In women, it is usually associated with cystitis or vaginitis (Bradshaw *et al*., 2006). Urethral discharge is usually present, along with dysuria and frequency. It is a syndrome seen in young sexually active women, who experience persistent or recurrent frequency, urgency, and dysuria, but excrete fewer than 10^5 colony forming units (CFU) of bacteria per ml in urine. The majority of patients with urethral syndrome have bacteriuria on some occasions; while at other times have low count bacteriuria. Approximately 90% of these women have pyuria, an important discriminatory feature of infection (Forbes, 2007).

1.3.2. Prostatitis

The term prostatitis has been used for various inflammatory conditions affecting the prostate including acute and chronic infections with specific bacteria and more commonly instances in which signs and symptoms of prostatic inflammation is present, but no specific organisms can be detected (Stamm, 2008). Both acute and chronic bacterial prostatitis is associated with urinary infection; prostatic secretions contain an excess of leukocytes and macrophages and bacteria can be cultured from the secretions (Catell, 2005). The hallmark of chronic bacterial prostatitis is

bacterial persistence in repeated urine cultures yielding the same organisms (Sobel and Kaye, 2005).

1.3.3. Cystitis

Symptoms of bladder infection relate to inflammation- frequency, urgency, and dysuria. Occasionally tenderness and pain over bladder area may be present. Urine may have offensive smell. Haematuria occurs in approximately one third of cases. Cystitis is localised infection, fever and other signs of systemic illness are usually not present (Forbes, 2007). Pus cells and bacteria may be detected by examination of uncentrifuged urine in most cases (Stamm, 2008). In majority of women, acute cystitis is an isolated event, never or very infrequently repeated (Catell, 2005).

1.3.4. Pyelonephritis

Pyelonephritis refers to inflammation of the kidney parenchyma, calices and pelvis, usually caused by bacterial infection (Forbes, 2007). Clinical diagnosis applied to patients with bacteriuria, who have loin pain, fever, and flank tenderness (Catell, 2005). Symptoms develop rapidly and symptoms of cystitis are sometimes present. Severe manifestations like necrotizing intrarenal and perinephric abscess can occur in compromised hosts like those having urinary tract obstruction, diabetes or other immunosuppressing conditions (Robert and Edwards, 1999).

Neonates and children below two years have non-specific symptoms including failure to thrive, vomiting and fever. Children between 2-5 years have localised symptoms like frequency, dysuria and abdominal or flank pain. Adults with lower UTI present with dysuria, frequency, urgency and occasionally suprapubic tenderness. Upper UTIs, particularly with acute pyelonephritis is accompanied by lower urinary tract symptoms in addition to flank pain, tenderness and fever (Alonto, 2007).

1.3.5. Asymptomatic bacteriuria

Asymptomatic bacteriuria is a significant number of bacteria in the urine that occurs without usual symptoms such as burning during urination or frequent urination. Asymptomatic bacteriuria has been alleged to be associated with several complications of pregnancy notably low birth weight, fetal loss, pre-eclampsia and maternal anaemia (Catell, 2005). Screening and treatment of asymptomatic bacteriuria is recommended for pregnant women, males undergoing transurethral resection of prostate and individuals undergoing urologic procedures (Forbes, 2007).

It is the presence of equal to or greater than 10^5 CFU/ml of same bacterial species in two consecutive midstream urine samples. In women the minimum prevalence is 2% to 4% in younger women and 10% in older women. In men it is rare until 55 years of age and approaches the rate in elderly women after this age. Prostatic hypertrophy and increased likelihood of instrumentation account for symptomatic bacteriuria in older men (Najar *et al.,* 2009; Thomas *et al.,* 2010).

Among young adults bacteriuria is 30 times more frequent in women than men. However, in the above 65 years of age, the ratio alters dramatically with progressive decrease in female to male ratio (Marques *et al.,* 2012). Bacteriuria, or the presence of bacteria in urine, is associated with both asymptomatic and symptomatic urinary tract infection and underpins much of the dynamic of microbial colonisation of the urinary tract (Ipe *et al.*, 2013).

1.4. Factors influencing UTIs

Anything that disrupts normal urine flow or complete emptying of bladder, facilitates access of organisms to bladder will predispose an individual to infection.

1.4.1. Sex

The female urethra appears to be particularly prone to colonisation with colonic Gram-negative bacilli, because of its proximity to the anus; it has short length and its termination beneath the labia. Sexual intercourse causes the introduction of bacteria into the bladder. Use of spermicidal compounds dramatically alters the normal introital bacterial flora and has been associated with marked increase in vaginal colonisation with *E.coli*. UTIs in healthy postmenopausal women are reported to be less common than in pre-menopausal women, probably due to lack of data for the latter. UTIs in young healthy men are very uncommon (Hooton, 2000; Grabe *et al.*, 2008). In male patients who are more than 50 years old and who have no history of heterosexual or homosexual insertive rectal intercourse, UTI is exceedingly uncommon. An important factor predisposing to bacteriuria in men is urethral obstruction due to prostatic hypertrophy (Stamm, 2008).

1.4.2. Age

Infections are rare in boys except in association with anatomic or functional abnormalities in the first year of life. Infections are also infrequent in the 2 to 13 years of old girls, but some young girls experience multiple repeated episodes of recurrent cystitis or pyelonephritis. UTI may be associated with dysfunctional voiding and bladder instability (Koff *et al.*, 1998). The presence of bacteriuria in childhood defines a population at higher risk for the development of bacteriuria in adulthood (Kunin, 1987). It has been observed that 7% of children with UTI develop renal scarring (Young, 2012).

1.4.3. Vesicoureteral reflux

Vesicoureteral reflux due to congenital abnormality, to bladder over distension or to unknown causes probably contributes to upper urinary tract infections via the ascending route. Clinical observations have demonstrated that infection may produce reflux especially in children (Feld and Mattoo, 2010).

1.4.4. Pregnancy

Symptomatic upper urinary tract infections are common during pregnancy due to decreased urethral tone, decreased urethral peristalsis and temporary incompetence of the vesicoureteral valves. UTI in pregnancy may be associated with an increased neonatal mortality (Jennifer *et al.*, 2012). Recently it was found that about 20% of patients had pyelonephritis as the cause of primary renal disease.

1.4.5. Neurologic bladder dysfunction

Interference with bladder enervation, as in spinal cord injury, tabes dorsalis, multiple sclerosis, diabetes and other diseases may be associated with UTI. Infection may be initiated by using of catheter for bladder drainage and is favoured by prolonged stasis of urine in the bladder (Stamm, 2008).

1.4.6. Obstruction

Any impediment to free flow of urine such as tumor, restriction, stone or prostatic hypertrophy results in hydronephrosis and a greatly increased frequency of UTI (Stamm, 2008).

1.4.7. Catheter associated UTIs

Catheter associated UTIs account for 40% of all nosocomial infections (Thomas *et al.,* 2010). With insertion of a catheter, bacteria may be pushed along the urethra into the bladder or with an indwelling catheter, may migrate along the track between the catheter and urethral mucosa, gaining access to the bladder. Most microorganisms causing endemic catheter associated UTIs derive from the patients own colonic and perineal flora or from the hands of health-care personal during catheter insertion or manipulation of the collection system (Maki and Tambyah, 2001). Factors associated with an increased risk of catheter associated UTI include, female sex, prolonged catheterisation, severe underlying disease, and disconnection of the catheter and drainage tube and lack of systemic antimicrobial therapy (Stamm, 2008). Using urethral catheterisation it has been shown that approximately 50% of women with asymptomatic bacteriuria had infection in their upper urinary tracts (Valerie, 2013).

1.5. Pathogenesis

Bacteria gain access to the urinary tract by the ascending route, the hematogenous route and lymphatic pathways. Once established in the bladder, bacteria may ascend the ureters, probably aided in many cases by vesicoureteral reflux or by peristaltic dilated ureters caused by intraluminal infection, an inflammation of the genitourinary tract musculature. Infection of the renal parenchyma by many species of Gram-positive bacteria, following *Staphylococcal* bacterimia or endocarditis, mycobacterial infection and *Candida* infection occurs by haematogenous route. Gram-negative infections rarely occur by haematogenous route (Sobel and Kaye, 2005).

1.6. Aetiologic agents of bacteriuria

The Enterobactericeae is responsible for about 80 percent of bacterial urinary tract infection. No unique symptoms complex is associated with any particular species. *E.coli* accounts for 80 percent of community-acquired urinary tract infections. *Klebsiella pneumoniae* cause about 5 percent of infection whereas *Enterobacter* species and *Proteus* species each cause 2 percent of infection. *Proteus* infections are often associated with renal infection. *Pseudomonas aeruginosa* are almost always hospital-acquired as a result of a failure of infection control practices, usually after urethral catheterisation or manipulation. *Staphylococcus saprophyticus* is the second most common pathogen isolated from young women in most surveys and accounts for about 10 percent of acute urethrocystitis**.** The most common group of bacteria responsible for urinary tract infections were *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter, Enterococci* bacteria, and *Proteus mirabilis* (Jepson and Craig, 2008).

1.6.1. *E.coli*

Escherichia coli are a bacterium commonly found in the large intestine of humans and other warm blooded animals. *Escherichia coli*, the most prevalent facultative Gram-negative bacillus in the human fecal flora, usually inhabit the colon as an innocuous commensal. According to the special pathogenicity theory (Orskov and Orskov, 1985), special properties enabling *E. coli* to overcome host defenses in a new environment, are necessary in order for it to escape the limitations of the colonic mileu and move into new niches devoid of competition from other bacterial species (Eisenstein and Jones, 1988). Strains of *E.coli* that cause disease outside of the gastrointestinal tract are referred to as extraintestinal pathogenic *E.coli* (ExPEC) and are divided into uropathogenic *E.coli* (UPEC) strains

causing neonatal meningitis and septicemic *E.coli* (Stecher and Hardt, 2008). UPEC is the most common pathotype of ExPEC and is found in patients with urinary tract infections (Katouli, 2010).

1.6.2. *Klebsiella pneumoniae*

Klebsiella pneumoniae is among the most common Gram-negative bacteria encountered by physician worldwide (Lin *et al.,* 2010). These bacteria have become important pathogens in nosocomial infections (Nordamann *et al.,* 2009), which have been well documented in the United States (Graybill *et al.,* 1973) and India (Mathur *et al.,* 1991). Epidemic and endemic nosocomial infections caused by *Klebsiella pneumoniae* are leading causes of morbidity and mortality. *Klebsiella pneumoniae* can also cause a urinary tract infection in children and adults. In the United States, *K. pneumoniae* accounts for 3-7% of all nosocomial bacterial infections, placing them among the eight most important infectious pathogens in hospitals (Sarathbabu *et al.,* 2012).

1.6.3. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a bacterium that is often encountered in urinary tract infection worldwide and has shown varied antibiotic susceptibility patterns. *Pseudomonas* is a large and complex genus of Gram-negative bacteria of importance, as it includes species with both clinical and environmental implication. The genus *Pseudomonas* first proposed by Migula in 1894 (Palleroni, 2005). *Pseudomonas aeruginosa* is a motile Gram-negative rod that belongs to the family Pseudomonadaceae. It is a leading causes of nosocomial infections, especially among critically ill admitted in intensive care unit, immunocompromised patients (Govan, 1998). *Pseudomonas aeruginosa* is widely distributed in nature, but has higher prevalence in hospital environment, as the wards encourage bacterial growth (Hugbo and Olurinola, 1992). The characteristic features

of *Pseudomonas aeruginosa* isolates that allows the persistence in hospital is the ability to acquire resistance to many of antibiotics, withstands physical conditions like temperature, high concentration of salts and antiseptics (Erdem, 1999).

Pseudomonas aeruginosa is an opportunistic pathogen that causes extensive morbidity and mortality in individuals who are immunocompromised or have underlying medical conditions such as urinary tract, respiratory tract and skin infections and primarily causes of nosocomial infections, and it is frequently resistant to commonly used antibiotics and disinfectants (Pollack, 2000).

1.6.4. *Proteus* species

Proteus species are members of the family Enterobacteriaceae (Caroline *et al.*, 2000). *Proteus* species are Gram-negative bacilli that thrive in soil, water and the intestinal tracts of mammals, are capable of swarming or swimming in a coordinated manner, on solid surfaces. Several species of *Proteus* bacteria are known to colonies and infect the human host, but the one most frequently linked with causing human disease is *Proteus mirabilis*. It is more commonly associated with urinary tract infections (UTIs) in those individuals with structural or functional abnormalities, especially ascending infections in patients undergoing urinary catheterisation (Sandra *et al.*, 2011).

1.6.5. *Enterobacter* species

Enterobacter is a genus of common Gram-negative, facultatively anaerobic, rod-shaped, non spore forming bacteria of the family Enterobacteriaceae. Several strains of these bacteria are pathogenic and cause opportunistic infections in immunocompromised hosts and in those who are on mechanical ventilation. The urinary and respiratory tracts are

the most common sites of infection. The genus *Enterobacter* is a member of the coliform group of bacteria (Cabral, 2010).

1.6.6. *Citrobacter*

The genus *Citrobacter* is a distinct group of aerobic Gram-negative bacilli from the Enterobacteriaceae family (Gill and Schutze, 1999). *Citrobacter* species are primary inhabitants of intestinal tract, often found in human feces. These organisms are isolated from clinical specimens like urine, pus, blood, and cerebrospinal fluid. Organisms of genus *Citrobacter* are Gram-negative straight rods, found singly or in pairs, and are motile by peritrichous flagellae (Doran, 1999).

1.6.7. *Acinetobacter*

Members of the genus *Acinetobacter* are ubiquitous, free living, aerobic, Gram-negative coccobacilli that prefer a moist environment and can be easily obtained from soil, water, food and sewage (Taneja *et al.*, 2011). They are usually considered opportunistic pathogens and cause nosocomial infections in hospitalised patients like bacteremia, pneumonia, meningitis and urinary tract infections (Bergogne *et al.*, 1996).

Multidrug-resistant *Acinetobacter baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism’s ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infection and an endemic, health care–associated pathogen (Fournier and Richet, 2006; Lisa *et al.*, 2008).

1.6.8. Coagulase-negative *Staphylococci* (CoNS)

Coagulase-negative *Staphylococci* (CoNS) are the most important pathogens in infectious disease. CoNS are reported as the third most widespread causative agent of nosocomial infections (Mohan *et al.,* 2002). CoNS are important and frequently encountered pathogens in hospital surroundings, and they account as a majority of all nosocomial infections (Von *et al.,* 2001). Infection is the major complication associated with the use of foreign bodies such as catheters (Heilmann and Peters, 2000). Only 16 Coagulase-negative species have been found in specimens of human origin. Among the CoNS, *Staphylococcus epidermidis* principal cause of infection, chiefly in hospitalised patients with indwelling foreign bodies and in immunocompromised patients (Piette and Verschraegen, 2009).

1.6.9. *Staphylococcus saprophyticus*

Staphylococci saprophyticus tends to cause infection in young women of a sexually active age (Schneider and Riley, 1996). *Staphylococcus saprophyticus* is implicated in 10-20% of urinary tract infections (UTIs). In females between the ages of about 17-27 it is the second most common causative agent of acute UTIs, after *Escherichia coli.* The bacteria may also reside in the urinary tract and bladder of sexually active females (Rupp *et al.,* 1992; Ishihara *et al.,* 2001). It is referred to as "honeymooner's" UTI due to its association with intercourse. *Staphylococcus saprophyticus* is a member of the Coagulase-negative *Staphylococci* (CoNS). Unlike most other CoNS, *S. saprophyticus* is rarely resistant to most antibiotics active against Gram-positive organisms (Kumari *et al.,* 2001).

1.6.10. *Staphylococcus aureus*

Staphylococcus aureus is a relatively uncommon cause of urinary tract infection in the general population (Robert *et al.,* 2006). Bacteriuria with *Staphylococcus aureus* is uncommon; it accounts for <2% of urinary tract infections. It is reported primarily in hospitalised older adults after they undergo surgery, catheterisation, or other invasive procedures. In the community, malignancy and other causes of obstructive uropathy are associated with *Staphylococcus aureus* bacteriuria. Many older patients have significant pyuria, but are afebrile and asymptomatic. Bacteremia from a urinary source develops in <5% of patients with *Staphylococcus aureus* bacteriuria (Thomas *et al.,* 2002). *S.aureus* bacteriuria more often occurs as a consequence of bacteremia or endocarditis.

1.6.11. *Enterococcus* species

Enterococci are Gram positive cocci, which are normal commensal of the gastrointestinal tract, genital tract, and anterior urethra. However, in recent years, it has gained importance as a nosocomial pathogen because of its antibiotic resistance (Rupali *et al.*, 2012). *Enterococci* are the commensal of the human intestinal flora. Sites less often colonised by these organisms include the oral cavity, genitourinary tract, and skin, especially in the perineal area. The main sites of colonisation in the hospitalised patients are soft tissue wounds, ulcers, and the gastrointestinal tract. *Enterococci* were traditionally regarded as low-grade pathogens, but have emerged as an increasingly important cause of nosocomial infections in recent years. The spectrum of disease varies from UTI, wound infection, soft tissue infection to bacteremia. It is the second most common cause of UTI and third most common cause of bacteremia. Urinary tract instrumentation or catheterisation, genitourinary pathology, prior use of antibiotics, prolonged hospitalisation is some of the predisposing factors for enterococcal infections (Sood *et al.*, 2008).

1.7. Antibiotic sensitivity tests

Antimicrobial chemotherapy has been a leading causes for the dramatic rise of average life expectancy in the twentieth century. However, disease causing microbes that have become resistant to antibiotic drug therapy are increasing public health problem (Todar, 2011). There are three mechanisms that can cause antibiotic resistance: prevention of interaction of drug with target organisms, decreased uptake due to either an increased efflux or a decreased influx of the antimicrobial agent and enzymatic modification or destruction of the compound (Bonilla and Muniz, 2001). Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognised as a serious problem by global medicinal and research community (Finch, 2004). Many factors play in the emergence of resistance (WHO, 2012) from poor utilisation of antimicrobial agents, to the transmission of resistant bacteria from patient to patient and from healthcare workers to patients and vice versa, to a lack of guidelines for an appropriate and judicious use of antimicrobial agents, to lack of easy-to-use auditing tools for restriction (Mahmoud and Hanan, 2012). In addition, there is a clear misuse of antimicrobial in the animal industry, those are the same agents used in humans. All these factors together led to the inevitable rise and emergence of resistance.

Clinical Laboratory and Standards Institute (CLSI, previously NCCLS, 2009) has published an abbreviated identification system for the laboratory identification of diverse pathogenic bacteria and yeasts. This includes both cultural and biochemical characters well suited for small and medium bacteriologic laboratories. This is a document developed through a consensus process that describes criteria for operating practice, procedure, or material for voluntary use. These tests allow reliable identification of organisms with a high degree of certainty, decreasing the necessity for time consuming tests. This, CLSI says, enhances timely patient care. CLSI is a

widely accepted organisation and laboratories in many parts of the world follow its recommendations for bacterial identification. The tests described in the document include tests/characters that have been validated by workers in the US. Expertise as a microbiologist and initial correctness in the interpretation of Gram stain and colony characters is essential for good results.

1.8. Candiduria

Candiduria is one of the most common symptoms of urinary tract infections caused by several species of *Candida*, which is a normal flora of human body. *Candida albicans* has played an important role in candiduria (Nayman *et al.,* 2011). *Candida* species are the most common cause of fungal infections leading to a range of life threatening invasive to non-life-threatening diseases (Jacqueline *et al.,* 2010). Urinary tract infections as a result of *Candida* species is becoming increasingly common in hospitalised setting particularly in intensive care units (Jain *et al.,* 2011). Epidemiological surveillance indicates that *Candida* species are now the most common pathogens causing nosocomial blood stream and urinary tract infection (Horvath *et al.,* 2003).

Urinary system infections are usually bacterial, however, fungal etiology, particularly *Candida* species are encountered in about 10% of these infections (Nayman *et al.,* 2011). *C. albicans* is still the most frequently isolated species in candiduria. The presence of *Candida* species in urine is a common clinical finding, particularly in hospitalised patients. *Candida* species accounts for almost 9 to 40% of nosocomial urinary tract infections (Jacqueline *et al.,* 2010). About 14 *Candida* species have been implicated in human infections, with *Candida albicans* being the most prevalent among the yeast isolates. The most frequently isolated species is *Candida albicans*, but *Candida tropicalis, Candida glabrata*, *Candida*

krusei, and *Candida parapsilosis* are also emerging as important etiologic agents of *Candida* infection (Krcmery and Barnes, 2002).

The frequency of infection of the urinary tract due to *Candida* species is increasing in parallel with the rapid advances of medical progress, and these infections are now among the most common problems facing physicians. Despite this fact, much remains to be learned regarding the pathogenesis, diagnosis, and management of blood borne (antegrade) kidney infections and ascending (retrograde) invasion of the urinary collecting system (John, 2011).

The susceptibility range of *Candid*a varies to antifungal drugs. *C. albicans* are usually sensitive to amphotericine B. However, several reports show that non-albicans are more resistant to antifungal, especially fluconazole (Saha *et al.,* 2008; Yanga *et al.,* 2008) believe that differences in sensitivity *Candida* species to antifungal are associated with geographical distributions.

Several brands of chromogenic media are available for rapid identification of yeast and bacteria (Cooke *et al.*, 2002). These special media yield microbial colonies with varying pigmentation secondary substrates that react with enzymes secreted by microorganisms (www.chromagar.com). These media are specific, allowing the organisms to be identified to the species level by their color and colonial characteristics. The manufacturer of CHROMagar *Candida* currently advertises its product as able to detect and differentiate many species, *C. albicans* by growth as light to medium green colonies, *C. tropicalis* by growth as steel blue colonies accompanied by purple pigmentation diffused into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose colored colonies with white edges, after incubation for 48 hours at 37°C, as also reported in several studies (Topley and Wilson, 2005). Use of chromogenic media in clinical microbiology laboratories for the isolation and

presumptive identification of important *Candida* species is easy to perform, requires less time and is cost effective too (Pfaller *et al.*, 1996; Willinger *et al*., 2001).

1.9. Molecular Characteristics of ESβL Producing *E.coli*

Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms have been found for every class of antibiotic agents. In recent years, increased incidence of resistance to beta-lactams among members of the family Enterobacteriaceae has been reported worldwide (Bradford, 2001). The Extended Spectrum of β-Lactamases (ESBL) are plasmid-mediated enzymes which are capable of hydrolysing and inactivating a wide variety of β-lactams including third generation cephalosporins, penicillins and aztreonam (Chaudhary and Aggarwal, 2004).

First plasmid mediated β-lactamase in Gram negative organisms was reported in 1965 from an *Escherichia coli* isolate belonging to a patient in Athens, Greece, named Temnoniera (hence designated TEM). Another common plasmid mediated β- lactamase found in *Klebsiella pneumoniae* and *E. coli* is SHV-1 (named after the sulfhydryl-variable-active site). There is no consensus on the precise definition of ESBLs. A commonly used working definition is that, ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins; first, second and third generation cephalosporins; and aztreonam by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). Production of extended spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases are the most common mechanisms of antimicrobial resistance in Gram negative bacilli (Taneja *et al.,* 2008).

ESBL-producing Gam negative bacteria are becoming a major global concern (Al-Jasser, 2006) and usually harbor plasmid-mediated enzymes of the TEM, SHV, OXA, PER, and CTX-M types (Deepti and Deepthi, 2010). The TEM-1/2 and SHV-1 broad-spectrum β-lactamases are the most prevalent secondary β-lactamases among clinical isolates of Enterobacteriaceae worldwide. Prevalence of ESBL producing strains also varies from one geographical region to another paralleling the misuse or overuse of beta- lactam drugs (Manchanda *et al.*, 2005). Resistance to beta lactam antibiotics is mainly due to the enzymes that hydrolyse the betalactam ring of the antibiotics, making it lifeless.

The infection of urinary tract by bacteria and fungi are important and serious problems in the clinical field. Moreover the bacterial urinary tract infection is the common danger disease in Pattukkottai area. Therefore, the present investigations were undertaken to study the prevalence and antimicrobial susceptibilities of bacteria and fungi isolated from patients with urinary tract infections in Pattukkottai area, Tamil Nadu, India.

1.10. OBJECTIVES OF THE STUDY

1.10.1. Main objective

To determine the antibacterial and antifungal susceptibility patterns of uropathogens and the molecular detection of the ESBL gene types prevailing in clinical isolates of mult-drug resistance *E.coli* isolated from patients suspected with urinary tract infection in various Hospitals at Pattukkottai area, Thanjavur district, Tamilnadu, India.

1.10.2. Specific objectives

1. To study the prevalence and the distribution of different bacterial pathogens isolated from patients with UTIs.

2. To isolate and characterise pathogens causing UTIs in patients.

3. To study the antibiotic sensitivity pattern of the urinary isolates, so as to provide a basic guideline in treating UTIs.

4. To isolate and characterise of the uropathogenic *Candida* species

5. To study the antifungal susceptibility pattern of *Candida* isolates from the UTIs

6. This study the molecular detection of the ESBL gene types prevailing in urine isolates of multi-drug resistance *E.coli* and describing the epidemiology.

2. REVIEW OF LITERATURE

2.1. GENERAL INFORMATION

Urinary tract infection (UTI) is the third most common infection experienced by humans after respiratory and gastro-intestinal infections. In fact, bacterial infections of the urinary tract are the most common cause of community acquired and nosocomial infections for patients admitted to hospitals in India. It is distressing and occasionally life-threatening. However, the prognosis and management of urinary tract infections depends on the site of infection and any predisposing factors (Najar *et al.*, 2009).

UTI may be defined as a condition in which bacteria are established and multiplying within the urinary tract. Diagnosis requires demonstration of bacteriuria. Exceptions to this include patients with pyogenic abscess of kidney or perinephric tissue, obstructed pyonephrosis or bacterial prostatitis in whom the urine may be sterile (Grabe *et al.*, 2008).

Some definitions are necessary because the infection of the urinary tract may result from microbial invasion of any of the tissues extending from urethral orifice to the renal cortex. Although the infection and resultant symptoms may be localised, the presence of bacteria in urine places the entire urinary system at risk of invasion by bacteria (Najar *et al.*, 2009).

2.1.1. Forms of bacteriuria

In healthy patients most uropathogens originate from rectal flora and enter the urinary tract via the urethra into the bladder (Handley *et al.*, 2002). This is known as the ascending route and uropathogens initially adhere to and colonise urothelium of the distal urethra. Enhancement of this route is exacerbated in patients with soiling around the perineum, in patients with urinary catheters and in females that use spermicidal agents (Foxman, 2003). Many studies have suggested that 95% of all urinary tract infections (UTIs) develop through an ascending route of infections, caused mostly by Gram-negative bacteria, while the other 5% develops by a descending route (hematogenous infection). The latter is usually caused by Gram-positive organisms (Bahrani *et al.*, 2002). Ascending route UTIs begin with the colonisation of bacteria in the periurethral area, followed by an upward progression of bacteria to infect the bladder. In patients with established cystitis up to 50% of infections may ascend into the upper urinary tracts and most episodes of pyelonephritis are caused by ascension of bacteria from the bladder through the ureter and into the renal pelvis (Najar *et al.*, 2009). Bacterial ascent is aided by conditions such as pregnancy and ureteral obstruction as these conditions inhibit ureteral peristalsis. Bacteria that reach the renal pelvis can penetrate the renal parenchyma through the collecting ducts and disrupt the renal tubules.

In healthy individuals infection of the kidney through the haematogenous route is uncommon. Occasionally, the renal parenchyma may be breached in patients with *Staphylococcus aureus* bacteraemia or *Candida* fungaemia that originate from oral sources in immunosuppressed patients. On rare occasions bacteria from adjacent organs may penetrate the urinary tract via the lymphatics (Figure 1). Conditions associated with the lymphatic route are retroperitoneal abscesses and severe bowel infections (Niall *et al.*, 2011).

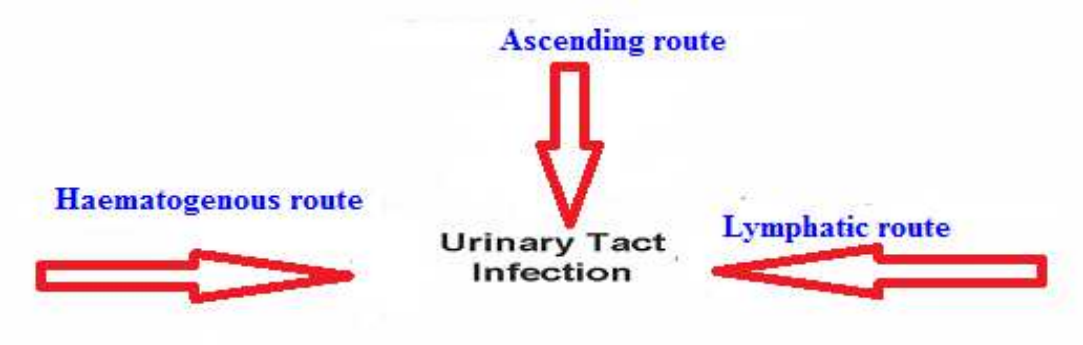


Figure 1. Route of urinary tract infections.

Urinary tract infections may arise from ascending, haematogenous or lymphatic routes. Ascending routes of infection are most common among patients with an established UTI.

2.1.2. Complicated and uncomplicated urinary tract infection

There is a general agreement that for the best management of patients with urinary tract infections, it is important to distinguish between complicated and uncomplicated infections (Najar *et al.*, 2009). UTIs can be classified as either complicated or uncomplicated depending on underlying host factors and on underlying uropathogens as illustrated in Table 1.

2.1.2.1. Complicated UTIs

A complicated urinary tract infection is a urinary infection occurring in a patient with a structural or functional abnormality of the genitourinary tract. Underlying host factors such as age, catheterisation, Diabetes mellitus and spinal cord injury predispose to complicated UTIs (Figure 2). In complicated UTIs less virulent uropathogens (that rarely cause disease in a normal urinary tract) can cause significant damage to an abnormal

urinary tract. Complicated UTIs are associated with elderly patients, development of infections due to instrumentation such as insertion of catheters, antimicrobial treatments, infections that are also associated with anatomic abnormalities of the genitourinary tract, infections in diabetic patients and urethritis due to inflammation of the prostate gland in men (Hooton, 2000; Kalra and Raizada, 2009).

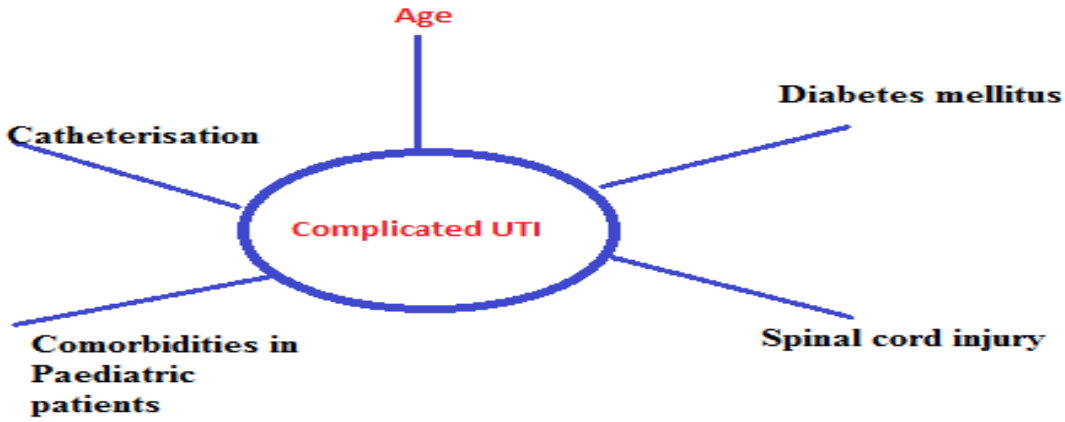


Figure 2. Predisposing factors for complicated UTIs.

Children with comorbidities are more likely to develop complicated UTIs and *Staphylococcus aureus* is the most frequently isolated microorganism in paediatric patients with indwelling catheters (Schlager, 2001). *Candida* and Coagulase-negative *Staphylococci* are associated with complicated UTIs after instrumentation of the paediatric urinary tract. Of note, Enterobacteriaceae are the most frequently isolated uropathogen in children with uncomplicated UTIs (Schlager, 2001). UTIs are among the top 10 complicating illnesses in patients with Diabetes mellitus with *E.coli, Klebsiella,* Group B *Streptococci* and *Enterococcus* among the common uropathogens. In fact, Group B *Streptococcus* and *Klebsiella pneumoniae* are 2-3 times more common in patients with diabetes mellitus than in patients without the condition (Ronald and Ludwig, 2001).

2.1.2.2. Uncomplicated UTIs

Uncomplicated urinary tract infections (UTIs) are among the most frequently encountered infections in the outpatient setting. After respiratory tract infections, they are the most common reason why antibiotics are prescribed. The uncomplicated urinary tract infections are including acute uncomplicated cystitis and pyelonephritis. Recently, the level of resistance of pathogens causing uncomplicated UTI has risen significantly (Gupta *et al.*, 2011).

Uncomplicated UTI may have a rather benign course, whereas complicated UTI has been associated with increased morbidity and mortality**.** Sexual intercourse is one of the most important risk factors associated with the risk of uncomplicated UTI**.** The increased risk caused by sexual intercourse appears to operate through a mechanical effect of introducing uropathogens into the bladder (Hooton *et al.,* 1991) and possibly through a trauma effect (Foxman *et al.*, 1997). The aetiology of uncomplicated UTIs has remained constant over the last 2 to 3 decades with *E. coli* accounting for the vast majority of cases. Previously, female patients with uncomplicated UTIs generally remained sensitive to a trimethoprim-sulfamethoxazole combination and the traditional approach to therapy had been an empirical short-course treatment with this antibiotic regimen (Hooton and Stamm, 1997). Unfortunately, a number of more recent studies have demonstrated increasing antimicrobial resistance among uropathogens causing uncomplicated cystitis and traditional antibiotic regimens (Gupta *et al.,* 1999).

For many years, pathogens associated with uncomplicated UTI have remained constant, with *E. coli* identified as the etiological agent in about 75-90% of infections (Hooton and Stamm, 1997). Five to ten percent of uncomplicated cases are caused by *S. saprophyticus* (Gupta *et al.*, 1999)

with *Klebsiella, Proteus, Enterococcus* and *Pseudomonas* species seen in smaller percentages. (Gupta *et al.,* 2001).

Table 1. Pathogens in complicated and uncomplicated UTIs.

Pathogens in complicated UTIs	**Pathogens in uncomplicated UTIs**
Escherichia coli	*Escherichia coli*
K. pneumoniae	*K. pneumoniae*
Enetrobacter cloacae	*Staphylococcus saprophyticus*
Enterococcus faecalis	*Enterococcus faecalis*
Serratia marcescens	
Proteus mirabilis	
Pseudomonas aeruginosa	
Group B streptococci	

Uncomplicated community acquired urinary tract infections is one of the most common bacterial infections that affect the patients in all age groups and both sexes (Wada and Gan, 2012). Uncomplicated UTI occurs in patients who have a normal, an obstructed genitourinary tract, who have no history of recent instrumentation, and whose symptoms are confined to the lower urinary tract. Uncomplicated UTIs are most common in young sexually active women with far fewer cases occurring in older women, those who are pregnant and in men.

2.1.3. Symptomatic and Asymptomatic bacteriuria

2.1.3.1. Asymptomatic bacteriuria

When a significant number of bacteria show up in the urine, this is called "bacteriuria" (Rahimkhani *et al.*, 2008). Finding bacteria in the urine can mean there is an infection somewhere in the urinary tract. The urinary tract is the system that includes:

- The kidneys, which make urine
- The ureters – thin tubes connecting the kidneys to the bladder
- The bladder, where urine can be stored
- The urethra - the final pathway to move urine from the bladder to outside the body.

In asymptomatic bacteriuria, large numbers of bacteria are present in the urine. However, the person has no symptoms of a urinary tract infection (asymptomatic means without symptoms). It is not clear why the bacteria don't cause symptoms. It may be that asymptomatic bacteriuria is caused by weaker (less "virulent") bacteria (Kutlay *et al.*, 2003). The condition does not always need to be treated.

Asymptomatic bacteriuria is most common in:

- Elderly women
- People with diabetes
- People with bladder catheters.

This is especially common in women as evidenced by a minimum prevalence of 2-4% in young and 10% in elderly women. The cumulative prevalence of asymptomatic bacteriuria in women increases about 1% per decade throughout life regardless of ethnicity and geographic locations. In contrast to women, the occurrence of asymptomatic bacteriuria in men is rare until after 55 years of age, at which time the prevalence increases per decade and approaches the rate in elderly women (Kammire, 2013). Prostatic hypertrophy and increased likelihood of instrumentation account for the bacteriuria in older men (Kunin, 1987). Differences between men and women in the rates of bacteriuria have been attributed to the shorter female urethra and its proximity to the vagina and rectal mucosa and their abundant microbial flora.

Asymptomatic bacteriuria is the presence of at least 10^5CFU/ml of the same urinary pathogen in consecutive voided urine specimens in asymptomatic women (Zanel *et al.,* 1990). Asymptomatic bacteriuria in young women is a strong predictor of subsequent symptomatic UTI (Hooton *et al.,* 2000). In pregnant women asymptomatic bacteriuria is a common finding and may lead to pyelonephritis (30%) and preterm labour. These events may be prevented by adequate antibiotic treatment. Antibiotic treatment was associated with a reduction in incidence of pre-term delivery or low-birth weight babies (Smaill, 2001). Infants with asymptomatic bacteriuria represent a low risk group with a tendency to spontaneous resolution usually within few weeks-months (Caramia and Fanos, 2002).

Asymptomatic bacteriuria is often defined as significant isolation of bacteria from urine that is consistent with infection, but lacks any signs of illness or symptoms that are usually associated with UTIs (Foxman, 2003; Johnson, 2008). Asymptomatic UTIs are often seen in diabetic females, patients with previous history of UTIs, women with cystoceles (hernias in urinary bladder), and in about 50% of geriatric women (Kass and Finland,

2002). Asymptomatic UTIs have been of great interest since these silent infections can result in sepsis or even death (Foxman, 2003).

2.1.3.2. Symptomatic urinary tract infection

These occur in all age groups. Among newborns, infants and boys are affected more than the girls. When urinary tract is the source of neonatal sepsis, serious underlying congenital anomalies are frequently present (Otajevwo, 2013). During childhood, persistent bacteriuria with or without repeated symptomatic episodes occurs in a small group (less than 2%) of school-aged girls. Such girls and also school-aged boys with bacteriuria should have a urological evaluation to detect correctable structural abnormalities when UTIs are documented (Kunin, 1987; Figure 3).

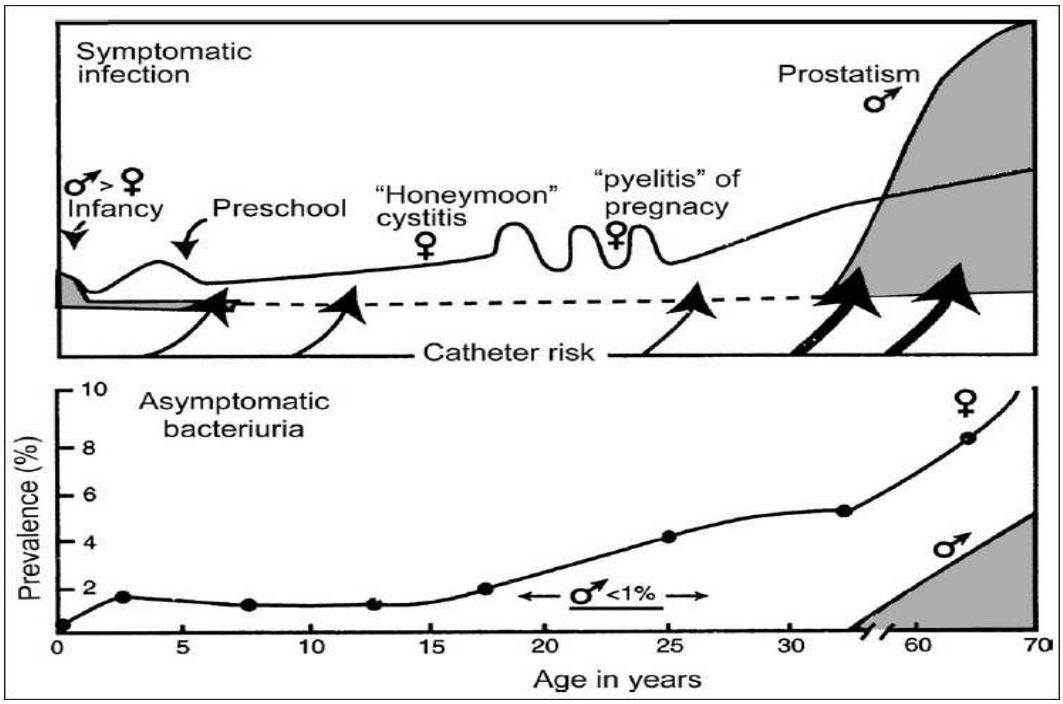


Figure 3. Distribution of symptomatic urinary tract infection and prevalence of asymptomatic bacteriuria by age and sex (Male- shaded area; Female-line) (Kunin, 1987)

Sexually active women have a markedly increased risk of cystitis. Vast majority of acute symptomatic infections involve young women. A prospective study demonstrated an annual incidence of 0.5-0.7 episodes per patient year in this group (Fihn, 2003). In the absence of prostatitis, bacteriuria and symptomatic UTIs are unusual in men. The risk of cystitis in young men due to uropathogenic *E.coli* increases because of lack of circumcision or having a partner with vaginal colonisation with such P-fimbriated *E.coli.* At any age, both sexes may develop symptomatic infections in the presence of risk factors that alter urinary flow. These include (Schrier, 2000). Congenital anomalies, renal calculi, ureteral occlusion (partial or total), vesico-ureteral reflux, residual urine in bladder, neurogenic bladder, urethral structure, prostatic hypertrophy, instrumentation of urinary tract, indwelling urinary catheters, catheterisation, urethral dilatation and cystocopy (Ramzan *et al.,* 2004; Al-Badr and Al-Shaikh, 2013).

2.1.4. Urine analysis

Urinalysis is a test that evaluates a sample of urine. Urinalysis is used to detect and assess a wide range of disorders, including urinary tract infection, kidney disease and diabetes (Patel, 2006.)

Microscopic bacteriuria, which is most conveniently assessed using Gram- stained, uncentrifuged urine, is found in over 90% of UTIs with colony counts of 10^5CFU/ml. or more and is highly specific finding (Jenkins *et al.,* 1986). When pyuria in voided urine specimens is carefully assessed using haemocytometer method and when UTI is defined as more than 10^2 CFU/ml, plus acute urinary symptoms, pyuria is a highly sensitive indicator of UTI. Pyuria and/or bacteriuria on microscopy are highly suggestive of UTI and are useful criteria to select specimens for direct

sensitivity testing. Pyuria is considered by some to be a poor predictor of infection.

Bacteriuria literally means ‘bacteria in urine’. The probability of the presence of infected urine in the bladder can be ascertained by quantifying the numbers of bacteria in voided urine or in urine obtained via urethral catheterisation (Walter *et al.*, 1989; Ipe *et al.*, 2013). Significant bacteriuria has been used to describe the numbers of bacteria in voided urine that usually exceed the numbers caused by contamination from anterior urethra (i.e., $\geq 10^5$ bacteria/ml). The implication is that in the presence of at least 10^5 bacteria / ml of urine, infection must be considered seriously (Smith, 2000).

The presence of white blood corpuscles (WBCs) in the urine is known as pyuria. It indicates inflammatory response of the urothelium to invading bacteria. Presence of > 10 WBCs / HPF is considered significant (Abrahamian *et al.*, 2013).

Routine follow up of cultures for test-of-cure are not recommended for patients who have been treated for asymptomatic bacteriuria, acute uncomplicated cystitis or acute uncomplicated pyelonephritis and for whom there is evidence of an appropriate clinical response to therapy (Winickoff *et al.*, 1989).

Urine cultures may not be necessary as part of the evaluation of outpatients with uncomplicated UTIs (Wing *et al.*, 2000). However urine cultures are necessary for outpatients, who have recurrent UTIs, experience treatment failures or have complicated UTIs (Mohsin and Siddiqui, 2010). Urine cultures are also necessary for inpatients that develop UTIs. The bacterial culture remains an important test in the diagnosis of UTI, not only because it helps to document infection, but also because it is necessary for determination of the identity of the infecting microorganisms and for

antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance (Wilson and Gaido, 2004).

Follow up cultures are recommended for patients with infections that do not respond to therapy, patients who have recurrent UTIs, patients who have anatomic or functional abnormalities of the urinary tract or patients who continue to have unexplained abnormal urinalysis findings (Wilson and Gaido, 2004).

Hanif (2006) demonstrated that out of one thousand pregnant women, 426 (42.6%) complained of one or more urinary symptoms. Diurinal and nocturnal frequency was the most commonly encountered symptom (87.32%), followed by irritative symptoms and voiding difficulties. Complete urine examination of symptomatic patients revealed<5 pus cells /HPF (high power field) in 322 cases and 6-20 pus cell/HPF in the remaining 104 cases.

2.1.5. UTIs according to different sex and age

The lesser prevalence of UTI in men than women is thought to result from a variety of factors, including; the greater distance between the anus and urethral meatus, the drier environment surrounding the male urethra, the greater length of male urethra, and the antibacterial activity of prostatic fluid (Lipsky, 1989). The four major risk groups for community acquired UTI are, school-aged girls, young women in their sexually active years, males with prostatic obstruction and the elderly (Stamm *et al*., 1989; Denise *et al.,* 2013).

The exact prevalence of UTIs is age and sex dependent. During the first year of life, UTIs are more common in males (Nguyen, 2011). However, the incidence of UTIs among males is low after age of one year and until approximately age of 60 years when enlargement of the prostate

interferes with emptying of the bladder. Therefore, UTI is predominantly a disease of females. Extensive studies have shown that the incidence of bacteriuria among girls age 5 through 14 is 1- 2%. This incidence increases to 5% in girls over age 10. The prevalence of bacteriuria in females increases gradually with time to as high as 10 - 20% in elderly women. In women between the ages of 20 and 40 whom have UTIs, as many as 50% may become reinfected with 1 year. The association of UTIs with sexual intercourse may also contribute to this increased incidence because sexual activity serves to increase the chances of bacterial contamination of the female urethra (Betty *et al.,* 1998).

The incidence of UTI is greater in women as compared to men, which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (Schaeffer *et al.,* 2001). Factors that may influence the risk of UTI include recent sexual intercourse (Nicolle *et al.,* 2001) use of a diaphragm with permicide and delayed postcoital micturation.

Urinary tract infections (UTIs) are a common cause of morbidity in children (Paterson, 2004). UTI causes significant illness in the first 2 years of life. Misdiagnosis very often leads to avoidable ill health and long-term renal damage. At least 8% of girls and 2% of boys have urinary tract infections in childhood, and between 30% and 40% have another episode within two years. Male to female ratio was 1:104. Nearly all UTIs are ascending infection.

Urinary tract infections (UTIs) are a common complication of pregnancy. Several anatomical and hormonal changes in pregnant women lead to ureter dilatation and urinary stasis (Briggs *et al.,* 2004). Symptomatic UTI occurs in 1% to 2% of pregnancies, while asymptomatic bacteriuria has been reported in 2% to 13% of pregnant women**.** Untreated UTIs can lead to complications, such as pyelonephritis, low-birth-weight

infants, premature delivery, and stillbirth, therefore, prompt treatment of symptomatic UTIs and asymptomatic bacteriuria is warranted in pregnant women.

The incidence of UTI in men's ages 15 to 50 years were very low**.** The female urethra is less than two inches long, and microorganisms traverse it readily. It is also closer to the anal opening than the male urethra and its contaminating intestinal bacteria. These considerations are reflected in the fact that the rate of urinary tract infections in women is eight times that of men (Susan, 2005).

2.1.6. Urinary tract infections

2.1.6.1. Types of urinary tract infection

A urinary tract infection is an infection involving the organs that produce urine and carry it out of the body. These structures include the kidneys, ureters (long, slender tubes connecting the kidneys with the bladder), bladder and urethra. Doctors often divide urinary tract infections into two types, lower tract infections and upper tract infections (Grabe *et al.*, 2008).

- **Lower tract infections in women:** Infection of the bladder is called cystitis (bladder infection). Bacteria normally found in the intestine are the main cause of lower urinary tract infections. These bacteria spread from the anus to the urethra and bladder, where they grow, invade the tissue and cause infection (Figure 4A).

- **Upper tract infections in women:** These involve the ureters and kidneys. These infections are called pyelonephritis or kidney infections. Upper urinary tract infections usually occur because

bacteria travel up from the bladder into the kidney. Sometimes, they occur when bacteria travel from other areas of the body through the bloodstream and settle in the kidney (Figure 4A).

- **Lower tract infections in men:** These include cystitis (bladder infection) and urethritis (infection of the urethra). Lower urinary tract infections commonly are caused by intestinal bacteria, which enters and contaminate the urinary tract from below, usually by spreading from the skin to the urethra and then to the bladder. Urethritis can be caused by microorganisms that are transmitted through sexual contact, including gonorrhea and Chlamydia. Another form of male urinary infection is prostatitis, which is an inflammation of the prostate (Figure 4B).

- **Upper tract infections in men:** These involve the ureters and kidneys and include pyelonephritis (kidney infection). Upper tract infections often occur because bacteria have traveled upward in the urinary tract from the bladder to the kidney or because bacteria carried in the bloodstream have collected in the kidney (Figure 4B).

Women are affected much more often than men because women have short urethras that allow relatively easy passage of bacteria into the bladder. Sexual intercourse can cause bacteria to spread upward into the bladder. Also, the use of contraceptive diaphragms and spermicides may change the normal bacterial environment around the urethra and make infection more likely (Donders, 2010).

In pregnant women, temporary changes in the physiology and anatomy of the urinary tract make expectant mothers prime candidates for cystitis and pyelonephritis. Kidney and bladder infections can pose a serious risk to pregnant women and their unborn children because they

increase the risk of premature contractions or delivery and sometimes death of the fetus or newborn infant (Fitz and Graziano, 2007).

Most cases of urinary tract infections occur in women. Of those that occur in men, relatively few affect younger men. In men older than 50, the prostate gland (a gland near the bottom of the bladder, close to the urethra) can enlarge and block the flow of urine from the bladder. This condition is known as benign prostatic hyperplasia or BPH. This condition can prevent the bladder from emptying completely, which increases the likelihood that bacteria will grow and trigger an infection. Cystitis is more common in men who practice anal intercourse and in those who are not circumcised (Najar *et al.*, 2009). Other factors that increase the risk of urinary infections include an obstruction, such as that caused by a partial blockage of the urethra known as a structure, and non-natural substances, such as rubber catheter tubes (as may be inserted to relieve a blockage in the urethra.

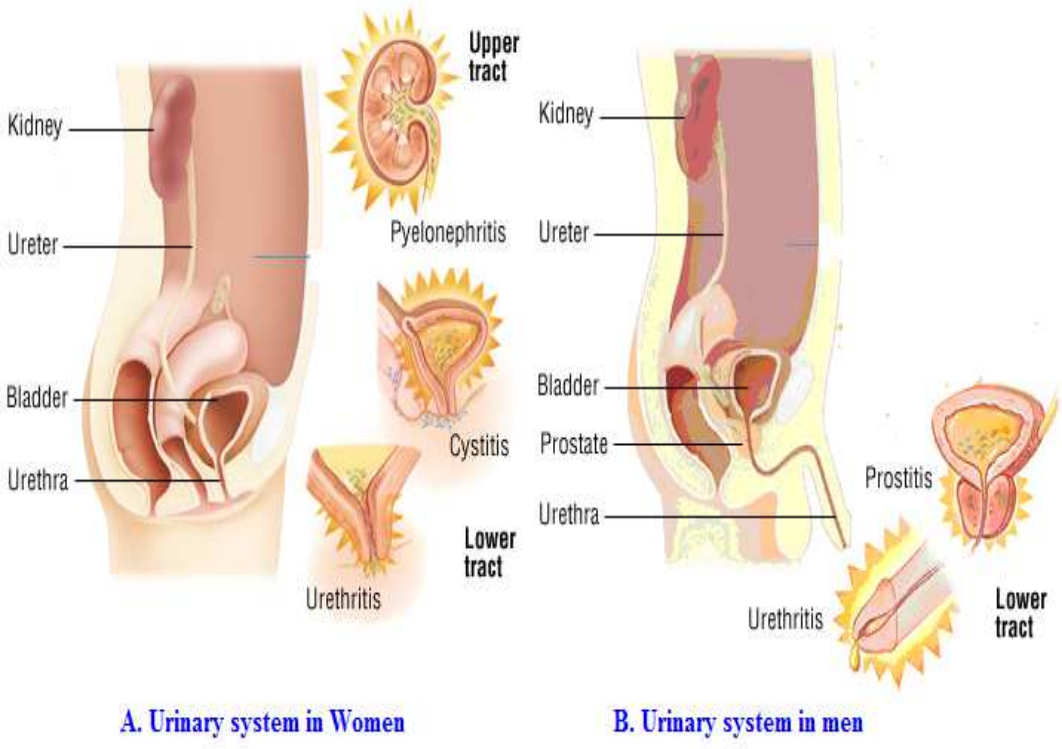


Figure 4. Urinary system of women (A) and men (B) (Grabe *et al.*, 2008).

2.1.6.2. Pyelonephritis

Acute pyelonephritis: It is a clinical syndrome characterised by flank pain, tenderness or both, and fever. It is often associated with dysuria, urgency and frequency. However, these symptoms can also occur in the absence of infection (in case of in renal infarction or renal calculus) (Yoshikawa *et al.*, 1996).

Chronic pyelonephritis: This may rise from either infection or metabolic disorders. It refers to pathologic changes in the kidney caused by infection only. However, identical pathologic alterations are found in several other entities, such as chronic urinary tract obstruction, analgesic nephropathy, hypokalemic nephropathy, vascular disease and uric acid nephropathy (Yoshikawa *et al.*, 1996; Orenstein and Wong, 1999). In chronic pyelonephritis, one or both kidneys contain gross scars, but even when involvement is bilateral, the kidneys are not equally damaged. This uneven scarring is useful in differentiating chronic pyelonephritis from diseases that cause symmetrical contracted kidneys- for e.g. chronic glomerulonephritis.

In severe pyelonephritis, the kidney is somewhat enlarged and discrete, yellowish, raised abscesses are apparent on the surface. The pathognomic histologic feature is suppurative necrosis or abscess formation within the renal substance (Orenstein and Wong, 1999).

Patients with acute pyelonephritis present with flank, low back or abdominal pain as well as fever, rigors, headache, nausea, vomiting and malaise. Symptoms and signs of cystitis may or may not be present. A wide spectrum of illness is encountered in person with acute pyelonephritis, ranging from mild disease to Gram-negative septicaemia.

These latter complications are usually associated with urinary tract obstruction, diabetes and immunosuppression (Ahmed, 2004).

Patients with pyelonephritis are usually unwell with back (lion) pain, high temperature and shaking episodes (rigors). Symptoms of cystitis may or may not be present as well. Pyelonephritis can permanently damage the kidney causing fibrosis (scaring). Repeated attacks of pyelonephritis can, overtime, cause the kidneys to stop working (Will *et al.,* 2005).

There are approximately 250,000 cases of acute pyelonephritis each year, resulting in more than 100,000 hospitalisations. The most common etiologic cause is infection with *Escherichia coli.* Urine cultures are positive in 90 percent of patients with acute pyelonephritis, and cultures should be obtained before antibiotic therapy is initiated. Outpatient oral antibiotic therapy with a fluoroquinolone is successful in most patients with mild uncomplicated pyelonephritis. Most cases of "community-acquired" pyelonephritis are due to bowel organisms that enter the urinary tract. Common organisms are *E. coli* (70-80%) and *Enterococcus faecalis.* Hospital-acquired infections may be due to coliforms and *enterococci*, as well as other organisms uncommon in the community (e.g. *K. pneumoniae Pseudomonas aeruginosa*). Most cases of pyelonephritis start off as lower urinary tract infections, mainly cystitis and prostatitis (Ramakrishnan and Scheid, 2005).

2.1.6.3. Cystitis

It has been used to describe the syndrome involving dysuria, frequency, urgency of micturition and occasionally suprapubic tenderness. However, these symptoms may be related to lower urinary tract inflammation caused by urethritis (Stephan, 2003).

Patients considered to have acute uncomplicated cystitis are those who present with a less than 1-week history of dysuria, pyuria, frequency, or urgency, alone or in combination, and non pregnant women; in these patients, *Escherichia coli* is the most common pathogen (Judith *et al.*, 2002) .

Acute cystitis generally occurs in women. Patients usually present with dysuria, frequency, urgency, voiding small amounts of urine, incontinence and suprapubic or pelvic pain (Ahmed, 2004). The typical symptoms of cystitis are frequency (frequent passing of urine) and dysuria (pain of stinging on passing urine). There may be a mild temperature and suprapubic discomfort. The urine may be cloudy or blood-stained (haematuria) (Will *et al.*, 2005). Cystitis is a common inflammation of the urinary tract bladder in females. Symptoms often include dysuria (difficult painful, urgent urination) and pyuria. Most cases are due to infection by *E.coli*; also secondary bacterial cause is the Coagulase negative *Staphylococcus saprophyticus* (Gerand *et al.*, 2007).

2.1.6.4. Recurrences of urinary tract infection

It may be, due to relapses or re-infections. Relapses of bacteriuria refer to a recurrence of bacteriuria with the same infecting microorganism that was present before therapy was started. This is caused by the persistence of the organism in the urinary tract (Schaeffer, 1990). Re-infection is a recurrence of bacteriuria with a microorganism different from the original infecting bacterium it is a new infection. Re-infection may occur with the same microorganism, which may have persisted in the vagina or feces. This can be mistaken for a relapse (Elder, 2004).

2.1.6.5. Urosepsis

Urinary tract infection (UTI) is the most frequent bacterial infection in infants younger than 90 days, and mainly affects uncircumcised male infants are at higher risk for infection with highly virulent uropathogenic *E. coli* strains than are females. Preputial colonisation may have a key role in the selection of such strains (Bonacorsi *et al*., 2000).

The term is commonly used to describe the sepsis syndrome caused by urinary tract infection. It includes clinical evidence of urinary tract infection plus two or more of the following:

Temperature- >38º C or < 36º C
Heart rate - > 90 beats/min
Respiratory rate- > 20/min or $PaCO_2$ < 32 mm of Hg
White blood count- > 12,000 cells/cumm (Paterson, 2004).

2.1.6.6. Chronic urinary tract infection

True chronic infection should really mean persistence of the same organism for months or years with relapses after treatment. Re-infection does not mean chronicity any more than repeated episodes of pneumonia indicate chronic pneumonia (Orenstein and Wong, 1999).

The symptoms associated with UTIs have been characterised according to the site of infections. Patients who have been diagnosed with acute cystitis usually present symptoms of micturition (urge to urinate frequently), dysuria (difficulty or painful discharge of urine), discomfort or pressure in the lower abdomen, cramping of pelvic area and strong odor of urine accompanied by the presence of white blood cells (pyuria) (Hooton, 2000 and Foxman, 2010). Patients with acute pyelonephritis usually present the same symptoms of lower UTIs lasting more than one week. In addition to these symptoms, patients with pyelonephritis also develop

nocturia (excessive urination at night), as well as chills, persistent fever, pain at the waist area, vomiting and nausea (Hooton and Stamm, 1997). Infections in the urinary tract can also be asymptomatic (Kass and Finland, 2002).

2.1.6.7. Catheter-Associated Urinary Tract Infection (CAUTI)

Catheter-associated bacteriuria is the most common health care associated infection worldwide and is a result of the widespread use of urinary catheterisation, much of which is inappropriate, in hospitals and long-term care facilities (Hooton *et al.,* 2010). CAUTI can lead to such complications as cystitis, pyelonephritis, Gram-negative bacteremia, prostatitis, epididymitis, orchitis in males, less commonly, endocarditis, vertebral osteomyelitis, septic arthritis, endophthalmitis and meningitis in all patients. Complications associated with CAUTI cause discomfort to the patient, prolonged hospital stay, and increased cost and mortality (Magill and Hellinger, 2012)

2.1.6.8. Prostatitis

Men become more susceptible to UTIs after 50 years of age, when they begin to develop prostate problems. Benign prostatic hyperplasia (BPH), enlargement of the prostate gland, can produce obstruction in the urinary tract and increase the risk for infection. In men, recurrent urinary tract infections are also associated with prostatitis, an infection of the prostate gland (Franco, 2005). Although only about 20% of UTIs occur in men, these infections can cause more serious problems than they do in women. Men with UTIs are far more likely to be hospitalised than women. Acute bacterial prostatitis is caused by uropathogens, presents with a tender prostate gland, and responds promptly to antibiotic therapy. Chronic

bacterial prostatitis is a subacute infection, may present with a variety of pelvic pain and voiding symptoms, and is characterised by recurrent urinary tract infections (Lipsky, 1999)

2.1.6.9. Suspected cancer

Bladder cancer is a common malignancy, worldwide; it is the seventh most prevalent cancer, accounting for 3.2% of all malignancies (Beaglehole *et al.*, 2004). Nitrate contamination of drinking water was reported as a risk of bladder cancer. Nitrates are endogenously reduced to nitrites, which through subsequent nitrosation give rise to highly carcinogenic nitroso compounds (Jankovic and Radosavljevic, 2007). Other etiological factors implicated in the development and progression of bladder cancer includes urinary tract infections (UTIs) including bacterial, parasitic, fungal, and viral infections; urinary lithiasis and pelvic radiation (Pasin *et al.*, 2008). Bladder cancer is a common malignancy in Egypt. A history of urinary tract infection can be considered as a risk factor for bladder cancer. *Escherichia coli* infection is responsible for 70% of urinary tract infection (El-Mosalamy *et al.*, 2012).

2.1.6.10. Urinary stones

The term ‘infection stones’ refers to calculi that following urinary tract infection (UTIs) caused by urease-producing Gram-negative organisms. They consist of magnesium ammonium phosphate, monoammonium urate and carbonate apatite. Alkaline urine is most favorable to their formation. Urinary tract obstruction, neurogenic bladder, voiding dysfunction, temporary or indwelling urinary catheters, distal renal tubular acidosis and medullary sponge kidney are considered the main risk factors for developing infection stones. The relationship between urinary stones and UTIs is well known and shows two different clinical pictures:

(1) stones that develop following UTIs (infection stones) which play a key role in stone pathogenesis, and (2) stones complicated by UTIs (stones with infection) which are metabolic stones that passively trap bacteria from coexistent UTIs and may consist of calcium or non-calcium (Miano *et al.*, 2007).

2.2. CAUSATIVE BACTERIAL AGENTS OF URINARY TRACT INFECTIONS

E. coli accounts for 85% of community acquired and 50% of hospital acquired urinary tract infections. Gram negative bacteria such as *Klebsiella* and *Proteus;* and Gram positive *Enterococcus faecalis* and *Staphylococcus saprophyticus* are causative agents for the remainder of community acquired infections (Kennedy *et al.*, 1965). The remainder of hospital acquired infections usually occurs after colonisation with *Klebsiella, Enterobacter, Citrobacter, Serratia, Pseudomonas aeruginosa, Providencia, E. faecalis, or S. epidermidis* (Kennedy *et al.,* 1965). Notably, the patient's age may influence they type of infective organism present with *Staphylococcus saprophyticus* now accounting for 10% of UTIs in young females compared to less than 1% in elderly female patients.

Escherichia coli predominate as the most common urinary pathogen accounting for at least 85% of community-acquired urinary tract infections **(**Sobel, 1991). *Escherichia coli* are the most common infecting agent in the urinary tract, targeting most frequently neonates, preschool girls, sexually active women and elderly women. While UTIs can be caused by other bacterial strains, including *Pseudomonas aeruginosa, K. pneumoniae Enterococcus* spp. and *Proteus mirabilis, Escherichia coli* is identified as the etiologic agent at least 75% of women who present symptoms of cystitis. *E. coli* also accounts for 90 to 100% of bacterial infection in the kidneys or acute pyelonephritis (Jones *et al.*, 1999).

During 1999, a total of 13,774 non hospital urine samples were analysed and a total of 2798 strains were isolated. About half of these were *E. coli* with *Proteus mirabilis, Enterococcus spp.* and *Klebsiella pneumonia* occurring in relatively small numbers. Non-glucose-fermenting Gram negative bacilli and other enterobacteria made up 27% of isolates; Gram positive cocci, 19% (*Staphylococcus* spp. 10% and *Streptococcus* spp. 9%) and yeasts, 8% (Rosa *et al.,* 2001).

Catheter-associated UTIs are common and often polymicrobial and the most causative agents include *Pseudomonas aeruginosa* and other nosocomial Gram-nagative rods, often with more resistant susceptibility profiles; *Enterococci* and *Candida* species (Judith *et al.,* 2002).

Escherichia coli causes 75 to 90 percent of episodes of acute uncomplicated cystitis, and *Staphylococcus saprophyticus* accounts for 5 to 15 percent, mainly in younger women, *Enterococci* and aerobic Gram-negative rods other than *E. coli*, such as *Klebsiella pneumoniae* and *Proteus mirabilis,* are isolated in the remainder of these cases (Ronald, 2002).

During 2001, 16500 urine samples were analysed, of which 4260 (25.8%) had significant bacteriuria and the pathogens which causing the predominant urinary tract infection are Gram negative rods as *E.coli*, *Klebsiella pneumoniae*, *Proteus* species, *Citrobacter* isolates, *Mycobacterium tuberculosis,* where as other isolates included other organisms as *Pseudomonas aeruginosa***,** *Enterococcus* species, *S.aureus* and *S.agalactiae,* which had very low incidence and were predominantly isolated from hospitalised patients (Raka *et al.,* 2004).

Urinary tract infection (UTI) is the second most common infectious presentation in community practice. All over the world, *Escherichia coli* accounts for 75% to 90% of UTI isolates, and *Staphylococcus*

saprophyticus accounts for 5% to 15% of cases of uncomplicated cystitis (Astal, 2005).

Zhanel *et al.* (2005) determined that the most common organisms were *Escherichia coli* (57.5%), *Klebsiella pneumoniae* (12.4%), *Enterococcus* spp. (6.6%), *Proteus mirabilis* (5.4%), *Pseudomonas aeruginosa* (2.9%), *Citrobacter* spp. (2.7%), *Staphylococcus aureus* (2.2%), *Enterobacter cloacae* (1.9%), Coagulase-negative *Staphylococci* (1.3%), *Staphylococcus saprophyticus* and *K. pneumoniae*. (1.2%), *Enterobacter aerogenes* (1.1%) and *Streptococcus agalactiae* (1.0%).

A variety of Enteropathogenic bacteria are known to cause UTI worldwide. *E. coli* being the predominant aetiological agent in community practice. Other bacterial agents include species of *Klebsiella, Enterobacter, Proteus, Pseudomonas, Staphylococcus, Streptococcus* and *Enterococcus faecalis* (Nerurkar *et al.,* 2012). The Honeymoon women were showing higher rate of UTI than other women in (87 from 95) (91.5 %) and (19 from 35) (54.3%) respectively. *E. coli* (46%) and *Staphylococcus aureus* (42%) were predominant isolate in honeymoon (Nada Khazal *et al.*, 2013).

E.coli is the most frequent infecting organism in acute infection. *Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, and Enterococci* species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population (Monali *et al.,* 2013). *E.coli* was the leading uropathogen 56.82% followed by *Klebsella* spp. 15.90%, *Pseudomonas* spp. *6.82%, Staphylococcus aureus 6.82%, Enterococcus* spp. *4.55% Candida* spp. *4.55%, Enterobacter* spp. *2.27% and Streptococcus* spp. 2.27% respectively. The most common uropathogen was *E.coli.* Higher prevalence of UTIs was observed in female population. The prevalence of UTIs was highest for age group of 21 to 40 years (Ghazi *et al.,* 2013).

Lewis *et al.,* (2013) reported that 460 women recruited, 425 MSU samples were processed and 204 UTI pathogens were identified in 201 samples. Most pathogens were Gram negative bacilli (182; 89.2%) and 22 (10.8%) were Gram positive cocci. *E. coli* were the most frequent GNB (160; 79.6%), while *Enterococcus faecalis* was the predominant GPC (8; 4.0%).

Poonam *et al.*, (2013) reported that Urinary tract infections (UTIs) are a common problem in women at all stages of life; this is particularly true of pregnant women. Isolated and diagnosed bacteria from pregnant women were: *Escherichia coli* (43.47%), *Staphylococcus aureus* (23.91%), *Proteus vulgaris* (19.56%), *Klebsiella pneumoniae* (5.43%), Coagulase-negative *Staphylococci* (7.6%). The isolated uropathogens showed resistant to ampicillin, cotrimoxazole, ciprofloxacin, and ceftazidime and sensitive to nitrofurantoin and cephotaxime. In conclusion, *E coli* were found to be the common cause of UTI among the pregnant women.

2.3. ANTIBIOTIC SUSCEPTIBILITY PATTERN TO ANTIMICROBIAL AGENTS

Widespread use of antimicrobial agents often leads to the selection of multi-drug resistant microorganisms. Acquired or emerging bacterial resistance to one or several antimicrobial agents is a global problem. Many microorganisms have become resistant to antimicrobial agents (Kader *et al.,* 2001).

The norfloxacin is more effective than cotrimoxazole when tested against Gram negative isolates. The activity of norfloxacin was 85.19 % where as cotrimoxazole has 42.86% activity against Gram negative urinary tract isolates. On the other hand, 65.15% and 47.88% Gram positive urinary tract isolates were sensitive to norfloxacin and cotrimoxazole respectively. This difference might to due to the increasing resistance of

microorganism to cotrimoxazole. The reason being the wide spread indiscriminate use of cotrimoxazole in varieties of infections by local quakes and general practitioners. Again, cotrimoxazole is very old chemotherapeutic agent while norfloxacin is comparatively new drug (Norrby *et al.,* 1987).

Nitrofurantoin showed the lowest resistance rate for *E. coli* and amoxicillin the highest. Among the Gram-negative isolates of the Enterobacteriaceae group, *Proteus* spp. showed the highest resistance to trimethoprim. The *E. coli* showed high rates of resistance to amoxicillin, amoxicillin-clavulanate and ciprofloxacin (Kader *et al.,* 2001).

The antimicrobial agent with the highest levels of activity against Gram-negative bacilli was amikacin which was restricted to hospital use while cefuroxime, ciprofloxacin, fosfomycin, gentamicin and nitrofurantoin showed acceptable levels of activity. Nitrofurantoin was active against all strains of *Staphylococcus aureus,* but there is a reduction in the activity of amoxycillin with clavulanate, cotrimoxazole and quinolones to *E.coli* (Rosa *et al.,* 2001*).*

The efficacy of trimethoprim is similar whether it is used alone or in combination with sulfamethoxazole and in some locales, such as the southeastern and western United States, including southern California, resistance to trimethoprim-sulfamethoxazole has become widespread and it detected in up to 18% of the pathogens cultured from the urine of women with acute cystitis (Manges *et al.,* 2001).

Uncomplicated acute pyelonephritis was treated by a fluoroquinolone or trimethoprim-sulfamethoxazole if the organism is susceptible to it **and** amoxicillin or amoxicillin-clavulanate is an alternative for infections caused by Gram-positive organisms, also nitrofurantoin, trimethoprim,

ciprofloxacin, or norfloxacin diminishes recurrences by 95% and may prevent pyelonephritis (Hooton, 2001).

Antibiotics used in therapy of UTI are usually able to reach high urinary concentrations, which are likely to be clinically effective. Fluoroquinolones are preferred as initial agents for empiric therapy of UTI in areas where resistance is likely to be of concern (Acar and Goldstein, 1997). This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens. Ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral and intravenous formulations. Ciprofloxacin has shown an excellent activity against pathogens commonly encountered in complicated UTIs.

Resistance rates in southern Europe and Bangladesh have been as high as 30 to 50 percent (Perfetto and Gondek, 2002). *E.coli* resistance was most common to ampicillin (29.8%) and sulfamethaxazole (14.1%) followed by trimethoprim (14.8%), trimethoprim sulfamethaxazole (14.1%) and nalidixic acid (5.4%) also resistance of *E.coli* to co-amoxiclav, mecillinam, cefadroxil, nitrfurantoin, fosfomycin, gentamicin and ciprofloxacin was <3% but in Portugal, co-amoxiclav resistance was (9.3%) as was resistance to the quinolones but nalidixic acid and ciprofloxacin were (11.6% and 5.8%, respectively). *Proteus mirabilis* was less resistance to ampicillin (16.1%) and more resistance to trimethoprim (25.5%) than *E.coli*, where as *Klebsiella pneumoniae* were more resistance to ampicillin (83.5%) and fosfomycin (56.7%). Other *Enterobacteriacae* were more resistance to the broad spectrum ß-lactams (ampicillin 45.9%, co- amoxiclav 21.3% and cefadroxil 24.6%), nitrofurantoin (40.2%) and fosfomycin (15.6%), but resistance development of *Staphylococcus saprophyticus* was rare (Kahlmeter, 2003).

Fluroquinolones are active against *Staphylococcus saprophyticus* and most typical Gram- negative uropathogens, but against only 60 to 70 % of *Enterococci*. Nitrofurantoin was considerably less active than trimethoprim-sulfamethoxazole or the fluoroquinolones against aerobic Gram-negative rods other than *E.coli* and it was inactive against *Proteus* and *Pseudomonas* species. The use of beta-lactams (e.g., ampicillin and amoxicillin) should be avoided because of frequent bacterial resistance to these agents and low cure rates. Amoxicillin-clavulanate may be somewhat more active, but generally is not recommended because of its high cost and frequent adverse gastrointestinal effects (Stephan, 2003).

The resistance rates for strains of *E. coli* isolated from the hospitalised patients were 61% to amoxicillin, 35% to amoxicillin-clavulanate, 47% to trimethoprim, 38% to ciprofloxacin, 31% to cephalexin and 13% to gentamicin. These rates were higher than those from the outpatients (52, 36, 40, 32, 29 and 5%) respectively, Out of the 42 (2%) multi-drug resistant *E. coli* and *Klebsiella pneumoniae*, 23 (1%) were found to be positive for extended spectrum beta lactamase. The resistance rate to ciprofloxacin among the isolates of *E. coli, K. pneumoniae*, *Proteus* spp. and *Pseudomonas* spp. was higher. Also, *Acinetobacter* spp. showed the highest rate of resistance to ciprofloxacin and gentamicin, while *Pseudomonas* isolates were resistant to ceftazidime and gentamicin (Kader *et al.,* 2001).

Forty-one medical centres (30 from the USA and 11 from Canada) participated, with each centre submitting up to 50 consecutive outpatient midstream urine isolates. Of the 1990 isolates collected, 75.1% (1494) were collected from the USA and 24.9% (496) were collected from Canada. Among all 1990 isolates, 45.9% were resistant to ampicillin, 20.4% to SMX/TMP, 14.3% to nitrofurantoin, 9.7% to ciprofloxacin and 8.1% to levofloxacin. Fluoroquinolone resistance was highest in patients >

or = 65 years of age. For the 1142 *E. coli* isolates, resistance rates were: ampicillin 37.7%, SMX/TMP 21.3%, ciprofloxacin 5.5%, levofloxacin 5.1% and nitrofurantoin 1.1%. For all 1990 isolates and for the 1142 *E. coli* only, resistance rates were significantly higher in US compared with Canadian medical centres (Zhanel *et al.*, 2005).

Escherichia coli were the most frequently isolated organism (41.5%), and it was highly susceptible to chloramphenicol, ciprofloxacin, ceftizoxime and amikacin (Al-Haddad, 2005). The most common causative agent was *Escherichia coli* (87% of cases) followed by *Klebsiella pneumoniae* (10%). Resistance to ampicillin (74.2%) and cotrimoxazole (61.3%) was significant in all isolates. Nitrofurantoin was the most active agent against *E coli* (2.2% resistant isolates), followed by amikacin (4.9%), ceftriazone (7.5%) and ciprofloxacin (12%). None of the isolates from Group I patient was resistance to ciprofloxacin and low resistance rate (7.1%) was noted for amikacin. In Group II patients, none of the isolates were resistance to amikacin, and ceftriaxone was the second most suitable antibiotics (resistance rate 2.2%). In group III patients, the lowest resistance rate was against nitrofurantoin (2.7%) (Yuksel *et al.*, 2006).

In India antimicrobial susceptibility pattern of uropathogens vary widely by region. High resistance rates to oral antibiotics have been observed, probably due to uncontrolled consumption of these antibiotics. Resistance to amikacin, piperacillin- tazobactum and meropenem are low, likely reflecting lower usage of these drugs. The worldwide trend of empirically treating community acquired UTI may not apply for specific geographical regions such as India, where decreased susceptibility rates are documented for common urinary pathogens (Kothari and Sagar, 2008).

E.coli showed variable antimicrobial resistance to different antibiotics. 92%, 86%, 80%, 62%, 47%, 20% and 4% of the isolates were found to be resistant to ampicillin, cotrimoxazole, ciprofloxin, gentamicin, nitrofuratoin and amikacin, respectively. The most effective *in vitro* agents were found to be amikacin followed by gentamicin (among the parenterals), and ciprofloxin among the orally given ones. A higher prevalence of UTIs was observed in the female population and *E. coli* showed no resistance to nitrofuratoin in age groups of 50+ and 70+ in both genders (Bashir *et al.,* 2008).

Escherichia coli, the predominant organism isolated, frequently was resistant to commonly prescribed oral antibiotics. Trimethoprim-sulfamethoxazole remains the best empiric antimicrobial therapy for a urinary tract infection, but nitrofurantoin should be considered if *E. coli* is identified (Das *et al.,* 2009).

E. coli isolates were mostly susceptible to nitrofurantoin (71.3%), followed by ciprofloxacin (68.1%); however, only 38.2% of *E. coli* isolates were susceptible to trimethoprim-sulfamethoxazole. Nitrofurantoin may be considered a first-line empiric antibacterial agent for urinary tract infections in outpatients in Tehran, Iran (Kashef *et al.,* 2010).

Most of the urinary isolates showed hundred percent resistant to ampicillin and high degree of resistance to nalidixic acid, nitrofurantoin, cotrimoxazole followed by ciprofloxacin and gentamicin. More than 50% of common pathogens were resistant to ceftriaxone. Uropathogens were more sensitive to cephotaxime, amikacin, ofloxacin and norfloxacin. Uropathogens resistant to 3rd generation cephalosporin are increasing. Irrational and repeated use of antibiotics is the main cause of increasing resistant organism of UTI (Acharya *et al.,* 2011).

More than 80% of the isolates were sensitive to amikacin and nitrofurantoin, while more than 70% was sensitive to norfloxacin, ciprofloxacin and levofloxacin. Very high rate of resistance was seen against cotrimoxazole (81.82%), amoxicillin (77.42%) and amoxi-clav (64.34%). *E. coli* showed high sensitivity to amikacin 98.91% (91), nitrofurantoin 93.48% (86). 75% of *E. coli* isolates were sensitive to minocycline, showing a good utility of this drug for the treatment of outdoor patients with urinary tract infections (Joshi *et al.*, 2011).

Esherichia coli was the most predominant isolate, 53.8% followed by *Klebsiella pneumoniae* 22.4% and *Pseudomonas aeruginosa* 7.6%. All isolates were fully sensitive to ofloxacin, and more than 94% was sensitive to cefuroxime. Apart from group D *Streptococcus*, the overall response to ampicillin by all isolates was less than 15%. The prevalence of multi-resistant *Pseudomonas aeruginosa* in community-acquired urinary tract infections is increasing. All *Pseudomonas aeruginosa* isolates were fully susceptible to cefuroxime and ofloxacin (Ahmad, 2012). It is recommended that cefuroxime and ofloxacin or both are used in the blind treatment of urinary tract infection while awaiting the culture and sensitivity results.

E. coli was the most common isolate (55%) followed by *Enterobacter* (13.3%) and *S. aureus* (11.76%). Susceptibility of *E. coli* to nitrofurantoin was 85.7%, gentamicin 82.5%, cefotaxime 81.9% and ciprofloxacin 78%. High resistance rate was observed among *E. coli* against ampicillin (100%). The sensitivity rates of *Enterobacter* isolates for ciprofloxacin, gentamicin and cefotaxime were 75, 76.4 and 77.7%, respectively. *S.aureus* strains were sensitive to gentamicin (84.2%), nitrofurantoin (75%) and ciprofloxacin (60%). Nitrofurantoin should be used for first line empirical treatment in UTI (Forouzan and Amir, 2013).

Ampicillin showed 100% drug resistance, sulphamethoxazole /trimethoprim (81.25%) and ciprofloxacin and chloramphenicol, both 43.8%. Multi-drug resistance showed in 81.25% isolates. Of those who had history of urinary tract infection, 77.77% exhibited resistance to three or more drugs. However, 96.87% susceptibility was seen to nitrofurantion, ceftriaxone (84.4%), cefotaxime (81.25%) and gentamicin (75.0%). Most uroisolates illustrated high-level of drug resistance (Mucheye *et al.,* 2013).

2.4. *CANDIDA* SPECIES FROM THE UTIs

Candida species are opportunistic pathogens and cause disease in hosts who are compromised by underlying local or systemic pathological processes. *Candida* species can cause a number of diseases ranging from localised mild infections to deep-seated candidiasis (Pfaller, 1996; Coleman *et al.,* 1998; Carrasco *et al.,* 2005; Meurman *et al.,* 2007). Since the 1980s, there has been a significant increase in the number of *Candida* infections, especially in hospitalised patients which regarded to several factors. Predisposing factors include immunosuppression, prolonged administration of antimicrobial agents, surgery, burns, and indwelling catheters intravenous drug use (Benedict and Colagreco, 1994).

Chromagar *Candida* media can be reliably used for isolation of yeasts. Use of this medium even allows mycology laboratories to identify rapidly clinically important species. Chromagar *Candida* culture will also enable the clinician to choose appropriate antifungal drugs and there by decreasing patients mortality and morbidity (Horvath *et al.,* 2003).

Modern intensive care unit technology in conjugation with increasing population of critically ill patients has substantially increased the incidence of candiduria with a mortality exceeding more than 70%. In view of this high mortality, efforts should be made to identify and evaluate intensive

care unit candiduria (Toya *et al.,* 2007). Several reports have indicated that candiduria is a very common infection in hospitalised patients and its incidence is linked to antibiotic usage, long stay in hospitals old age (Weinberger *et al.,* 2003; Dalen *et al.,* 2005).

Candiduria is a common finding. Yeasts can be detected in urine that is contaminated during collection, in patients who have bladder colonisation, and in patients who have upper urinary tract infection that developed either from retrograde spread from the bladder or hematogenous spread from a distant source. Most patients with candiduria are asymptomatic. The rate of development of complications is not known, but appears to be low; candidemia rarely results from asymptomatic candiduria unless obstruction is present or instrumentation of the urinary tract is done (Kauffman, 2005).

The microbiology of candiduria is changing, with 50% of urinary isolates now belonging to non-*Candida albicans* species. The presence of yeast in the urine is associated with increased mortality, especially in patients in ICUs with much comorbidity. However, mortality is not often directly attributable to invasive candidiasis. Nevertheless, candiduria may be a marker for serious underlying illness (Kauffman, 2005).

Many of researchers believed that candiduria is not a marker for disseminated candidiasis (Bukhary, 2008). As a result, detection asymptomatic candiduria from bladder and renal infection is problematic. Nevertheless, candiduria in hospitalised patients in intensive care unit (ICU) can be a relevant marker for systemic candidiasis (Kristina *et al.,* 1999).

Asymptomatic candiduria: For asymptomatic candiduria in a previously healthy individual, the finding should be verified by obtaining a second carefully collected urine specimen. For many patients, the finding of candiduria will not be replicated. For those who have persistent candiduria, treatment of the underlying conditions or removal of risk factors is usually sufficient to clear candiduria and no antifungal agent is needed. Antifungals have not been shown to have a benefit in the absence of clinical evidence of infection of the kidney or collecting system (John, 2011).

Symptomatic candiduria: Symptomatic *Candida cystitis*, in most instances, will respond to fluconazole because the drug is concentrated in urine; remains a highly active antifungal agent against most species of *Candida*, especially *C.albicans*; and is well tolerated and inexpensive. The pharmacokinetics of other azoles is not favorable (Brammer, 1990) and the newer ones remain expensive. For refractory bladder infections, flucytosine, which is also concentrated in urine, may be considered; however, potential toxicity to the bone marrow is a major drawback. Treatment with ≥1 intravenous doses of amphotericin B (AmB) deoxycholate is a third option, because the drug has prolonged urinary excretion and is a potent antifungal compound. Bladder irrigation has been used to treat this disease, but has a high relapse rate and is a strategy rarely needed except for persistent cystitis due to fluconazole-resistant organisms (Glew, 2005).

Several reports showed that the frequency of urinary tract infection (UTI) due to yeasts has increased during the last decades (Laverdiere *et al.*, 2007; Saha *et al.*, 2008). Prolonged hospitalisation, long stay in ICU, urinary tract abnormality, immunocompromised patients, antibacterial therapy with broad spectrum for long time and prophylaxis by antifungal

agents are presented as more important risk factors for UTI (Nayman *et al.,* 2011; Dalen *et al.,* 2005).

Uma chaudhary in their in her study on Rapid identification and antifungal susceptibility pattern of *Candida* isolates from critically ill patients with Candiduria, showed high prevalence rates in females. Most common species isolated were *C.albicans* (34%), *C. dubliniensis* (31%), *C. krusei* (19%), *C. tropicalis* (15%) and *C. glabrata* (1%), with highest susceptibility to Amphotericin B (Chaudary *et al.,* 2009).

In a study by Manisha Jain on Candiduria in catheterised intensive care unit patients, non- *Candida albicans* species emerged as the predominant pathogens and were responsible for 71.4% of nosocomial fungal urinary tract infection. *C.tropicalis* accounted for 52.9% of cases where as *C.albicans* was seen in 28.6% of cases. Candiduria was also seen in extremes of ages (Jain *et al.,* 2011).

Vinitha Mohandas in her study on Candidial isolates from urine samples and its virulence factors showed *C.krusei* (50%) followed by *C.albicans* (25.5%). Biofilm production, Proteinase activity and Phospholipase activity was detected 73%, 80% and 44.14% respectively (Mohandas and Ballal, 2011).

2.5. ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA* SPECIES

Out of 250 high vaginal swabs, *Candida* species were isolated in 100 (40%) of cases. Out of 100, *C. albicans* 30 (30%), *C.tropicalis* 21 (21%), *C. parapsillosis* 10 (10%), *C. parakrusi* 8 (8%), *C.glabrata* 8 (8%) and *C. krusei* 3 (3%) were isolated. In vitro antifungal activity indicated clotrimazole (MIC 16 and 8 μg/ml) effective against 68 (70%) of *Candida*

spp., fluconazole (MIC 64 and 32 μg/ml) effective against 29 (36.2%) and nystatin disc (100 units) was 51 (63.5%) effective. *C. albicans* was mainly isolated. Clotrimazole was more effective as compared to fluconazole and nystatin (Fouzia and Rakhshanda, 2010).

In a study on randomised double blinded study of treatment with Fluconazole and placebo by Sobel *et al.* (2000) among Candiduria patients stated that oral fluconazole is safe and effective in treating symptomatic Candiduria patients. The susceptibility decreased to fluconazole although voriconazole, Amphotericin B and flucytosine continue to show good efficacy and concluded that there is gradual increase in antifungal antifungal resistance in India (Adhikary and Joshi, 2011).

Feglo reported that out of 528 samples tested 67 yielded yeasts giving a prevalence of 12.7%. *Candida albicans* was the commonest species isolated with a prevalence of 33 (49.3%) followed by *Candida glabrata* 12 (17.9%), *Candida tropicalis* 8 (11.9%), *Candida dubliniensis* 4 (6%), *Candida krusei* 3 (4.5%) and *Candida sake* 2 (3%), whilst *Candida guilliermondii* and *Candida parapsilosis* prevalence was 1 (1.5%) each and *Cryptococcus neoformans* prevalence was 3 (4.5%). All the isolates were susceptible to flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole except *C. albicans, C. glabrata, C. tropicalis and C. krusei* all having about 79% susceptibility to flucytosine, amphotericin B, and itraconazole (MICs 0.125-8mg/l). All the *Candida krusei* isolated were resistant to fluconazole (MICs ≥ 64mg/l). Generally yeast resistance ranges from 4.5% to 22.2% to flucytosine, amphotericin B, fluconazole and itraconazole (Feglo and Narkwa, 2012).

The most frequently isolated species were *Candida albicans*, which showed good (100%) susceptibilities to 5-fluorocytosine (5-FC) and fluconazole (FLCZ) but not to voriconazole (VRCZ), followed by *C. glabrata*. ICU was the greatest source of *Candida* positive samples, and

the most relevant underlying diseases of ICU patients were pneumonia followed by renal failure and post-liver transplantation status. Combined isolation with other bacteria was seen in 27 cases (42.9%) in 2009, 25 (33.3%) in 2010 and 31 (31.3%) in 2011 and comparatively often seen in non-ICU patients. Other *Candida* species than *C.albicans* showed significantly decreased susceptibility to FLCZ over these 3 years (*P*=0.004). One hundred (97.1%) of 103 ICU cases were given antibiotics at the time of *Candida* isolation, and the most often used antibiotics were cefazolin or meropenem. In conclusion, *C. albicans* was representatively isolated in *Candida* UTI and showed good susceptibilities to 5-FC, FLCZ and VRCZ, but other *candidas* than *C. albicans* showed significantly decreased susceptibility to FLCZ in the change of these 3 years (Kayo *et al.*, 2013).

Candiduria presents as an increasingly common nosocomial infection, which may involve urinary tract. Spectrum of disease is varying from asymptomatic candiduria to clinical sepsis (Jacqueline *et al.*, 2010). Disease is most commonly caused by *Candida albicans.* Zahra *et al.*, (2013) studied those 402 patients with the age range <1-14 years were sampled (59.2% males and 40.8% females). Prevalence of *Candida* among enrolled patients was found to be 5.2% (71.4% males and 28.6% females). In this study *C. albicans* was identified in 19 cases as the most common yeast followed by nine *C. glabrata* and one *C. krusei*. Urine cultures were yielded more than 10000 CFU/ml in 14.3% of the cases followed by 600-10000 CFU/ml (28.5%) and 100-600 CFU/ml (57.2%). Antifungal susceptibility testing revealed that only one isolate of *C. glabrata* and seven isolates of *C. albicans* was resistant to nystatin and ketoconazole, respectively. However, all tested isolates were resistance to fluconazole (Zahra *et al.*, 2013).

2.6. MOLECULAR CHARACTERISTICS OF ESBL PRODUCING *E.COLI*

2.6.1. Bacterial resistance to antibacterial drugs

The discovery of antibiotics in the late 1920s, and their subsequent use in treating and preventing infections beginning in the 1940s, is undoubtedly one of the great medical breakthroughs in the last 100 years (Davies and Davies, 2010). In the early years of antibiotic treatment, many scientists and doctors believed that infectious disease had been triumphed once and for all (Davies, 2007). And, while it is true that antibiotics have largely nullified several diseases and infections that were once very difficult to treat, there is reason to be concerned that this may not always be the case. Less than a decade after the first antibiotics were introduced in medicine, evidence of bacterial strains resistant to those antibiotics began to surface (Davies and Davies, 2010). Shortly thereafter, scientists uncovered evidence that bacteria were not only capable of developing resistance to one antibiotic, but to multiple antibiotics that were also transferable to sensitive strains (Davies, 2007). The rise of multi-drug resistant (MDR) bacteria is a result of unscrupulous antibiotic use in medicine and agriculture over the last several decades (Mazel and Davies, 1999; Cocchi *et al.,* 2007).

Today, MDR bacteria provide numerous challenges and problems for healthcare providers, including increases in hospital-acquired infections, reduced treatment options, higher morbidity and mortality rates, and healthcare cost increases due to longer hospital stays (Ducel *et al.,* 2002). MDR bacteria may be resistant to a couple of antibiotics, several classes of antibiotics, and in some cases every antibiotic. Even MDR bacteria that are resistance to only a couple of antibiotics can greatly complicate treatment.

Often, such bacteria are resistance to the primary antibiotic preferred for treatment, requiring the use of secondary and tertiary drugs instead, which may be less effective and more toxic to the patient (CDC, 2012). The growing problem of MDR infections is made even more concerning by the fact that new discoveries of antimicrobial agents have been few and far between in recent years (Coates *et al.,* 2011; Davies, 2007). Over the last five decades, only two new classes of antibiotics have reached the market, and current information suggests that no new antibiotic classes will be introduced in the near future. Without the continuous introduction of new antibiotics, as was seen during the first 20 years of their use, the threat of a return to the pre-antibiotic era is very real (Coates *et al.,* 2011).

Antimicrobial resistance among UPEC continues to rise in the United States, contributing to greater difficulty in the management of UTI (Gupta *et al*., 2001; Perfecta and Gondek, 2002). Studies carried out in France by Leflon *et al.* (2000) and in the United States by Kaye *et al.* (2004) have shown a steady increase in resistance to most commonly used antimicrobial drugs for treatment of UTI caused by *E.coli.* The changing etiology of UTI and increasing antibiotic resistance require periodic monitoring and possibly modification of empirical regimens.

The Antimicrobial Availability Task Force (AATF), established by the Infectious Diseases Society of America, identified several particularly problematic pathogens, one of which included extended-spectrum beta-lacatamase (ESβL)-producing Enterobacteriaceae **(**Talbot *et al.,* 2006). ESβLs are enzymes produced by bacteria that confer resistance to multiple antibiotic classes, namely cephalosporins, penicillins, monobactams, and beta-lactamases. Over 500 different ESβLs have been identified, the most common belonging to the CTX and CMY gene families. Infections caused by ESβL producers usually must be treated with a carbapenem

(e.g. imipenem, meropenem). Recently however, ESβL-producing Gram-negative bacteria have been identified that are also resistant to the carbapenem class of antibiotics (Talbot *et al.*, 2006; CDC, 2012).

Urinary tract infections are among the most common infections encountered in clinical practice. Study was conducted to detect extended-spectrum beta lactamase (ESBL) type of resistance in urinary isolates in North Eastern Region of India. In all, 171 isolates of Gram-negative bacilli were detected of which 42 isolates produced ESBL. So the detection rate of ESBL in the study was 24.56%. The ESBL-producing isolates were 19 (28.78%) in males and 23 (21.9%) in females, and this difference was not found to be significant (P >0.05). In 97.61% of isolates, associated resistance was observed for ampicillin and cotrimoxazole. Ciprofloxacin and gatifloxacin showed coresistance of 69.04% and 71.42%, respectively. Associated resistance for amoxicillin with clavulanic acid and piperacillin/tazobactum was 38.09% and 35.71%. All the isolates of Enterobacteriaceae producing ESBL were 100% sensitive to imipenem (Das and Borthaku, 2012).

Chaudhary and Murthy (2013) reported that three hundred urine specimens were studied. Significant bacteriuria was present in 35% of specimen. The most common pathogens isolated were *Escherichia coli* 52.4%. The resistance pattern of uropathogens was for amikacin 19.04%, nitrofurantoin 40%. We found 55% Gram-negative uropathogen harbored the ESBLs. Majority of ESBLs seen in *Klebsiella pneumoniae* 60% and *Escherichia coli* 55%. The ESBLs producing *Escherichia coli* were highly susceptible to imipenum, 90.90% and meropenem, 94.45%. Screening of multidrug resistant bacteria especially GNB poses considerable therapeutic challenges in critical care patients because of the production of ESBLs.

2.6.2. Bacterial mechanisms of antibiotic resistance

In addition to adding and deleting genes on the chromosome, bacteria can change phenotype through the gain or loss of plasmids (Leflon *et al*., 2000). Antibiotic resistance, for example, can be transferred via plasmid and has been demonstrated to be transmitted across bacterial species (Sahm *et al.,* 2001). An outbreak would follow the path of the plasmid (horizontal and vertical gene transfer) rather than clonal spread in the path of a particular bacterial clone. The identification of uropathogenic factors and their mode of transmission between pathogens would greatly assist understanding of UTI epidemiology and pathogenesis and ability to prevent disease via vaccination or other strategies (Foxman and Riley, 2001). These types of studies require epidemiologic methods to collect appropriate sample isolates from well-defined populations and to make appropriate inferences about the findings based on laboratory analyses. The genotypic characterisation of pathogens therefore has become an important objective in epidemiologic investigations of infectious agents.

2.6.3. Detection of Extended Spectrum Beta Lactamase

The increased prevalence of Enterobacteriaceae producing ESBL creates a great need for laboratory testing methods that accurately identify the presence of these enzymes in clinical isolates (Bradford, 2001). Although most ESBL confer resistance to one or more of the oxyimino-β-lactam antibiotics, the β-lactamase does not always increase the MICs to high levels to be called resistant by the Clinical Laboratory Standards Institute (CLSI) interpretive guidelines (Wayne, 2008). The sensitivity and specificity of a susceptibility test to detect ESBL vary with the cephalosporin tested. Several ESBL detection tests that have been proposed are based on the Kirby-Bauer disc diffusion test methodology (Bradford, 2001).

These ESBL enzymes are plasmid borne and have evolved from point mutations altering the configuration of the active site of the original and long known β lactamases designated TEM-1, TEM-2, and SHV-1 (Bradford, 2001). The activity of these enzymes is limited to ampicillin, penicillin, and carbenicillin. Initially these bacteria contained a single ESBL gene, but later multiple ESBL genes are commonly present in a single strain, further complicating the process of detecting them and identifying an appropriate treatment regimen. To date, more than 90 TEM-type and more than 25 SHV-type β lactamases have been identified (Joumana and George, 2003). The AmpC β-lactamases hyper producing Enterobacteriaceae have been reported worldwide, but few data are available about their prevalence in human clinical specimens. These enzymes are numerous, and they mutate continuously in response to the heavy pressure of antibiotic use, leading to the development of ESBL (Joumana and George, 2003).

Lack of awareness, resources and facilities to conduct ESBL identification, contribute to the spread of multi-drug resistance in most Enterobacteriaceae organisms. It has been observed that while antibiotics revolutionised the treatment of infectious diseases in the 20th century, resistance threatens to render these drugs ineffective in the 21st century (Salyers *et al*., 2004).

Rodrigues *et al.,* (2004) conducted a study for the detection of beta lactamases in nosocomial Gram negative clinical isolates. Out of 286 isolates 53% were ESBL producers. Inducible Amp C lactamase production was detected in 7% of the isolates. ESBL was detected in 30.18% of *K. pneumoniae* by PCDDT (phenotypic confirmatory disc diffusion test) and 27.3% by DDST (double disc synergy test). They concluded that detection of ESBL production by PCDDT is a simple and cost effective test. Out of 70 isolates screened for ESBLs, 20% were ESBL

producers of which *Enterobacter* 28.5%, *K. pneumoniae,* 21.2% and *E. coli* 19.2%. Three dimensional tests detected 85.7% ESBL producers whereas only 14.2% were detected by Double disc synergy test.

Out of 9076 isolates 31.9% *E. coli,* 26.2% *P. mirabilis,* 15.1% *K. pneumoniae,* 7.5% *Enterobacter aerogenes* and 7.1% *Providencia stuartii* were ESBL producers. Extended spectrum β-lactamase enzymes (ESBL) are modified β-lactamase enzymes mainly derived from TEM1/2, SHV-1 and CTX-M plasmid mediated enzymes, which hydrolyse expanded spectrum cephalosporin to varying degrees (Thomson *et al*., 2007). The extended spectrum β-lactamase enzymes are widespread all over the world, but the prevalence and phenotypic characteristic among clinical isolates may vary between geographical areas (Sirot *et al.,* 1991; Jacoby *et al.,* 2006). The original TEM was first discovered in *E.coli* isolates in a patient named Temoniera in Greece, but it spread rapidly to other bacteria. The production of plasmid mediated ESBL have emerged as an important mechanism of resistance to β-lactam antibiotics among *E.coli* (Kaye *et al.*, 2004).

Parul *et al.,* 2008 reported that resistance to broad spectrum β lactams, mediated by extended spectrum beta lactamase (ESβL) and AmpC βL enzymes is an increasing problem worldwide. Presence of these in clinical infections can result in treatment failure if one of the second or third generation cephalosporins is used. In this study, a total of 250 *Escherichia coli (E. coli)* isolates were subjected to double disc test and AmpC disc test for the detection of ESβL- and AmpC βL-producing strains, respectively. Prevalence of ESβL- and AmpC βL-producing strains among *E.coli* isolates, over a 3-month-period in the hospital-based population of Jaipur, was 64.80% (162/250). AmpC βL producers were 24.00% (60/250) and co-existence of ESβL and AmpC βL was detected in 8.00% (20/250) of the isolates.

Hassan *et al.,* (2013) studied that the overall prevalence of ESBL-producing isolates was 4.8% (253/5256). Most isolates (80%) were from the inpatient department. The ESBL phenotype was more frequently detected in *K. pneumonia*. CTX-M genes were the most prevalent ESBL genes, detected in 82% of the studied isolates. The ESBL producers demonstrated a high multidrug resistance rate (96.6%). In transconjugation assay, the same ESBL gene pattern was transmitted from 29.7% of *K. pneumoniae* donors to the recipient strain, and the latter exhibited concomitant decreased aminoglycosides and cotrimoxazole susceptibility. The presence of ESBL screen-positive but confirmatory-negative isolates (8.9%). Phenotypic tests for the production of AmpC β-lactamase tested positive in 52% of these isolates.

3. MATERIALS AND METHODS

3.1. STUDY OF PREVALENCE OF URINARY TRACT INFECTIONS

3.1.1. Study area and specimen collection

Study area: Pattukkottai is located along the southeast coast of India in the East-central region of Tamil Nadu. The coast of the Bay of Bengal is just 12 km away from this town. A total of 2400 midstream urine samples were collected into sterile screw-capped containers containing few crystals of boric acid as preservative from outpatients who had visited the private hospital, Pattukkottai to see doctors with various complaints which were diagnosed tentatively as symptoms of urinary tract infection (UTI). The consulting doctors had then referred the patients to the Gangasaras Diagnostic and Research laboratory for urine mcs (microscopy, culture and sensitivity) investigation for the purpose of making definite diagnosis. Recruited outpatients were instructed on how to collect the samples. All collected samples were appropriately labeled and processed immediately. The study was carried out between January 2012 and June 2013. Samples recovered from inpatient and outpatients of the hospitals were received from various specialties like Medicine, Surgery, Obstetrics and Gynecology, Pediatrics, Orthopedics, Nephrology and Intensive Care Units. Patient's history and provisional diagnosis of the infection were obtained from hospital records. Pattukkottai is located along the southeast coast of India in the East-central region of Tamil Nadu. The coast of the Bay of Bengal is just 12 km away from this town.

Method of specimen collection: Midstream urine samples were collected. Every patient was given a sterile wide mouth container and explained the proper method of collection of urine to avoid contamination. Male patients were instructed to clean their genital area before voiding. Female patients were instructed to clean the vulva and perineum with soap and water, dry the area. They were told to provide 10 ml of urine sample. Collected urine samples were processed without delay. The specimens were processed according to standard bacteriological methods and identified by standard conventional methods.

Inclusion criteria

Both male and female patients having clinically suspected UTI were included in the study. Clinical diagnostic criteria – dysuria, frequency, urgency and fever.

Exclusion criteria

- Patients on long term antibiotic therapy prior to or during the investigation.
- Persons who are HIV positive.
- Patients having malignancies.

3.1.2. Wet film examination:

All samples that recorded significant bacterial counts were subjected to urine microscopy test to detect presence of five pus cells per high power focus using X40 objective microscopically. All samples that were positive for significant bacterial count and also recorded five pus cells and above were then cultured on laboratory media. Similarly presence of bacteria,

casts, crystals, RBCs was noted. Another drop of uncentrifuged urine sample was placed on a clean slide and was allowed to air dry. This smear was Gram stained and examined under oil immersion. Presence of at least one organism per field was considered significant.

3.2. ISOLATION AND IDENTIFICATION OF BACTERIA CAUSING UTIs.

3.2.1. Isolation of bacteria and colony count

A standard bacteriological loopful of each urine sample (0.01ml) was spread over the surface of sterile Nutrient agar, MacConkey agar and Blood agar plates (Himedia). After inoculation, the plates were left on the bench for sometime in order to allow the urine to be absorbed into the agar medium. The plates were then inverted and incubated at 37^{o}C for 18-24 hours. The number of bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria per milliliter of urine. A significant bacterial count was taken as any count equal to or in excess of 10^{5} per milliliter.

Media for culture: Nutrient agar, MacConkey agar and Blood agar are usually sufficient for the recovery of the organisms.

3.2.1.1. Nutrient agar

Beef extract	: 3.0g
Yeast extract	: 3.0g
Peptone	: 5.0g
Sodium chloride	: 5.0g
Agar	: 15g

The contents were suspended in one liter of distilled water bring to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes, pH was adjusted at 7.4 ± 0.2 before sterilisation.

3.2.1.2. MacConkey Agar

Pancreatic digest of gelatin	: 17.0g
Pancreatic digest of casein	: 1.5g
Peptic digest of animal tissue	: 1.5g
Lactose	: 10.0g
Bile salts	: 1.5g
Sodium chloride	: 5.0g
Neutral red	: 0.03g
Crystal violet	: 0.001g
Agar	: 13.5g
Distilled water	: 1000 ml

The contents were suspended in one liter of distilled water. Mix thoroughly heat with frequent agitation and boil for 1 min to completely dissolve the powder and autoclaved at 121ºC for 15 minutes.

3.2.1.3. Blood haemolysis Test

Blood agar supports the growth of most common pathogen. Sterile defibrinated sheep blood: 7 ml.

Nutrient agar (melted) :100ml

Nutrient agar first sterilised and cooled to 40–50°C. Than sterile sheep blood was added and mixed well. Quantity of 15 ml of blood agar poured on each Petridis.

Blood hemolysis test (Collee *et al*., 2007): This test was used to detect the ability of bacteria to produce hemolycin and as indicated by hemolysis. Tested bacteria were examined by streaking on plate of blood agar and incubated at 37°C for 48 hrs. The appearance of a clear zone around the colony indicates β-hemolysis while the presence of green-color indicates α-hemolysis.

3.2.2. Gram's staining

Gram stain was the key step and was carried out according to Harrigan and McCance (1966). Smears were prepared from 18-20 hours old culture and allowed to air dry and heat fixed. Smears were then subjected to Gram staining as per the following procedure.

- Slide flooded with crystal violet and allowed to react for 1 min.
- Slides were then rinsed with distilled water, flooded with iodine and allowed to react for 1 min.
- The smears were again rinsed with distilled water and decolourised with acetone alcohol by adding drop by drop until crystal violet failed to wash off from the smear.
- The smears were again rinsed in distilled water and counter stained with safranin for 1 min.

- Slides rinsed again with distilled water, blot dried and observed under oil immersion objective of light microscope (Olympus CH 20i).

Reagents used for Gram's staining:

A. Crystal violet solution (g/L)

Crystal violet	: 2.0g
Ethyl alchol,95%	: 20 ml
Ammonium oxalate	: 0.8 ml
Distilled water	: 100 ml

B. Iodine solution

Potassium iodide	: 2.0g
Iodine crystals	: 1.0 g
Distilled water	: 100 ml

C. Decolourised

Acetone	: 50 ml
Ethyl alcohol, 95%	: 20 ml

D. Safranin Solution- Counter stain

Safranin	: 0.25g
Ethyl alcohol	: 10 ml
Distilled water	: 100 ml

Safranin was dissolved in alcohol, then water added and filtered.

.3.2.3. Nutrient agar for slants

Peptone	: 5 gm
Yeast Extract	: 3gm
Beef Extract	: 3gm
Nacl	: 5gm
Agar	: 15 gm
Distilled water	: 1000 ml

The ingredients were suspended in one liter of distilled water, heated till boiling to dissolve them completely, distributed in 250ml conical flasks and sterilised by autoclaving at 121°C for 15 minutes and poured as slants in sterilised test-tubes. Test isolate was inoculated lightly over the slant avoiding the media on which it was grown and incubated at 37°C upto 96 hours. All the strains were maintained in nutrient agar slants/deeps.

3.2.4. Biochemical tests for identification of bacteria

The following biochemical tests were carried out to determine the identity of the bacterial isolate.

3.2.4.1. Catalase test (Cappuccino and Sherman, 2001)

The principle of this test depend up on the basis that the breakdown of hydrogen peroxide to oxygen and water is mediated by the enzyme catalase. When a bacterial strain that produces catalase is grown in nutrient broth or on an agar slope to which about 1ml. of H_2O_2 (10 vol) is added, introduced into hydrogen peroxide, elaboration of bubbles of oxygen, the

gaseous product of the enzyme activity, is manifested immediately and after 5 minutes. The catalase test was carried out follows:-

1. With sterile loop, a small amount of pure growth was transferred from the agar plate onto the surface of a clean, dry glass slide.

2. A drop of 3% hydrogen peroxide (H_2O_2) was placed immediately onto a portion of a colony on the slide.

3. Evolutions of bubbles of gas were observed, indicating a positive test.

This test is mainly used to differentiate *Saphylococci* from *Sreptococci* when Gram positive cocci are grown in urine culture and also in the identification of aerobic Gram-negative bacilli.

Positive control – *Staphylococcus aureus*

Negative control – *Streptococcus* species

3.2.4.2. Gelatin liquefaction (Collee *et al*., 2007; Wolfgang *et al*., 1998**)**

Tubes containing gelatin media were stabbed once of the center with inoculating needle then, incubated at 37°C for 24 hours, then the tubes incubated at 4°C for half hr., the liquefied media indicate the positive test.

3.2.4.3. Lactose test

The lactose-utilising properties of Gram-negative bacilli can be directly evaluated from MacConkey agar by observing the red pigmentation of the colonies.

3.2.4.4. Indole production test (Wolfgang *et al*., 1998)

Peptone Water

Peptone	: 20.0 gm
Sodium chloride	: 5 gm
Distilled water	: 100 ml

The ingredients were dissolved in distilled water and pH was adjusted to 7.6. It was distributed into small test tubes in 2 ml quantities and autoclaved. It is used as a base for sugar fermentation test and also used to test for indole production by Enterobacteriaceae.

Kovac's indole reagent

Isomyl alcohol (3 methyl-butanol)	: 150.0ml
Dimethylamine benzaldhyde	: 10.0g
HCl (concentrated)	: 50.0ml

P-dimethylaminobenzaldhyde was dissolved in the isoamyl alcohol. Concentrated HCl was added and mixed well and transferred to clean brown bottle and stored at 2-8°C. The organism was inoculated in peptone water and after incubation at 37°C for 48 hours; the presence of indole is detected by adding kovac's reagent in positive case ring was seen.

Positive Control – *E. coli.*

Negative Control – *K. pneumoniae*.

3.2.4.5. Methyl red test (MR)

The tested organisms were inoculated in glucose phosphate peptone and incubated at 37°C for 24 hours , then about 5 drops of the methyl red reagent were added , mixed and the test was read immediately . Production of bright red colour indicates mixed acid fermentation. Negative test was indicated by the production of yellow colour.

Positive control – *Escherichia coli.*

Negative control – *K.pneumoniae*

Methyl red – Voges Proskauer broth MR-VP

Peptone	: 7g
Glucose	: 5g
Dipotassium phosphate	: 5g
Distilled water	: 1000 ml

Dissolved the ingredients in distilled water by gentle heating and sterilise at 121°C for 15 min.

3.2.4.6. Voges-Proskauer test

The tested organism was inocubated in 2 ml sterile glucose phosphate peptone water and incubated at 37°C for 48 hours. One ml of 40% potassium hydroxide and 3 ml of 5% solution of alpha naphthol in absolute alcohol were added. Shake the tube gently to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10-15

minutes; the development of pink colour was positive reaction. Yellow colour indicated negative reaction.

Positive control –*K.pneumoniae*

Negative control – *Escherichia coli.*

3.2.4.7. Citrate utilisation test (Baron *et al.,* 1994)

Contents:-

Magnesium sulphate	: 0.2g
Ammonium dihydrogen phosphate	: 0.2g
Sodium ammonium phosphate	: 0.8g
Sodium citrate tribasic	: 2.0g
Sodium chloride	: 5.0g
Bromothymol blue	: 0.08g
Agar	: 15.0g

The ingredients were suspended in one liter of distilled water, heated till boiling to dissolve them completely, distributed in 250 ml conical flasks and sterilised by autoclaving at 121°C for 15 minutes and poured as slants in sterilised test-tubes. Test isolate was inoculated lightly over the slant avoiding the media on which it was grown and incubated at 37°C upto 96 hours. A streak of growth and development of blue colour was positive. No growth and original green colour was negative test.

Positive control – *K. pneumoniae.*

Negative control – *E. coli*

3.2.4.8. Oxidase test (Collee *et al.*, 2007)

The oxidase test determines the presence of oxidase enzymes.

a) Media

Use either nutrient agar or tryptic soy agar plates to streak cultures and produce isolated colonies. From these, the inoculum was obtained for oxidase testing on impregnated filter paper.

The tryptic soy agar medium consists of

Tryptone	: 15.0g
Soyton	: 5.0g
Sodium chloride	: 5.0g
Agar	: 15.0g
Water (reagent-grade water)	:1000 ml

pH should be 7.3± 0.2 after sterilisation at 121°C for 15 min.

b) Reagents

Tetramethyl p-phenylenediamine dihydrochloride 1% aquous solution freshly prepared or refrigerated for no longer than 1 week. Impregnate a filter paper strip (Whatman No.1) with this solution.

c) Procedure

Removed some of a colony from agar with a platinum wire, and smeared on the test strip. A dark purple or violet colour that developed within 10 seconds indicated a positive oxidase test.

Positive control – *Pseudomonas aeruginosa*

Negative control – *Escherichia coli*

3.2.4.9. Coagulase test (Cheesbrough, 2000)

Coagulase is an enzyme that clots blood plasma by catalysing the conversion of a soluble protein (fibrinogen) to an insoluble protein (fibrin). This test is performed on Gram-positive, catalase positive species to identify the Coagulase positive *Staphylococcus aureus.* Coagulase is a virulence factor of *Staphylococcus aureus.* The formation of clot around an infection caused by these bacteria likely protects it from phagocytosis

Plasma was diluted 1:10 in physiological saline and 0.5ml of the diluted plasma was placed in a small tube. A colony of the test organism was emulsified in the diluted plasma. The tubes were incubated at 37°C and checked every 30 min for up to 4 hours for clot formation by tilling the tube.

Also, a colony of bacteria is emulsified in a drop of plasma on a glass slide. Bacterial clumping within 2 minutes indicates the presence of bound coagulase and constitutes a positive test result.

Positive control – *Staphylococcus aureus*.

Negative control – *Staphylococcus epidermidis*.

3.2.4.10. Urease test (Wolfgang *et al*., 1998)

Urease contents:-

Peptone	: 30.0g
HCl	: 5.0g
Potassium dihydrogen phosphate ($2H_2O$)	: 1.0g
Phenol red (0.25%)	: 2.0ml
Agar	: 20.0g
Glucose	: 100ml
Urea (20%)	: 100ml
Distilled water	: 1000 ml

Phenol red (0.25%) was prepared by dissolved 0.25g in 50ml of absolute ethanol and 50ml of dissolved water was added and mixed well.

Methods

The dry ingredients were mixed in water and heated to 100°C to dissolve the chemicals and allowed to cool to 50-55°C and the phenol red solution was added and mixed well, dispensed in screw capped bottles and sterilised in autoclave at 121°C for 15 minutes with caps loosened, when the medium has cooled. A Christensen's urea agar inoculated with the organism and incubated at 37°C for 18 hours. The development of deep pink colour in the upper half of the slop was an indication of positive test.

Positive control – *P. vulgaris*

Negative control – *E. coli*

3.2.4.11. Triple sugar iron agar (TSI) (Enan *et al.,* 1996).

Contents

Sucrose	: 10.0g
Beef extract	: 3.0g
Yeast extract	: 3.0g
Peptone	: 15.0g
Proteose peptone	: 5.0g
Lactose	: 10.0g
Glucose	: 1.0g
Ferrous sulfate	: 0.2g
Sodium chloride	: 5.0g
Sodium thiosulfate	: 0.3g
Agar	: 12.0g
Phenol red	: 0.024g
Distilled water	:1000 ml

Triple sugar iron medium (Himedia) was prepared by dissolving 65gms of dehydrated TSI medium in 1000 ml of distilled water and autoclaved at 121°C for 15 minutes. The medium was poured in test tubes in such a way that, a butt with a slant was formed. It was inoculated with the help of a straight inoculation wire. A well isolated colony of test organism was touched with the tip of the sterile inoculation wire and stabbed through the center of the TSI butt and then streaked over the

surface of the slant. The tube was incubated at 37°C for 18-24 hours. It detects ability of organism to ferment sugar (glucose-lactose-sucrose). Development of pink colour indicates alkaline and yellow colour indicates acid reactions. Reactions are read as follows;

Acid slant/acid butt (A/A) – glucose, sucrose, and/or lactose fermented

Alkaline slant/acid butt (K/A) – only glucose fermented, peptones utilised

Alkaline slant/no change in the butt (K/NC) – No fermentation of glucose, lactose or sucrose, peptone utilised.

A black precipitate in the butt indicates H_2S production.

Bubbles or cracks in the tube indicate production of CO_2 or H_2.

3.2.4.12. Oxidation / fermentation (OF) test (Collee *et al.,* 2007)

Oxidation/fermentation media (Hi-Media) was prepared by dissolving 45.52 grams dehydrated media in1000 ml of distilled water, poured into tubes and sterilised by free steaming for 30 minutes. It is a semi-solid medium containing sugars like glucose, lactose or sucrose. The isolate to be tested was inoculated to a depth of 1cm in to the O/F agar by stabbing 4-5 times with a sterile, cool straight wire containing the colony to be identified. Two tubes were inoculated; one of the tubes was overlaid with sterile liquid paraffin to detect fermentation. Tubes were incubated at 37°C up to seven days. Acid production is indicated by the change of colour from blue to yellow. If the organism was oxidative, yellow colour develops from top downwards in the tube which is not overlaid with liquid paraffin. Fermentative organisms change to yellow colour from bottom of the tube, or throughout the tube, in both tubes.

Fermentative – *Escherichia coli*

Oxidative – *Pseudomonas aeruginosa*.

3.2.4.13. Nitrate reduction test

Nitrate broth was prepared by dissolving 0.2 gm of potassium nitrate and 5 gm of peptone in 1000 ml of distilled water. Two drops of overnight broth culture of the test organism was added to 5 ml of nitrate broth in a test tube with a Durham's tube and incubated at 37°C for 48 hours. Testing was done after 24 hours after obvious growth was detected. Durham's tube was observed for production of gas. Then, 5 drops each of nitrate reagent solution A (sulphanilic acid in acetic acid) and solution B (α-naphthylamine in acetic acid) were added. Development of red colour indicated reduction of nitrate to nitrite. Negative tests were confirmed by detection of nitrate in the media by adding zinc powder. Development of red colour after addition of zinc powder confirms negative test.

Positive control – *E.coli*
Negative control –*Acinetobactor*

3.2.4.14. Bile esculin Agar

Bile esculin agar media was prepared by dissolving 64.5 grams of dehydrated media (Hi-Media) in 1000ml of distilled water, sterilised by autoclaving at 121°C for 15 minutes and poured as slopes in tubes. The test isolate was inoculated onto the surface of the slope. Inoculated tubes were incubated at 37°C for 48 hours. Blackening of the media indicates growth in the presence of 40% bile and esculin hydrolysis.

3.2.4.15. Growth in 6.5% NaCl

Nutrient broth with 6.5% NaCl was inoculated with two or three colonies of the isolate and incubated at 37°C for 3 days. Turbidity seen at the end of incubation indicates growth in 6.5% NaCl.

Positive control – *Enterococcus faecalis*.

Negative control – *Viridans streptococcus.*

3.2.4.16. Sugar fermentation media (Wolfgang *et al*., 1998)

Peptone water media containing 1% sugar were used. Peptone water was prepared by dissolving 15 gms of peptone and 5gm of sodium chloride in 1000 ml of distilled water. Ten ml of Andrade's indicator and desired sugar to a final concentration of 1% were incorporated in to the peptone water. The medium was poured into test-tubes containing Durham's tubes and sterilised by free steaming for 30 minutes. Broth culture of the test organisms were inoculated into the sugar media using sterile Pasteur pipette. The tubes were incubated at 37°C for 24h. Development of pink colour indicates acid production due to fermentation of the test sugar and displacement of medium from Durham's tube indicates gas production. Sugars used in the test for identification were glucose, lactose, sucrose, maltose, mannitol and arabinose.

3.2.5. Chromagar media for the isolation and differentiation of uropathogens (Odds and Bernaerts, 1994; Okulicz *et al.*, 2008).

Contents

Peptone	: 17.0g
Yeast extract	: 17.0g
Chromogenic mix	: 1.0g
Agar	: 15.0g
Deionised water	: 1000 ml
pH	: 7.0 ± 0.2

For the 100°C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, removed from the oven, stirred gently, then returned to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam). Cooled in a water bath to 45-50°C, swirling or stirring gently. Poured into sterile petri dishes or tubes and allowed to gel and dry. Prepared media plates can be kept for one day at ambient temperature.

Interpretation Chart

Microorganisms - Typical colony appearance on Chromagar

E.coli	: Dark pink to reddish
Enterococcus	: Turquoise blue
Klebsiella, Enterobacter, Citrobacter	: Metallic blue
Proteus	: Brown halo
Pseudomonas	: Cream, translucent
S. aureus	: Golden, opaque, small
S. saprophyticus	: Pink, opaque, small

The samples inoculated on Chromagar media, growth was observed after 24 and 48 hours of incubation. Isolates were identified by colony's colour and morphology.

3.3. ANTIBIOTIC SUSCEPTIBILITY TESTING

All bacterial strains isolated were tested for antibiotic sensitivity by standardisation disc diffusion technique. The agar diffusion disc technique described by Bauer *et al.,* 1966 was applied.

3.3.1. Muller-Hinton Agar

Contents:-

Beef extract	: 30.0g
casamino acids	: 17.5g
Starch	: 1.5g
Agar	: 17.0g
Deionised water	: 1000 ml

The contents were suspended in one liter of distilled or deionised water and boiled to dissolved completely; sterilised at 121ºC for 15 minutes, and pH was adjusted to 7.2±0.4. Immediately after autoclaving, it was allowed to cool in a 45 to 50ºC water bath. Freshly prepared and cooled medium was poured into glass or plastic, flat- bottomed Petri dishes on a level, horisontal surface to give a uniform depth of approximately 4 mm.

The agar medium was allowed to cool to room temperature and, unless the plate is used the same day, otherwise stored in a refrigerator (2 to 8ºC). Plates were used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimise drying of the agar. A representative sample of each batch of plates was examined for sterility by incubating at 30 to 35º C for 24 hours or longer. The pH of each batch of Mueller-Hinton agar was checked when

the medium was prepared. The agar medium with a pH between 7.2±0.4 at room temperature after gelling was used.

3.3.2. Inoculation of test plates

At least three to five well isolated colonies of the same morphological type were selected from an agar plate. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4-5 ml of a suitable broth medium. A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by evenly spreading 100µl inoculums with the help of a sterilised swab onto the entire surface of the agar plate. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculam from the swab. The dried surface of Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60º each time to ensure an even distribution of inoculam. As a final step, the rim of the agar was swabbed. The lid was left apart for 3-5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the antibiotic discs.

3.3.3. Application of Discs to Inoculated Agar Plates

Kirby-Bauer disc diffusion method of antimicrobial susceptibility testing is the most practical method and is still the method of choice for diagnostic microbiology laboratories. The Kirby-Bauer method recommended by the CLSI guidelines (2009) was used for antimicrobial susceptibility testing. Antimicrobial disc susceptibility tests were performed for 628 study isolates. The accuracy and reproducibility of the test are dependent on maintaining a standard set of procedures as described here.

Antimicrobial susceptibility testing of all bacterial species was carried out by the disc diffusion technique using a commercially available disc (Himedia- CLSI, 2009). The antimicrobial sensitivity of the test strains to sixteen antibacterial drugs was done using the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966; Forbes *et al.*, 2002; Macfaddin, 2000; Sharma, 2008). The antibiotics used were amoxicillin (AX, 30µg), ampicillin (AM, 10µg), cefotaxime (CTX, 30µg), ceftazidime (CAZ, 30µg), ceftriaxone (CT,30µg), cephalexin (CL,30µg), ciprofloxacin (CIP, 5µg), nalidixic acid (NA,30µg), norfloxacin (NX, 10µg), ofloxacin (OF, 5µg), amikacin (AK, 30µg), gentamicin (GEN, 10µg), tobramycin (TB, 10µg), imipenem (IPM, 10µg), nitrofurantoin (NIT, 300µg) and cotrimoxazole (COT, 30µg).

Antimicrobial discs were dispensed onto the surface of the inoculated agar plate and were pressed down to ensure complete contact with the agar surface distributed evenly so that they were no closer than 24 mm from centre to centre. The plates were inverted and placed in an incubator set to 37ºC within 15 minutes after the discs were applied. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data (Table 2).All the strains were maintained in nutrient agar slants/deeps. All strains of *E.coli* were also stored on 15% glycerol-supplemented Luria-Bertani medium at 80ºC for further molecular test.

Table 2. Antimicrobial drugs and their sensitivity profiles (CLSI, 2009).

Antimicrobial agent	**Symbol**	**Concentration**	**Resistant mm**	**Intermediate mm**	**Susceptible mm**
Amoxicillin	AX	30 µg	≤ 11	12 - 13	≥14
Ampicillin	AM	10 µg	≤13	14 – 16	≥17
Cefotaxime	CTX	30 µg	≤ 14	15 - 22	≥23
Ceftazidime	CAZ	30 µg	≤14	15 – 17	≥18
Ceftriaxone	CT	30 µg	≤13	14 – 20	≥21
Cephalexin	CL	30 µg	≤ 14	15 - 17	≥18
Ciprofloxacin	CIP	5 µg	≤ 15	16 - 20	≥21
Nalidixic acid	NA	30 µg	≤ 13	14 - 18	≥19
Norfloxacin	NX	10 µg	≤ 12	13 - 16	≥17
Ofloxacin	OF	5 µg	≤ 14	15 - 21	≥22
Amikacin	AK	30 µg	≤ 14	15 - 16	≥17
Gentamicin	GEN	10 µg	≤ 12	13 - 14	≥15
Tobramycin	TB	10 µg	≤ 12	13 - 14	≥15
Imipenem	IPM	10 µg	≤ 13	14 - 15	≥16
Nitrofurantoin	NIT	300 µg	≤ 14	15 - 16	≥17
Cotrimoxazole	COT	30 µg	≤ 10	11 - 15	≥16

3.4. ISOLATION AND CHARACTERISATION OF THE PATHOGENIC *CANDIDA* SPECIES

3.4.1. Characterisation of *Candida* Species

Twenty two isolates of *Candida* species were used for microscopy analysis through Gram staining and culture on Chromagar media, Sabouraud dextrose agar medium, supplemented with 50 mg mL-1 of chloramphenicol. The cultures were incubated at 37^{o}C, for 24-48h, under aerobic conditions.

3.4.1.1. Sabouraud Dextrose Agar (SDA)

Glucose 40g, Peptone 10g, Agar 20g, Distilled water 1 Liter. The ingredients were steamed to dissolve, and then the medium was autoclaved at 121º C for 15 minutes and was dispensed in plates. Finally, it was left to cool to 50º C before the addition of Chlramphenicol at a concentration of (40 mg/ Liter).

3.4.1.2. Chromagar media used for isolation of *Candida* species

CHROMagar *Candida* (Himedia) was prepared according to the manufacturer's instructions. CHROMagar *Candida* is composed of (per litre): peptone (10g), glucose (20g), agar (15g), chloramphenicol (0.5g) and “chromogenic mix” (2g). Twelve grams of CHROMagar *Candida* powder which was added to 250 ml of sterile distilled water in a sterile Erlenmeyer flask. The suspension was completely dissolved by boiling (<100°C) and mixing. The medium dose not require sterilisation by autoclave, therefore

after cooling in a water bath to 45°C the agar was poured into sterile petri dishes. After allowing cooling, the plates were stored at 4°C prior to use.

3.4.1.3. Germ tube test

Small portion of an isolated colony was suspended in a test tube containing 0.5 ml of human serum then incubated at 37ºC for 2 hours then examined microscopically at 30 minutes intervals up to 2 hours for the presence of germ tube.

3.4.1.4. Sucrose assimilation

One colony of *Candida* isolate was added to 5 ml of distilled water to make suspension. Five drops of *Candida* suspension was added to yeast nitrogen base agar after cool at 45ºC then poured into plates. Filter paper discs impregnated with saturated sucrose solution were placed on the surface of agar, and then incubate at 27-30ºC up to 48 hours. Positive growth indicated by growth of *Candida* around the assimilated sucrose.

3.5. ANTIFUNGAL SUSCEPTIBILITY TESTING

Antifungal susceptibility testing was carried out using the disc diffusion method following the National Committee for Clinical Laboratory Standards institute (CLSI, 2004) guidelines, using fluconazole (25µg), itraconazole (50µg), ketoconazole (10µg), and amphotericin B (20µg) antifungal discs. Supplemented Mueller-Hinton agar [Mueller-Hinton agar + 2% glucose and 0.5 g/mL methylene blue dye, (GMB medium)] was used for performing the antifungal susceptibility testing.

3.5.1. Preparation of inoculam

Inoculum was prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Saboured Dextrose Agar (SDA agar) incubated at 35- 37ºC. Colonies were suspended in 5 ml of sterile 0.85% saline.

3.5.2. Susceptibility test procedure

1. Prepared plates with Mueller Hinton Agar +2% glucose and 0.5 µg/ml methylene blue dye (GMB) medium for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.

2. The prepared inoculum streaked in the entire agar surface of the plate with the cotton swab three times, turning the plate at 60º angle between each streaking. The inoculum allowed to drying for 5-15 minutes with lid in place.

3. The discs were applied using aseptic technique. Deposit the discs with centers at least 24 mm apart.

4. Inverted the plates and placed in an incubator set to 35- 37ºC within 15 minutes after the discs were applied.

5. Examined all plate after 20-24 hours of incubation.

6. Measured the zone diameter to the nearest whole millimeter at the point at which there is prominent reduction in growth.

3.6. PLASMID ANALYSIS-MOLECULAR CHARACTERISATION OF ESBL PRODUCING *E.COLI*

3.6.1. Clinical isolates

A total of 100 highly Multi Drug Resistant (MDR) *E.coli* culture isolates were selected from urine specimens for ESBL activity and molecular characteristics test. Only a single positive culture was included in the analysis.

3.6.2. Double disc synergy test

E. coli that exhibited resistance to third generation cephalosporins were screened to detect ESBL production by DDST. Cefotaxime 30g was placed at a distance of 15mm edge to edge from a centrally placed augmentin disc containing 20g of amoxicillin+10g of clavulanic acid. Plates were incubated at 37°C for 18-24 hours and the pattern of zone inhibition was noted. Isolates that exhibited a distinct shape/size with potentiation towards amoxicillin + clavulanic disc were considered potential ESBL producers (CLSI, 2002).

3.6.3. Preparation of genomic DNA

Genomic DNA was purified by phenol extraction method (Sambrook *et al.*, 2001). The DNA was stored at -20ºC. The samples were run on 0.8% Agarose gel and stained with Ethidium bromide. The stained gel was examined under UV light to look for the presence of DNA bands of particular size using a molecular weight marker; λ DNA Hind III double digests.

3.6.4. PCR amplification for β-lactamase encoding genes

PCR analysis for β-lactamase genes of the family TEM was carried out. Primers obtained from Medox, (Chennai) used for bla TEM amplification were 5′- CTTCCTGTTTTGCTCACCCA -3′ and 5′ TACGATACGGGAGGGCTTAC - 3′. For PCR amplification, the parameters were, 94°C for 1 minute initial denaturation, and 30 cycles of 94°C for 30 seconds, 63°C for 1 minute, 72°C for 1 minute and a final extension in 72°C for 7 minutes. The amplified products were resolved in 1.5% agarose gel. The gel was visualised by staining with ethidium bromide. A 100bp ladder molecular weight marker was used to measure the molecular weights of amplified products.

3.6.5. Molecular typing of *E. coli* DNA by RFLP

The DNA samples were digested by using XbaI restriction enzyme. The digestion mix constitutes 5µl of Restriction enzyme, 7µl of Restriction assay buffer, 18µl of sterile water. The mixture was distributed into each 0.5ml tubes. 10µl of the DNA sample was then added to each vial. The digestion tubes were spun again for a few seconds for complete settlement. The tubes were then kept at 37°C for 4 hours. The digested product were then loaded and run on 1.5% agarose gel containing ethidium bromide and the fragments were visualised under UV transilluminator.

4. RESULTS

4.1. STUDY OF THE PREVALENCE OF UTIs

4.1.1. Study population

A total of 2400 midstream urine samples were processed from patients having clinically suspected Urinary Tract Infections (UTIs) attending various hospitals in pattukkottai area from January 2012 to June 2013. Out of the 2400 specimens, 650 (27.1%) were culture positive and 1750 (72.9%) specimens were negative (Table 3).

Table 3. Result of urine culture among study population (n=2400).

Growth and Sex	No. of patients	Percentage (%)
Growth	650	27.1
No growth	1750	72.9
Male	980	40.8
Female	1420	59.2

4.1.2. Distribution of uropathogens according to age groups and gender

The age and sex-wise distribution of male and female patients is given in Table 4. Among them 980 (40.8%) were males and 1420 (59.2%) were female patients (Table 3). Out of the 1420 samples collected from females 454 (69.8%) showed growth. Of the 980 male urine samples 196

(30.2%) grew uropathogens in culture. Among females, children of 0-10 years of age group had 15.5% Urinary tract infections, 11-20 years of age group had 10%, 21-30 years of age group had above 16.2%. 31-40 years of age group had 10.8%. 41-50 years of age group had above 7.7%. 51-60 years of age group had above 4.3%. 61-70 years of age group had above 3.0%. Above 71 years of age group had above 2.3% infection (Table 4). Male children of 0-10 years of age group had 9.2%, 11-20 years of age group had 2.8%, Similar to females, males of above 21-30 years of age group showed approximately, more than 6.2% infection, 31-40 years of age group had 3.4%, 41-50 years of age group had above 2.6%, 51-60 years of age group had 2.0%. 61-70 years of age group had 2.2%. Above 71 years of age group had 1.8% infection (Table 4). There is significant difference of positive growth between female and male patients ($P<0.05$).

Table 4. Distribution of uropathogens according to age groups and sex.

Age groups in years	**No of Females infected**	**%**	**No of males infected**	**%**
0-10 (Children)	101	15.5	60	9.2
11-20	65	10.0	18	2.8
21-30	105	16.2	40	6.2
31-40	70	10.8	22	3.4
41-50	50	7.7	17	2.6
51-60	28	4.3	13	2.0
61-70	20	3.0	14	2.2
Above 71	15	2.3	12	1.8
Total	454	69.8	196	30.2

4.2. ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA FROM UTIs

4.2.1. Sources of isolated strains with percentage

Table 5 and Figure 5 showed that the sources of the isolated strains from 650 patients with different diseases of urinary tract infections. urinary tract infection (396) with the percentage 60.9%, urinary cystitis (63) with the percentage 9.7 %, urethritis (51) with the percentage 7.9%, catheter (50) with the percentage 7.7%, pyelonephritis (28) with the percentage 4.3%, suspected cancer (25) with the percentage 3.8%, prostatitis (20) with the percentage 3.1%, stone (10) with the percentage 1.5 % and ureteric stone (7) with the percentage 1.1%.

Table 5. The sources of isolated strains from UTIs.

Clinical Diagnosis	**No of isolated uropathogens**	**Percentage %**
Urinary tract infection	396	60.9
Cystitis	63	9.7
Urethritis	51	7.9
Catheterisation	50	7.7
Pyelonephritis	28	4.3
Suspected cancer	25	3.8
Prostatitis	20	3.1
Stone	10	1.5
Ureteric Stone	7	1.1

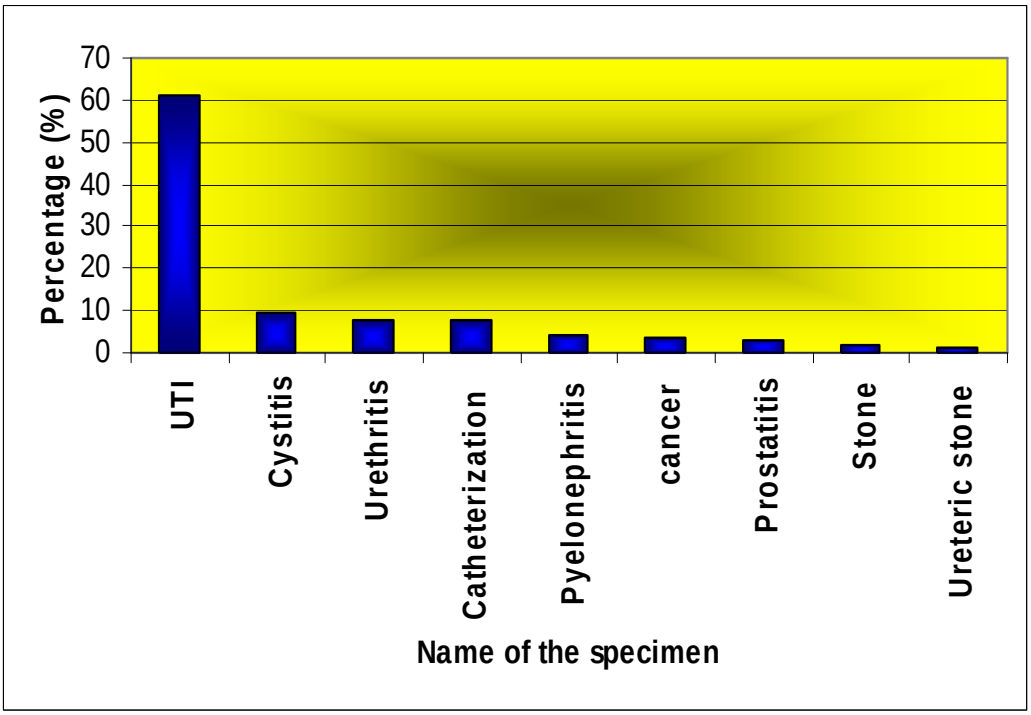


Figure 5. The sources of isolated strains from UTIs**.**

4.2.2. Correlation between pyuria, Gram's stain and culture in UTIs

Microscopy to detect pyuria: Among 650 samples showing bacterial growth in culture, microscopy of wet mount revealed 260 (40%) samples with 0- 3 pus cells/HPF, 170 (26.2%) samples with 4-6 pus cells/HPF and 220 (33.8%) samples with >6 pus cells/HPF. The details of wet mount microscopy findings along with results of Gram's staining are given in Table 6 and Figure 7(g).

Gram's staining and microscopy: Microscopy of 1750 Gram's stained uncentrifuged urine samples did not show any bacteria. These samples also failed to grow any uropathogens in culture. Another 205 samples also were negative for bacteria by microscopy, but grew bacteria in culture. However, 445 samples were positive both by microscopy and culture. 125 (19.2%) culture positive samples showed >6 pus cells/HPF but no bacilli on Gram's staining. 95 (14.6%) of culture positive samples showed both >6 pus cells/HPF and bacilli in Gram's staining. All the samples which showed bacilli in Gram's staining were

culture positive. 50 (12·08%) culture positive samples showed 4-6 pus cells / HPF, but no bacilli were observed upon Gram's staining. 30 (40·58%) culture positive samples had 0 – 3 pus cells /HPF and no bacilli seen on Gram's staining (Table 6).

Table 6. Correlation between pyuria, Gram's stain and culture in UTIs.

Gram's staining	Growth in culture	Number of pus cells/HPF			Total
		0-3/hpf	4-6/hpf	>6/hpf	
Negative	Nil	1558	140	52	1750
	Present	30	50	125	205
Positive	Present	230	120	95	445

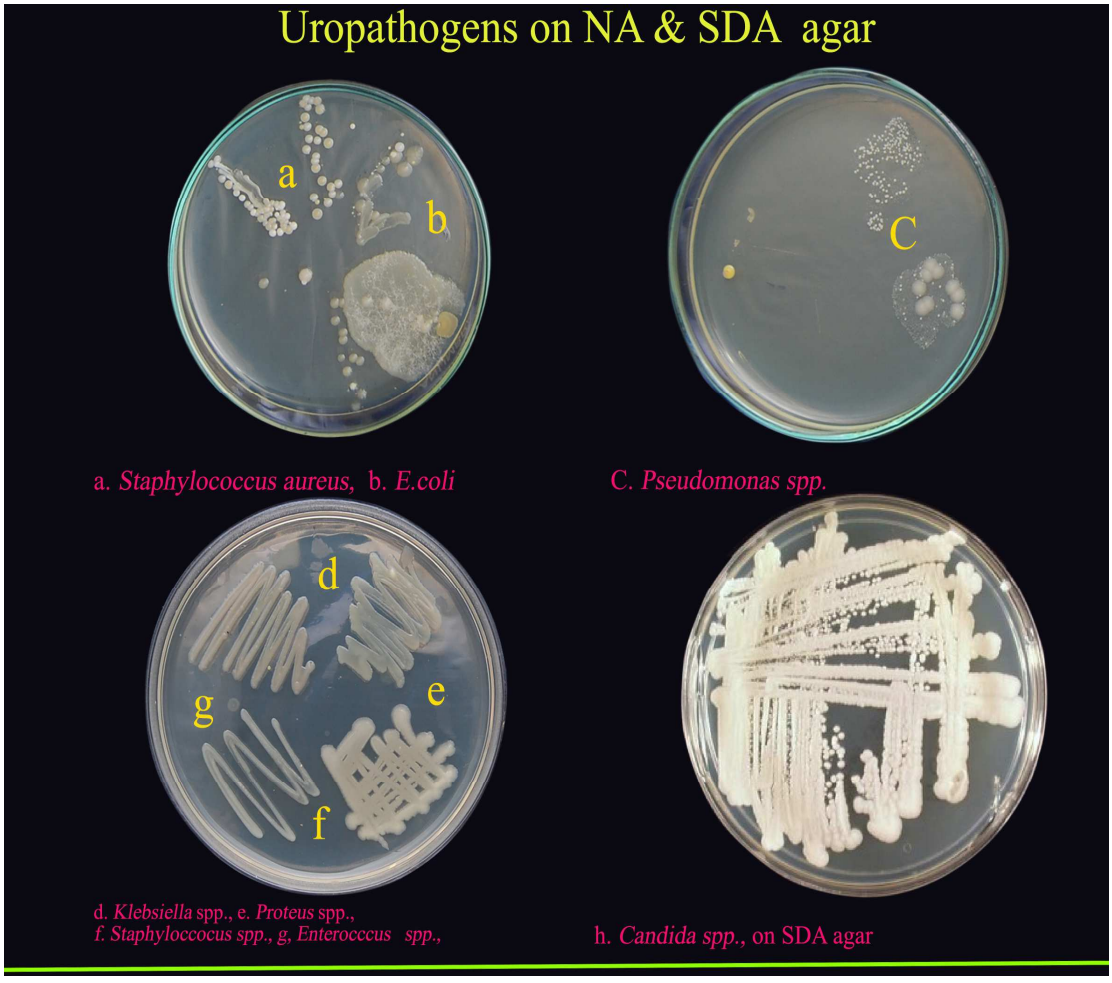


Figure 6. Isolation of bacteria on Nutrient agar (a-g) and isolation of *Candida* species on Sabouraud dextrose agar (h).

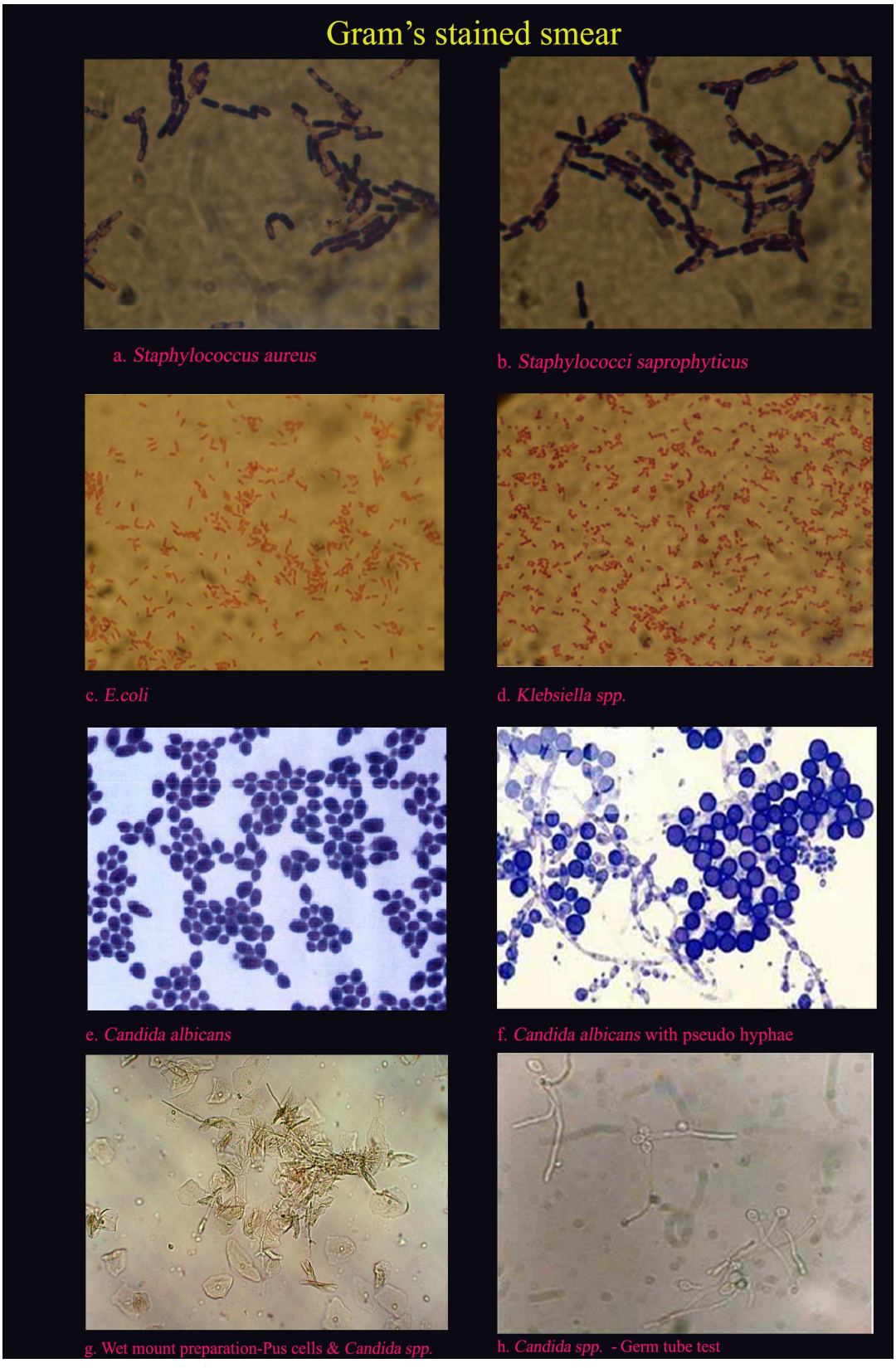


Figure 7 shows Gram's staining: a. *Staphylococcus aureus*, b. *Staphylococcus saprophyticus*, c. *E.coli*, d. *K. pneumoniae* e and f. *Candida albicans*, g. Direct microscopy- pus cells, RBC, Epithelial cells and *Candida* species, h. Germ tube test for identification *candida albicans*.

4.2.3. Uropathogens identified on Chromagar media according to pigment reactions

The colony characteristics and colour of the different microorganisms detected are described in Table 7. *E. coli*, *Proteus* spp. and *Enterococci* grow on this medium in typical differentiated colonies. *Acinetobacter* spp. were also easily differentiated and distinguished from *Pseudomonas* isolates. The similarity of colours produced by *Klebsiella*, *Enterobacter* and *Citrobacter* spp. prevents differentiation among them, and additional biochemical tests were done for final identification. The results showed that overnight incubation is optimal for the growth response of microorganisms on Chromagar medium. Longer incubation of up to 72 hours confirmed the results and deepened the colony colours **(**Table 7; Figure 8).

Table 7. Urine isolates presumptively identified on Chromagar media according to pigment reactions.

Organisms	**Morphology and/or colour (18-48 hours incubation)**
E. coli	Small, pink-purple
K. pneumoniae	Mucoid, *a* metallic blue
Proteus spp.	Pale brown
Pseudomonas	Green
Enterobacter spp.	Metallic blue
Citrobacter spp.	Metallic blue
Acinetobacter spp.	Nontransparent, cream, white
CoNS	Colourless, small, undifferentiated
Enterococcus spp.	Tiny blue, dry
S. saprophyticus	Small translucent; opaque
S. aureus	Small, colourless
Candida species	Creamy, wet convex

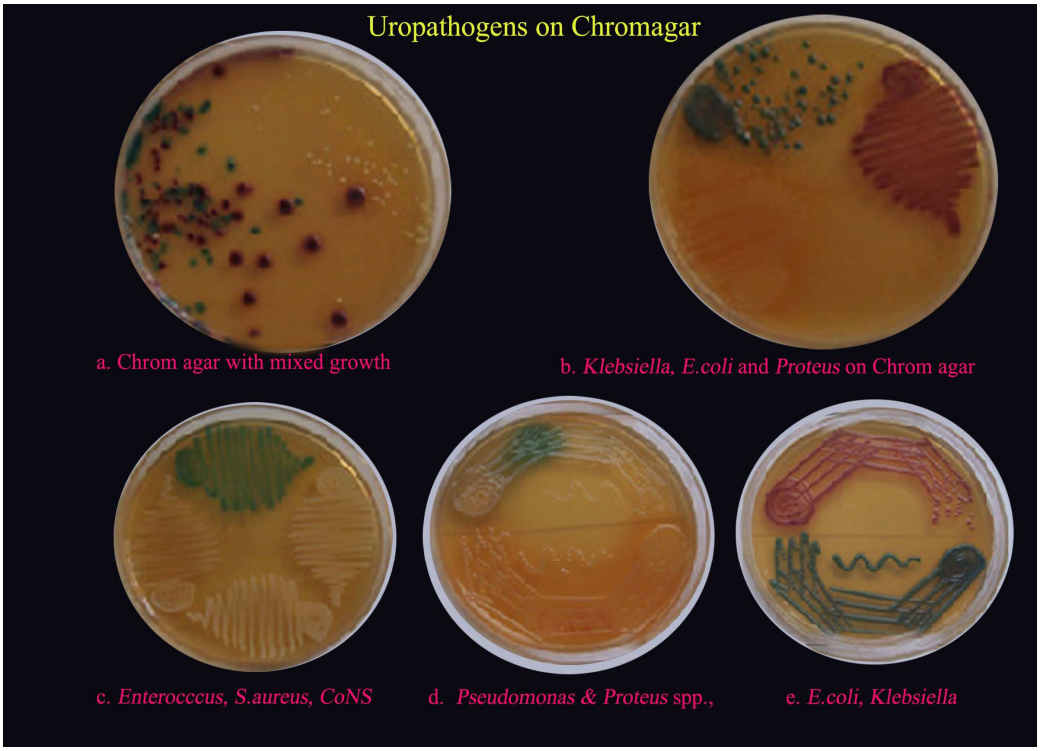


Figure 8. Identification of bacterial species on Chromagar media.

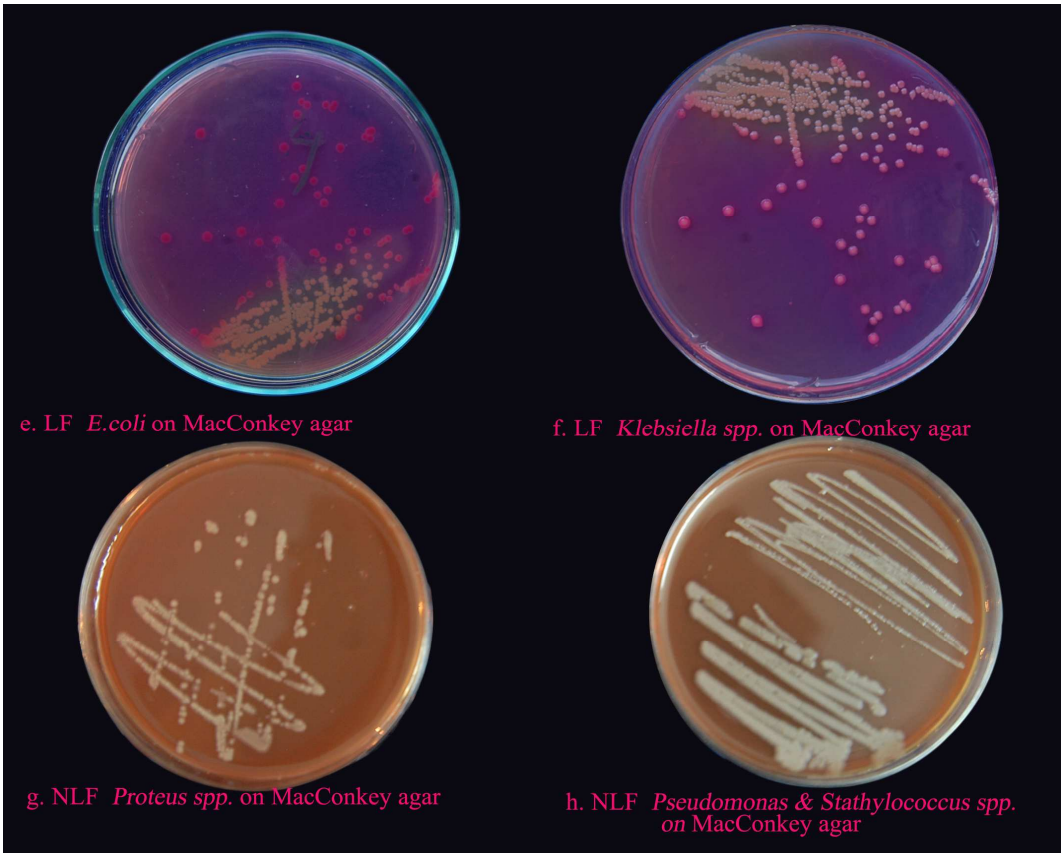
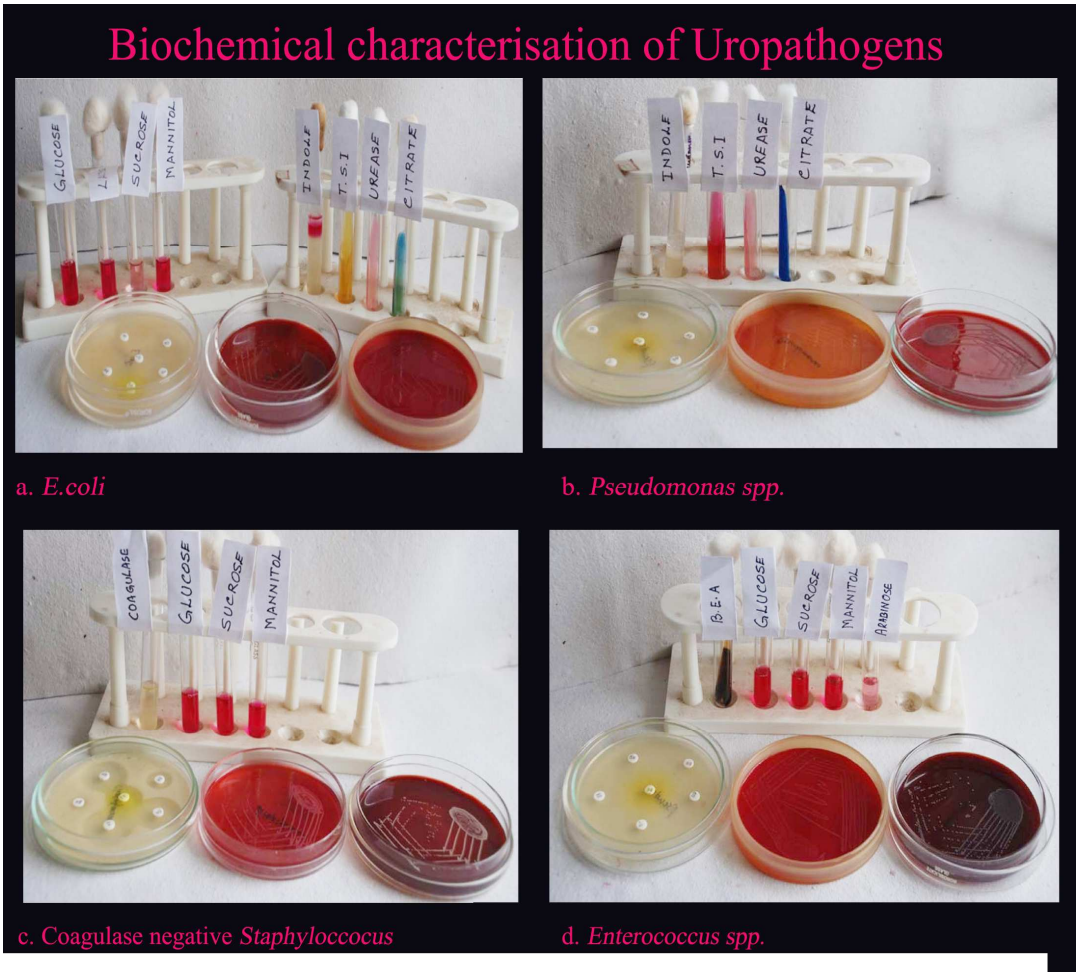


Figure 9. Identification of bacteria on MacConkey agar and Blood agar.

Table 8. Identification of uropathogens by Gram's staining, SBA and MA agar.

Organisms	**Gram's staining**	**Sheeb Blood Agar (SBA)**	**MacConkey Agar (MA)**
E. coli	GNB	B-haemolysis	LF, Pink, rough
K. pneumoniae	GNB	-	LF, Pink, mucoid
Proteus spp.	GNB	-	NLF, Colorless.
Pseudomonas spp.	GNB, Pigment and fruit odour	-	NLF, Colorless.
Enterobacter spp.	GNB	-	-
Citrobacter spp.	GNB	-	-
Acinetobacter spp.	GNB, diplobacilli	-	NLF
CoNS	GPC in clusters	No haemolysis, white pigment	No growth to slight growth (pale pink).
S.saprophyticus	GPC in clusters	No haemolysis, white pigment	
Enterococcus spp.	GPC, Oval cocci in pairs and short chains	No haemolysis	
S. aureus	GPC in clusters	B-haemolysis	
Candida species	Gram positive budding yeast with no pseudohyphae, mucoid colony on SDA, capsule +ve in indian ink preparation, Urease +ve.		



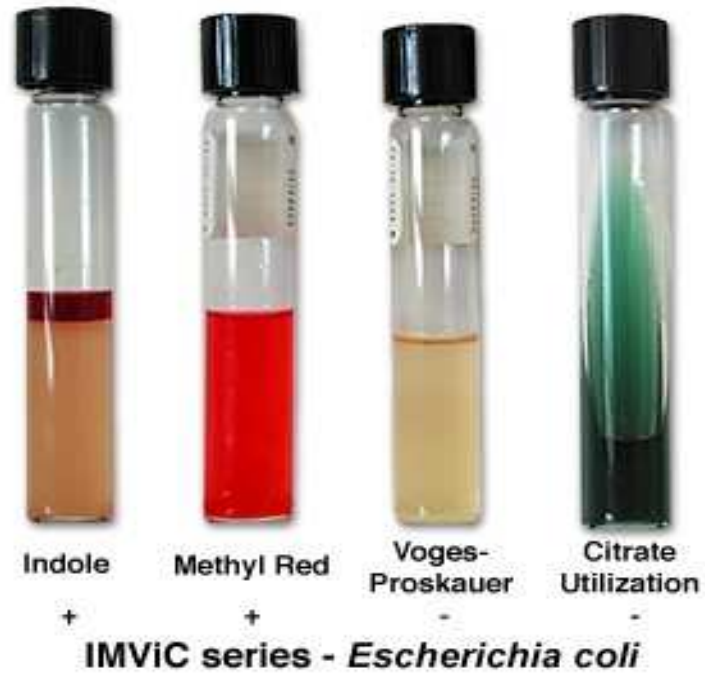


Figure 10. Identification of bacteria through biochemical testing and selective media

Table 9. Biochemical identification of bacteria isolated from UTIs.

Bacteria	**Catalase**	**Oxidase**	**Coagulase**	**Indole**	**MR**	**VP**	**Citrate**	**Urease**	**TSI**	**Nitrate**
Enterococcus spp.	+	Nd	-	Nd	Nd	Nd	Nd	Nd	Nd	Nd
S. aureus	+	Nd	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd
S. saprophyticus	+	Nd	-	Nd	Nd	Nd	Nd	Nd	Nd	Nd
CoNS	+	Nd	-	Nd	Nd	Nd	Nd	Nd	Nd	Nd
E. coli	+	-	Nd	+	+	-	-	-	A/AG	+
K. pneumoniae	+	-	Nd	-	-	+	+	+	A/AG	+
P. aeruginosa	+	+	Nd	-	-	-	+	+	Nd	+
Proteus spp.	+	-	Nd	-	+	-	+	+	K/A H_2S	+
Acinetobacter spp.	+	-	Nd	-	-	+	+	+	Nd	-
Citrobacter spp.	+	-	Nd	-	+	-	+	-	A/A H_2S	+
Enterobacter spp.	+	-	Nd	-	-	+	+	-	A/A	+

MR: Methyl Red test; VP: Voges-Proskauer test; TSI: Triple Sugar Iron test; V: variable; A/A: Acid in slant and butt; A/AG H_2S: Acid in slant and butt with H_2S Gas production; A/AG: Acid in slant and butt with Gas production; K/A H_2S: Alkali in slant and butt with H_2S Gas production; Nd: not done; +: positive; -: negative.

4.2.4. Distribution of isolates among positive urine specimens

The distribution of isolates among positive uring specimens are presented in the Table 10.

Table 10. Frequency of bacterial isolates from urine samples (n = 2400). Data are reported as number of isolates and percentages of total.

Microorganisms	**Frequency**	**Percentage**
Escherichia coli	355	54.6%
K. pneumoniae	72	11.2%
Pseudomonas spp.	68	10.5%
Proteus spp.	44	6.8%
Enterobacter spp.	10	1.5%
Citrobacter spp.	4	0.6%
Acinetobacter spp.	2	0.3%
GNB Total	**555**	**85.4%**
CoNS	27	4.1%
Enterococcus spp.	24	3.7%
S. saprophyticus	15	2.3%
S.aureus	7	1.1%
GPC Total	**73**	**11.2%**
Candida spp.	22	3.4%
Total	650	100%

Out of total 2400 samples were collected, only 27.1% of the cases showed growth of bacteria and fungi. 650 uropathogens belonging to 12 different species were isolated (Table 10). The most common isolates in this study have been the Gram negative bacilli which accounts for 85.4% of the total positive isolates. In the Gram negative bacilli, the predominant isolates from UTI were 355 strains of *E.coli.* The frequency of other uropathogens in descending order were 72 strains of *K. pneumoniae* 68 strains of *Pseudomonas* spp., 44 strains of *Proteus* spp., 10 strains of *Enterobacter* spp., 4 strains of *Citrobacter* spp., 2 strains of *Acinetobacter* spp. In the Gram positive bacteria the main organisms identified were 27 strains of CoNS, 24 strains of *Enterococcus* spp., 15 strains of *Staphylococcus saprophyticus*, 7 strains of *S. aureus* and 22 numbers of *Candida* spp. Thus, *E. coli* (54.6%) was the maximally isolated UTI causing bacterium, followed by *K. pneumoniae* (11.2%), *Pseudomonas* spp. (10.5%), *Proteus* spp. (6.8%), CoNS (4.1%), *Enterococcus* spp. (3.7%), *Staphylococcus saprophyticus* (2.3%), *Enterobacter* spp. (1.5%), *S. aureus* (1.1%), *Citrobacter* spp. (0.6%) and *Acinetobacter* spp. (0.3%). Table 10 shows the detailed frequency of all the isolates identified.

4.3. ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES FROM THE UTIs

4.3.1. Characterisation and susceptibility pattern of *E.coli*

In the Gram negative bacilli, the most predominant isolate from UTI were 355 strains of *E.coli* (54.6%) included for this study.

Biochemical Tests: All Gram negative, raised, entire, circular, motile, lactose, glucose fermenting, indole positive, methyl red positive, voges-proskauer negative, citrate negative and urease negative bacilli strains were identified as *Escherichia coli.* While on MacConkey's agar, lactose fermenting (pink) colonies was detected. On the TSI test *E.coli*

strains produced acids both in butt and slant along with gas production (Figure 6(b), 7(c), 8(b), 9(c) and 10(a); Table 7, 8 and 9).

This experiment was carried out to study the susceptibility of the bacterial isolates collected from urine specimens toward different 16 antibiotics. The percentages of susceptibility of *E.coli* isolates to the antibiotics which are commonly used to treat *E.coli* infections are shown in Table 11. The lowest percentage of susceptibility was manifested against amoxicillin (8.2%) followed by ampicillin (11.3%), cephalexin (11.5%), nalidixic acid (22.8%), cotrimoxazole (23.7%), whereas more susceptibility was observed with imipenem (93.5%) and amikacin (88.2%) followed by nitrofurantoin (87%), tobramycin (76.9%), ciprofloxacin (69.0%), norfloxacin (67.6%), ofloxacin (64.5%), gentamicin (62.5%), cefotaxime (58.0%), ceftazidime (54.7%) and ceftriaxone (47.0%) respectively (Figure 12, 13 and 14).

Three aminoglycoside antibiotics, amikacin (11.8%), tobramycin (23.1%) and gentamicin (37.5%) were moderately resistant to species of pathogens used. Similarly, resistance patterns of the beta-lactam group, ampicillin (88.7%) and amoxicillin (91.8%) antibiotics were almost equally resistant to the isolated *E.coli*. Imipenem resistance patterns were 6.5 of *E.coli.* Further, the cephalosporin antibiotics, ceftriaxone (53%), ceftazidime (45.3%) and cefotaxime (42.0%) were moderately resistant and cephalexin (88.5%) was highly resistant to species of pathogens used. Similarly, the fluoroquinolone group, ofloxacin (35.5%), norfloxacin (32.4%) and ciprofloxacin (31%) were low resistant and nalidixic acid (77.2%) were highly resistant to species of pathogens used. Among these four antibiotics, nalidixic acid was recorded to be more resistant to these pathogens. Lastly, detailed antibiograms of two stand-alone antibiotics, cotrimoxazole and nitrofurantoin were recorded. Nitrofurantoin was found very low resistance for 13% of *E.coli*. The resistance percent values of the cotrimoxazole were 76.3% (Figure 11).

Table 11. Antibiotic susceptibility pattern of *E. coli.*

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	20 (5.6)	9 (2.6)	29 (8.2)	326 (91.8)
Ampicillin	30 (8.5)	10 (2.8)	40 (11.3)	315 (88.7)
Cefotaxime	190 (53.5)	16 (4.5)	206 (58.0)	149 (42.0)
Ceftazidime	188 (53.0)	6 (1.7)	194 (54.7)	161 (45.3)
Ceftriaxone	163 (45.9)	4 (1.1)	164 (47.0)	188 (53)
Cephalexin	39 (10.9)	2 (0.6)	41 (11.5)	314 (88.5)
Ciprofloxacin	239 (67.3)	6 (1.7)	245 (69.0)	110 (31.0)
Nalidixic acid	78 (22.0)	3 (0.8)	81 (22.8)	274 (77.2)
Norfloxacin	237 (66.8)	3 (0.8)	240 (67.6)	115 (32.4)
Ofloxacin	227 (63.9)	2 (0.6)	229 (64.5)	126 (35.5)
Amikacin	297 (83.7)	16 (4.5)	313 (88.2)	42 (11.8)
Gentamicin	212 (59.7)	10 (2.8)	222 (62.5)	133 (37.5)
Tobramycin	269 (75.8)	4 (1.1)	271 (76.9)	82 (23.1)
Imipenem	327 (92.1)	5 (1.4)	332 (93.5)	23 (6.5)
Nitrofurantoin	303 (85.3)	6 (1.7)	309 (87.0)	46 (13.0)
Cotrimoxazole	81 (22.8)	3 (0.9)	84 (23.7)	271 (76.3)

Results are expressed as a percentage of 355 *E.coli* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial

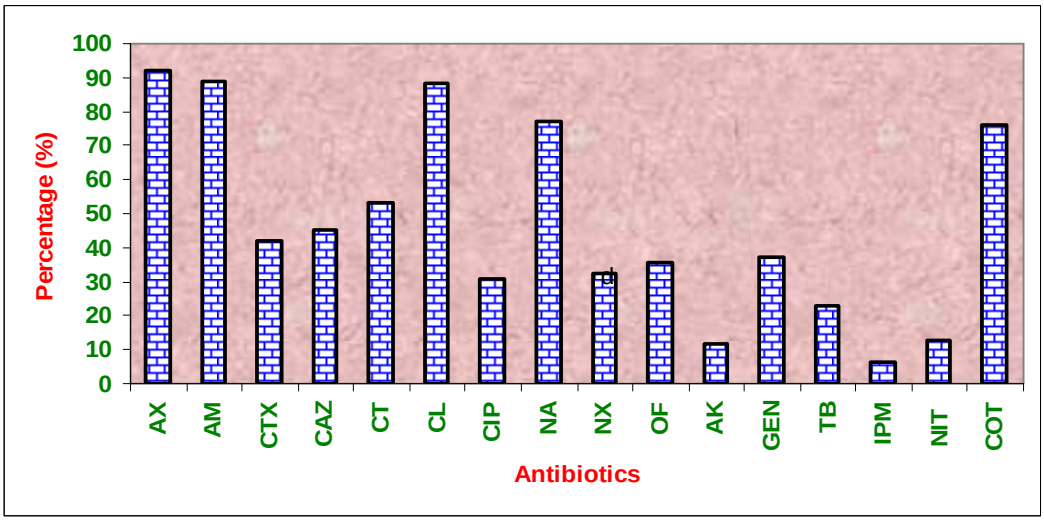


Figure 11. Antibiotic resistance of *E. coli* strains from patients with UTIs.

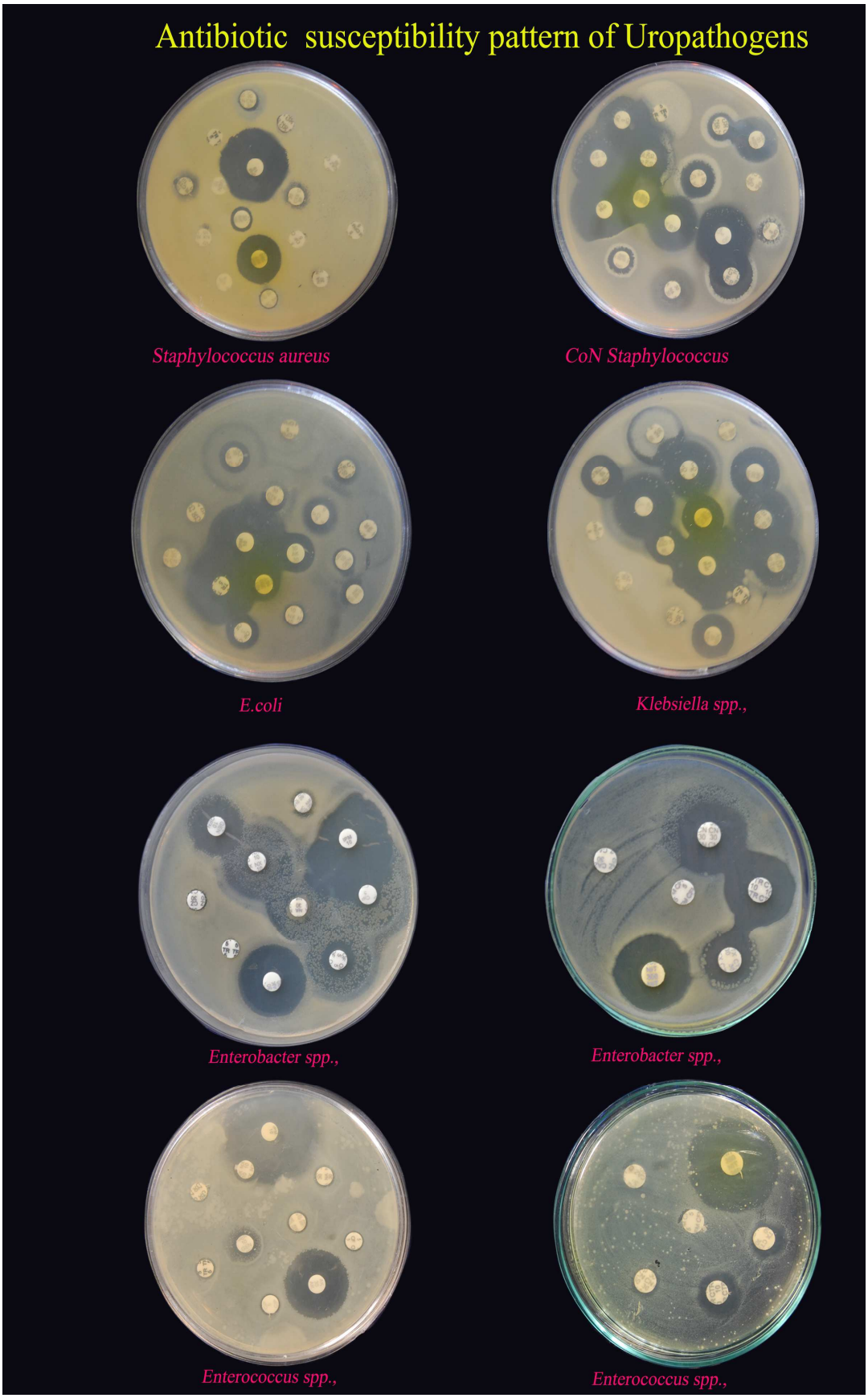


Figure 12. Antibiotic susceptibility pattern of bacteria isolated from UTIs.

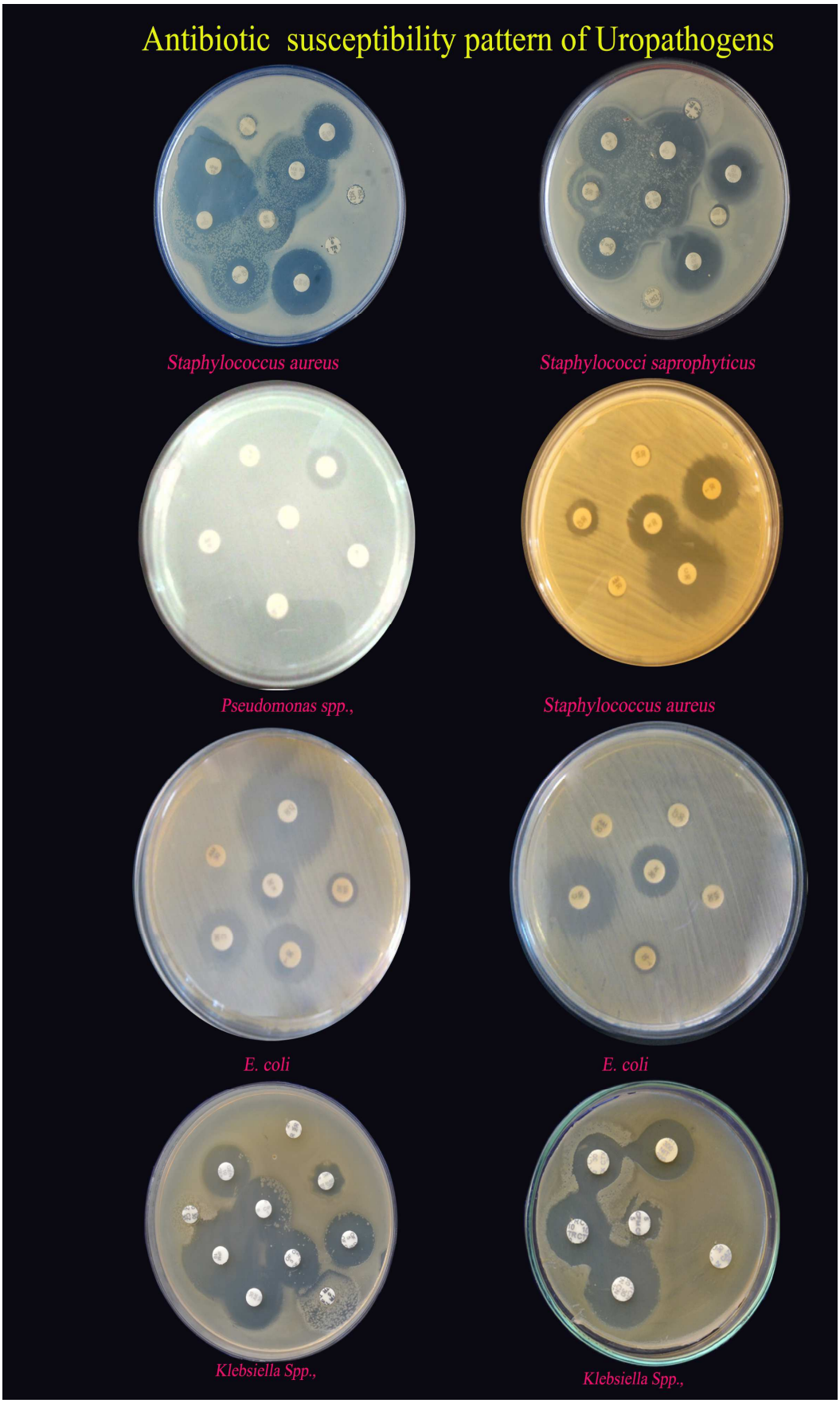


Figure 13. Antibiotic susceptibility pattern of bacteria isolated from UTIs.

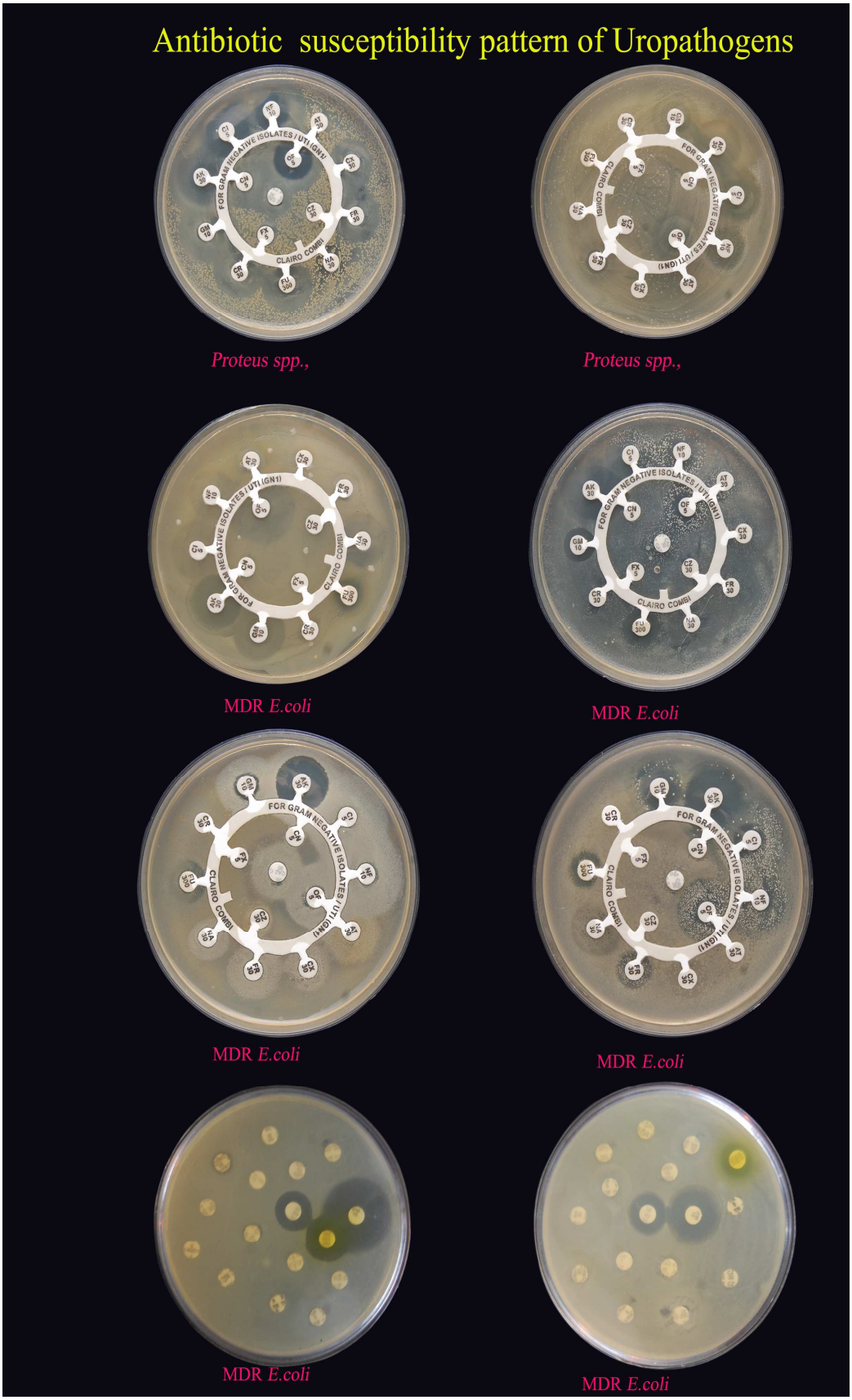


Figure 14. Antibiotic **s**usceptibility pattern of *Proteus* spp. and MDR *E.coli* isolated from UTIs.

4.3.2. Characterisation and susceptibility pattern of *K. pneumonia.*

Biochemical Tests: Gram negative, mucoid, non-motile, lactose, glucose fermenting, voges-proskauer positive, urease positive, citrate positive and indole negative bacilli strains were identified as *Klebsiella pneumoniae.* While on MacConkey's agar, lactose fermenting (pink) mucoid colonies was detected. On the TSI test, *Klebsiella pneumoniae* produced acids both in butt and slant along with gas production (Table 7, 8 and 9; Figure 6(d), 7(d), 8(b, c) and 9(f).

The percentages of susceptibility of *Klebsiella pneumoniae* to the antibiotics which are commonly used to treat *Klebsiella* infections are shown in Table 12. The lowest percentage of susceptibility was manifested against amoxicillin (11.1%) followed by ampicillin (16.7%), cotrimoxazole (29.2%), cephalexin (30.6%) and nalidixic acid (49.4%), whereas more susceptibility was observed with amikacin/imipenem (86.1%), followed by gentamicin/tobramycin (80.6%), ofloxacin (79.2%), ciprofloxacin (76.4%), norfloxacin (75%), cefotaxime (66.7%), ceftriaxone (66.7%), ceftazidime (54.2%) and nitrofurantoin (50%) respectively (Figure 12 and 13).

Three aminoglycoside antibiotics, amikacin (13.9%), and gentamicin, tobramycin (19.4%) were moderately resistant to species of pathogens used. Similarly, resistance patterns of the beta-lactam group, ampicillin (83.3%) and amoxicillin (88.9) are detailed in Table 12. These two antibiotics were almost equally resistant to the isolated *Klebsiella pneumoniae*. Imipenem resistance patterns were 13.9% of *Klebsiella pneumoniae.*

Table 12. Antibiotic susceptibility pattern of *K. pneumoniae*

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	6 (8.3)	2 (2.8)	8 (11.1)	64 (88.9)
Ampicillin	10 (13.9)	2 (2.8)	12 (16.7)	60 (83.3)
Cefotaxime	43 (59.7)	5 (7.0)	48 (66.7)	24 (33.3)
Ceftazidime	35 (48.6)	4 (5.6)	39 (54.2)	33 (45.8)
Ceftriaxone	48 (66.7)	0	48 (66.7)	24 (33.3)
Cephalexin	19 (26.4)	3 (4.2)	22 (30.6)	50 (69.4)
Ciprofloxacin	55 (76.4)	0	55 (76.4)	17 (23.6)
Nalidixic acid	32 (49.4)	0	32 (49.4)	40 (50.6)
Norfloxacin	52 (72.2)	2 (2.8)	54 (75.0)	18 (25.0)
Ofloxacin	56 (77.8)	1 (1.4)	57 (79.2)	15 (20.8)
Amikacin	62 (86.1)	0	62 (86.1)	10 (13.9)
Gentamicin	58 (80.6)	0	58 (80.6)	14 (19.4)
Tobramycin	57 (79.2)	1 (1.4)	58 (80.6)	14 (19.4)
Imipenem	59 (81.9)	3 (4.2)	62 (86.1)	10 (13.9)
Nitrofurantoin	34 (47.2)	2 (2.8)	36 (50.0)	36 (50.0)
Cotrimoxazole	20 (27.8)	1 (1.4)	21 (29.2)	51 (70.8)

Results are expressed as a percentage of 72 *K. pneumoniae* susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

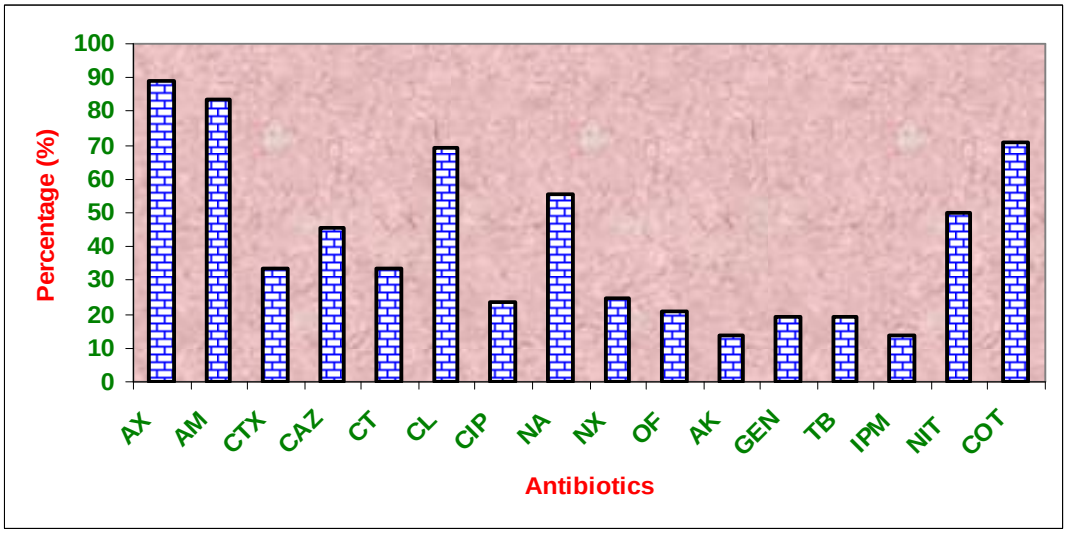


Figure 15. Antibiotic resistance of *Klebsiella* strains from patients with UTIs.

Further, resistance-percent values of UTI bacteria to cephalosporin antibiotics, Cefotaxime/ceftriaxone (33.3%), ceftazidime (45.8%) were moderately resistant and cephalexin (69.4%) was highly resistant to species of pathogens used. Similarly, resistance-percent values of UTI bacteria to antibiotics of the fluoroquinolone group, ofloxacin (20.8%), ciprofloxacin (23.6%), norfloxacin (25%) were low resistant and nalidixic acid (50.6%) were moderately resistant to species of pathogens used. These antibiotics were resistant to *Klebsiella pneumoniae* in the order: nalidixic acid, norfloxacin, ofloxacin and ciprofloxacin. Among these four antibiotics, nalidixic acid was recorded to be more resistant to these pathogens. The antibiograms of two stand-alone antibiotics, cotrimoxazole (50%) and nitrofurantoin (70.8%) were recorded (Figure 15).

4.3.3. Characterisation and susceptibility pattern of *Pseudomonas* spp.

The rate of isolation of *Pseudomonas* species from urine samples was found to be 2.8% from 2400 samples.

Biochemical Tests: All isolates of *Pseudomonas* species were Gram negative bacilli, actively motile and show different colonies large and small ones with irregular translucent edges and dark center. All strains were oxidase positive, produce acid by oxidation of sugars not by fermentation, produce gelatin liquefaction and formed slime with surface pellicles when grown in nutrient broth. Large, flat, opaque, aerobic, irregular colonies having grape-like smell, yellow-green pyocyannin pigment producing colonies on common culture media, oxidase positive colonies which grow at 42^{o}C were identified as *Pseudomonas aeruginosa* (Figure 6(c), 8(d), 9(b) and 10(b); Table 7, 8 and 9).

Table 13. Antibiotic susceptibility pattern of *Pseudomonas* spp.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	0 (0.0)	0 (0.0)	0 (0.0)	68 (100)
Ampicillin	0 (0.0)	0 (0.0)	0 (0.0)	68 (100)
Cefotaxime	46 (67.6)	4 (5.9)	50 (73.5)	18 (26.5)
Ceftazidime	39 (57.4)	2 (2.9)	41 (60.3)	27(39.7)
Ceftriaxone	24 (35.3)	2 (2.9)	26 (38.2)	42 (61.8)
Cephalexin	10 (14.7)	2 (2.9)	12 (17.6)	56 (82.4)
Ciprofloxacin	24 (35.3)	2 (2.9)	26 (38.2)	42 (61.8)
Nalidixic acid	5 (7.4)	0 (0.0)	5 (7.4)	63 (92.6)
Norfloxacin	10 14.70	3 (4.4)	13 (19.1)	55 (80.9)
Ofloxacin	24 (35.3)	1 (1.5)	25 (36.8)	43 (63.2)
Amikacin	60 (88.2)	0 (0.0)	60 (88.2)	8 (11.8)
Gentamicin	46 (67.6)	1 (1.5)	47 (69.1)	21 (30.9)
Tobramycin	59 (86.8)	0 (0.0)	59 (86.8)	9 (13.2)
Imipenem	39 (57.3)	1 (1.5)	40 (58.8)	28 (41.2)
Nitrofurantoin	19 (27.9)	1 (1.5)	20 (29.4)	48 (70.6)
Cotrimoxazole	0 (0.0)	0 (0.0)	0 (0.0)	68 (100)

Results are expressed as a percentage of 68 *Pseudomonas* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

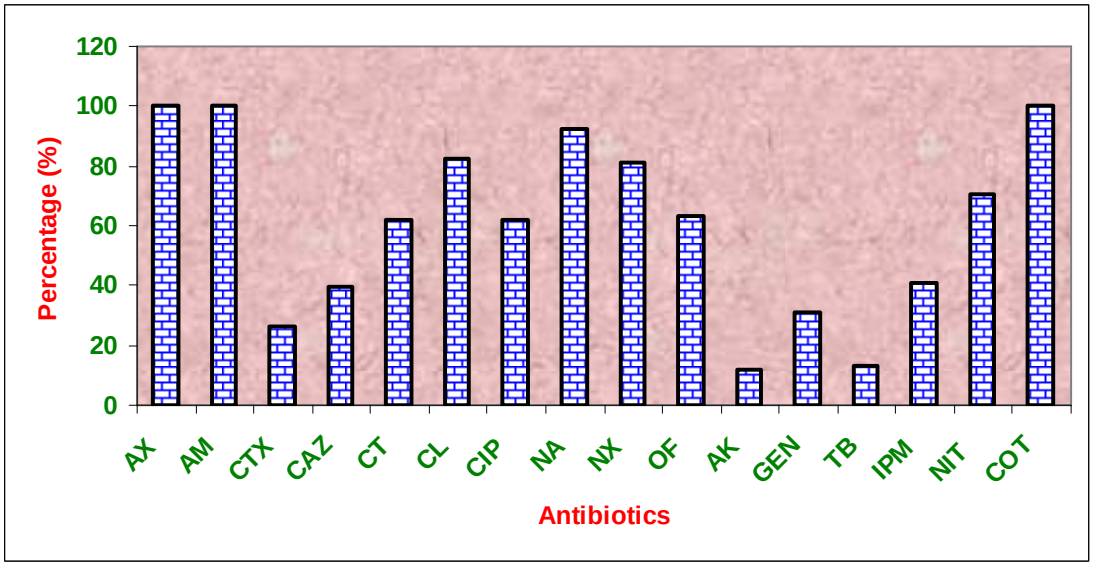


Figure 16: Antibiotic resistance of *Pseudomonas* strains from patients with UTIs.

This constitutes 10.5% (68) of the *Pseudomonas* species, which were highly sensitive towards amikacin (88.2%), tobramycin (86.8%), cefotaxime (73.5%), gentamicin (69.1%), ceftazidime (60.3%) and imipenem (58.8%). However, they show a low sensitivity rate towards ceftriaxone (38.2%), ciprofloxacin (38.2%), ofloxacin (36.8%), nitrofurantoin (29.4%), norfloxacin (19.1%), cephalexin (17.6%), nalidixic acid (7.4%), amoxicillin (0%), ampicillin (0%) and cotrimoxazole (0%) (Table 13 and Figure 16).

4.3.4. Characterisation and susceptibility pattern of *Proteus* spp.

Gram negative, swarming, fish odour colonies on sodium chloride-containing media, indole negative and urease positive strains were identified as *Proteus mirabilis*. On the TSI test, *Proteus* strains produced acids in both butt and slant along with H_2S gas (Figure 6(c), 8 (e), 9(g); Table 7, 8 and 9).

Proteus isolates exhibited highest susceptibility against amikacin 100%, gentamicin 100%, tobramycin 90.9%, ciprofloxacin 88.7%, imipenem 88.6%, ceftriaxone 84.1%, cefotaxime 81.8%, ofloxacin 79.5% and norfloxacin 75%. Other agents showed less sensitivity to nalidixic acid 65.9%, ceftazidime 54.6%, nitrofurantoin 45.5%, ampicillin 24%, cephalexin 22.7%, amoxicillin 20.5% and cotrimoxazole 20.5% (Table 14, Figure 14 and 17).

Table 14. Antibiotic susceptibility pattern of *Proteus* spp.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	4 (9.1)	5 (11.4)	9 (20.5)	35 (79.5)
Ampicillin	3 (6.8)	6 (13.6)	9 (24.0)	35 (79.6)
Cefotaxime	36 (81.8)	0 (0.0)	36 (81.8)	8 (18.2)
Ceftazidime	23 (52.3)	1 (2.3)	24 (54.6)	20 (45.4)
Ceftriaxone	37 (84.1)	0 (0.0)	37 (84.1)	7 (15.9)
Cephalexin	8 (18.2)	2 (4.5)	10 (22.7)	34 (77.3)
Ciprofloxacin	38 (86.4)	1 (2.3)	39 (88.7)	5 (11.3)
Nalidixic acid	28 (63.6)	1 (2.3)	27 (65.9)	15 (34.1)
Norfloxacin	32 (72.7)	1 (2.3)	33(75.0)	11 (25)
Ofloxacin	35 (79.5)	0 (0.0)	35 (79.5)	9 (20.5)
Amikacin	44 (100)	0 (0.0)	44 (100)	0 (0.0)
Gentamicin	43 (97.7)	1 (2.3)	44 (100)	0 (0.0)
Tobramycin	39 (88.6)	1 (2.3)	40 (90.9)	4 (9.1)
Imipenem	39 (88.6)	0 (0.0)	39 (88.6)	5 (11.4)
Nitrofurantoin	19 (43.2)	1 (2.3)	20 (45.5)	24 (54.5)
Cotrimoxazole	9 (20.5)	0 (0.0)	9 (20.5)	35 (79.5)

Results are expressed as a percentage of 44 *Proteus* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial

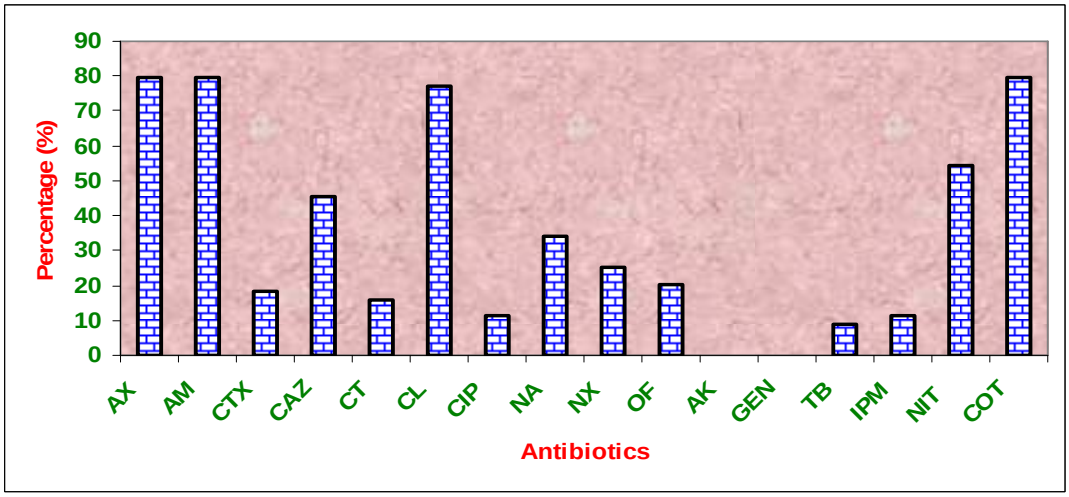


Figure 17. Antibiotic resistance of *Proteus* strains from patients with UTIs.

4.3.5. Characterisation and susceptibility pattern of *Enterobacter* spp.

The isolate from urine produced smooth yellow-colored colonies, 2-3 mm in diameter on blood agar and moist, pink colonies on MacConkey's agar after 24 hours incubation at 37°C. Strains and colonies that were Gram negative, motile, non-sporing, lactose fermenting, indole negative, methyl red negative, voges-proskaeur positive and citrate positive were confirmed to be *Enterobacter* spp. It fermented glucose, lactose and mannitol with the production of acid and gas. The organism was identified as *Enterobacter* (Table 7, 8 and 9).

All *Enterobacter* showed 100% sensitivity to amikacin, tobramycin and imipenem, and highly sensitive to ciprofloxacin 90%, gentamicin 90%, ceftriaxone 80%, ofloxacin 80%, cefotaxime 70%, norfloxacin 70%, nalidixic acid 60%, ceftazidime 50%, cotrimoxazole 50%, whereas more than 50% of these isolates were less sensitive to cephalexin 40%, nitrofurantoin 40%, amoxicillin 30% and ampicillin 10% (Table 15; Figure 12 and18).

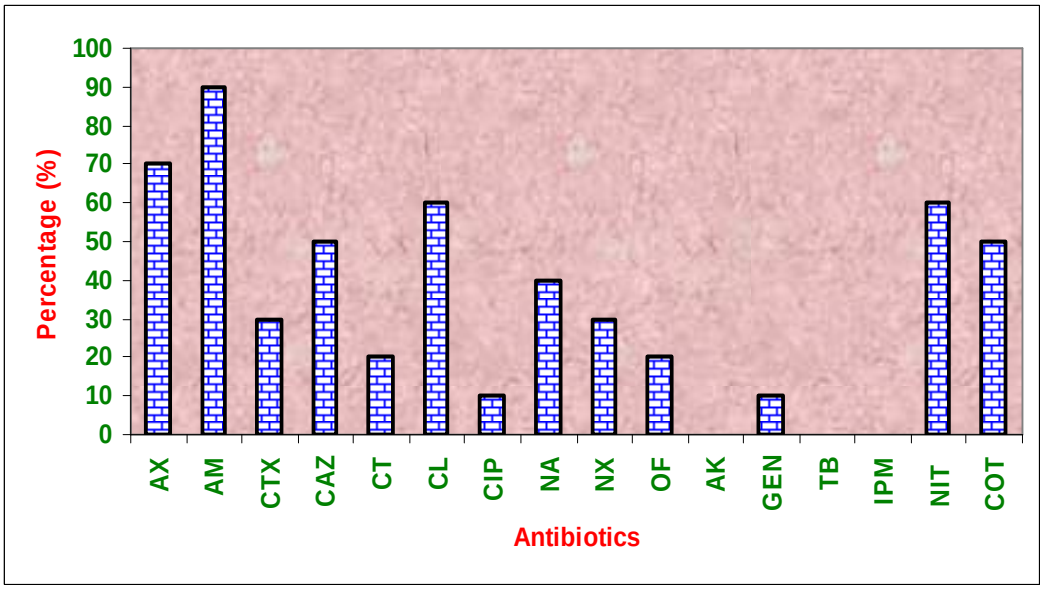


Figure 18. Antibiotic resistance of *Enterobacter* strains from patients with UTIs.

Table 15. Antibiotic susceptibility pattern of *Enterobacter* spp.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	2 (20)	1 (10)	3 (30)	7 (70)
Ampicillin	1 (10)	0 (0.0)	3 (10)	9 (90)
Cefotaxime	7 (70)	0 (0.0)	7 (70)	3 (30)
Ceftazidime	5 (50)	0 (0.0)	5 (50)	5 (50)
Ceftriaxone	7 (70)	1 (10)	8 (80)	2 (20)
Cephalexin	2 (20)	2 (20)	4 (40)	6 (60)
Ciprofloxacin	8 (80)	1 (10)	9 (90)	1 (10)
Nalidixic acid	6 (60)	0 (0.0)	6 (60)	4 (40)
Norfloxacin	7 (70)	0 (0.0)	7 (70)	3 (30)
Ofloxacin	8 (80)	0 (0.0)	8 (80)	2 (20)
Amikacin	10 (100)	0 (0.0)	10 (100)	0 (0.0)
Gentamicin	8 (80)	1 (10)	9 (90)	1 (10)
Tobramycin	10 (100)	0 (0.0)	10 (100)	0 (0.0)
Imipenem	10 (100)	0 (0.0)	10 (100)	0 (0.0)
Nitrofurantoin	4 (40)	0 (0.0)	4 (40)	6 (60)
Cotrimoxazole	4 (40)	1 (10)	5 (50)	5 (50)

Results are expressed as a percentage of 10 *Enterobacter* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

Citrobacter spp. was identified by its colony characteristics on MacConkey's agar and results obtained from the biochemical tests; it produced light pink-coloured late-lactose–fermenting (LLF) colonies after an 48 hours of incubation on MacConkey's agar; particularly, it was found

positive to catalase, MR, citrate and nitrate tests, whereas negative to oxidase, indole, VP and urease tests. On the TSI, the bacterium produced both acid and H_2S gas during growth (Table 7, 8 and 9).

Acinetobacter spp. was identified on colony characteristics on nutrient agar and MacConkey agar and from results obtained from adopted biochemical procedures: it grew as colourless, smooth, opaque, raised and pinpoint colonies on Nutrient agar, but as colourless, smooth, opaque, raised and non-lactose-fermenting (NLF) colonies on MacConkey's agar; it was found positive to catalase, VP and citrate tests, whereas negative to oxidase, indole, MR and nitrate tests (Table 7, 8 and 9).

4.3.6. Characterisation of Gram Positive Bacteria

Further, Gram- positive bacteria as medium to large, smooth, entire, slightly raised, creamy yellow, green/beta-haemolytic colonies on blood agar, found positive to catalase and coagulase tests were confirmed to be *S. aureus.* Further, bile esculin producing colonies, negative to catalase and coagulase tests were taken as *Enterococcus* spp., which produced grayish, round, small colonies without any haemolytic zones on blood agar (Figure 6(a,f), 7(a,b), 8(c), 9(c) and 10(c); Table 7, 8 and 9).

4.3.7. Antibiotic susceptibility pattern of CoNS

Coagulase negative *Staphylococcus* (CoNS) were showed high rate susceptibility to ciprofloxacin 100%, amikacin 100%, gentamicin 100%, tobramycin 100%, nitrofurantoin 100%, imipenem 96.3%, ceftriaxone 70.4%, ofloxacin 63%, norfloxacin 59.3%, cephalexin 55.6% and amoxicillin (51.9%), but more than half of CoNS showed less sensitivity against cefotaxime 37%, ceftazidime 37%, cotrimoxazole 37%, ampicillin 14.8% and nalidixic acid 0% (Table 16). More than 50% of Coagulase *Staphylococcus* strains were resistant to the most of commonly used antibiotics (Figure, 12 and 19).

Table 16. Antibiotic susceptibility pattern of CoNS.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	14 (51.9)	0 (0.0)	14 (51.9)	13 (48.1)
Ampicillin	3 (11.1)	1 (3.7)	4 (14.8)	23 (85.2)
Cefotaxime	8 (29.6)	2 (7.4)	10 (37.0)	17 (63.0)
Ceftazidime	9 (33.3)	1 (3.7)	10 (37.0)	17 (63.0)
Ceftriaxone	18 (66.7)	1 (3.7)	19 (70.4)	8 (29.6)
Cephalexin	15 (55.6)	0 (0.0)	15 (55.6)	12 (44.4)
Ciprofloxacin	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Nalidixic acid	0 (0.0)	0 (0.0)	0 (0.0)	27 (100)
Norfloxacin	16 (59.3)	0 (0.0)	16 (59.3)	11 (40.7)
Ofloxacin	15 (55.6)	2 (7.4)	17 (63)	10 (37.0)
Amikacin	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Gentamicin	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Tobramycin	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Imipenem	26 (96.3)	0 (0.0)	26 (96.3)	1 (3.7)
Nitrofurantoin	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Cotrimoxazole	9 (33.3)	1 (3.7)	10 (37.0)	17 (63.0)

Results are expressed as a percentage of 27 CoNS isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial

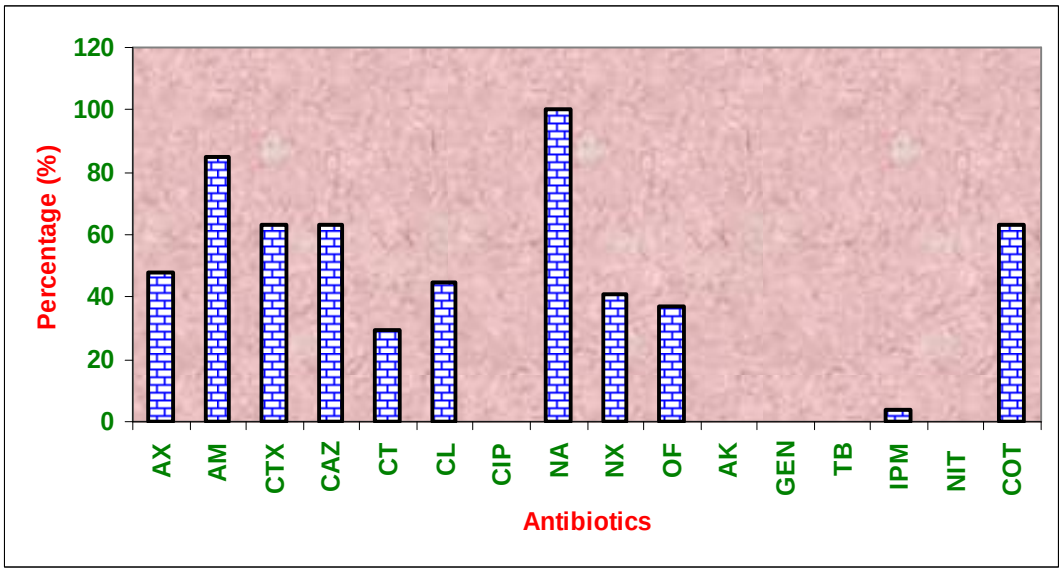


Figure 19. Antibiotic resistance of CoNS strains from patients with UTIs.

4.3.8. Antibiotic susceptibility pattern of *Enterococcus* spp.

Enterococci were fully susceptible to ceftriaxone 100%, amikacin 100%, gentamicin 100%, tobramycin 100%, followed by cefotaxime 83.3%, ciprofloxacin 83.3%, imipenem 83.3%, nitrofurantoin 79.2%, ofloxacin 75%,; but it was low sensitivity to ampicillin 54.2%, cephalexin 54.2%, norfloxacin 54.2%, amoxicillin 50%, ceftazidime 41.7%, cotrimoxazole 41.7% and nalidixic acid 0% (Table 17; Figure 12 and 20).

Table 17. Antibiotic susceptibility pattern of *Enterococcus* spp.

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	11 (45.8)	1 (4.2)	12 (50.0)	12 (50.0)
Ampicillin	13 (54.2)	0 (0.0)	13 (54.2)	11 (45.8)
Cefotaxime	20 (83.3)	0 (0.0)	20 (83.3)	4 (16.7)
Ceftazidime	10 (41.7)	0 (0.0)	10 (41.7)	14 (58.3)
Ceftriaxone	24 (100)	0 (0.0)	24 (100)	0 (0.0)
Cephalexin	13 (54.2)	0 (0.0)	13 (54.2)	11 (45.8)
Ciprofloxacin	20 (83.3)	0 (0.0)	20 (83.3)	4 (16.7)
Nalidixic acid	0 (0.0)	0 (0.0)	0 (0.0)	24 (100)
Norfloxacin	13 (54.2)	0 (0.0)	13 (54.2)	11 (45.8)
Ofloxacin	17 (70.8)	1 (4.2)	18 (75.0)	6 (25.0)
Amikacin	24 (100)	0 (0.0)	24 (100)	0 (0.0)
Gentamicin	24 (100)	0 (0.0)	24 (100)	0 (0.0)
Tobramycin	24 (100)	0 (0.0)	24 (100)	0 (0.0)
Imipenem	20 (83.3)	0 (0.0)	20 (83.3)	4 (16.7)
Nitrofurantoin	19 (79.2)	0 (0.0)	19 (79.2)	5 (20.8)
Cotrimoxazole	10 (41.7)	0 (0.0)	10 (41.7)	14 (58.3)

Results are expressed as a percentage of 24 *Enterococcus* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial

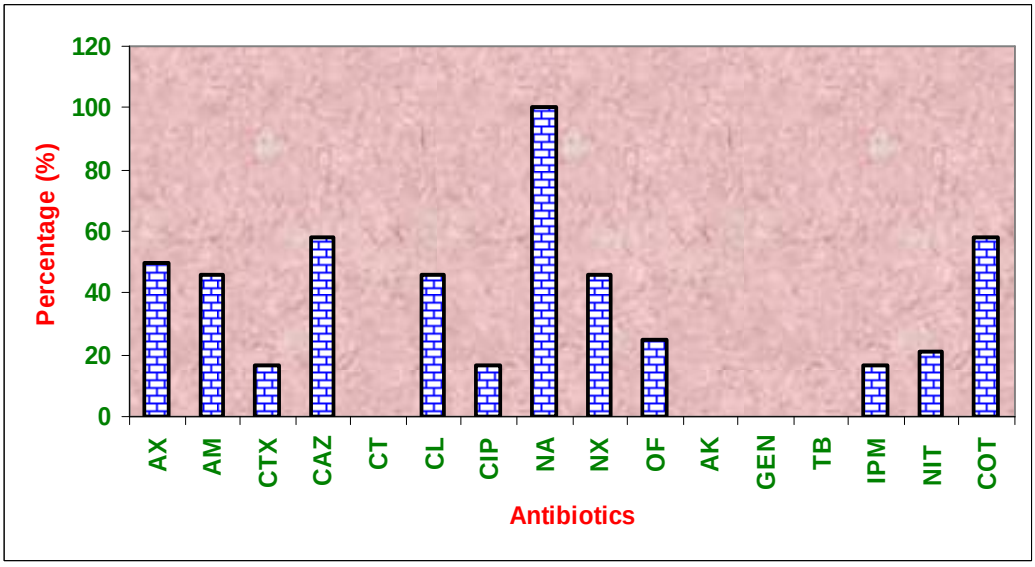


Figure 20. Antibiotic resistance of *Enterococcus* strains from patients with UTIs.

4.3.9. Antibiotic susceptibility pattern of *Staphylococcus saprophyticus*

Staphylococcus saprophyticus was 100% sensitive to amikacin, gentamicin, tobramycin, imipenem, while it was highest sensitive to ofloxacin 89.7%, ciprofloxacin 86.7%, norfloxacin 80%, nitrofurantoin 73.4%, cotrimoxazole 53.3%, ceftazidime 46.7%, cefotaxime 40%, ceftriaxone 40% and cephalexin 26.7%. *Staphylococcus aureus* was 100 % resistant to three antibiotics amoxicillin, ampicillin and nalidixic acid (Table 18; Figure 13 and 21).

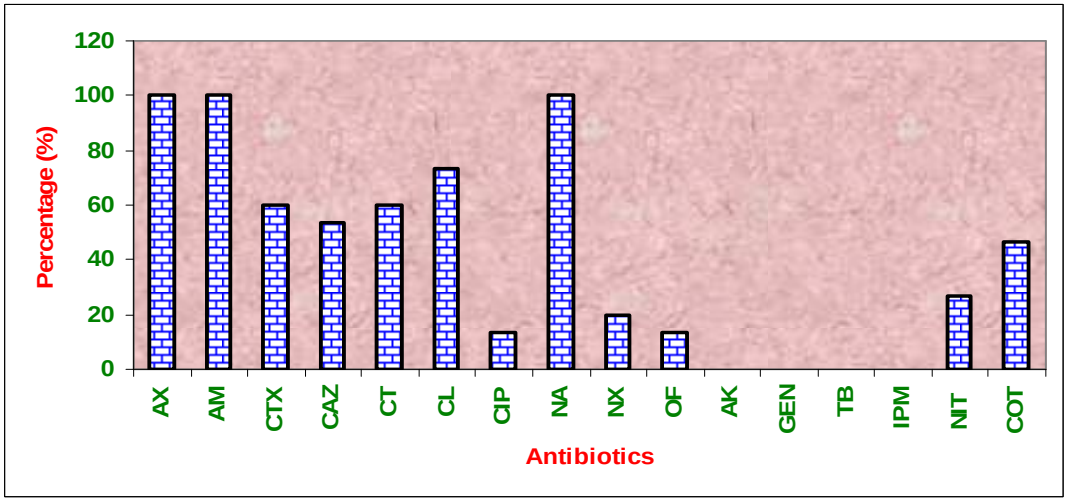


Figure 21. Antibiotic resistance of *S.saprophyticus* from patients with UTIs.

Table 18. Antibiotic susceptibility pattern of *Staphylococcus saprophyticus.*

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	0 (0.0)	0 (0.0)	0 (0.0)	15 (100)
Ampicillin	0 (0.0)	0 (0.0)	0 (0.0)	15 (100)
Cefotaxime	6 (40.0)	0 (0.0)	6 (40.0)	9 (60.0)
Ceftazidime	7 (46.7)	0 (0.0)	7 (46.7)	8 (53.3)
Ceftriaxone	6 (40.0)	0 (0.0)	6 (40.0)	9 (60.0)
Cephalexin	3 (20.0)	1 (6.7)	4 (26.7)	11 (73.3)
Ciprofloxacin	13 (86.7)	0 (0.0)	13 (86.7)	2 (13.3)
Nalidixic acid	0 (0.0)	0 (0.0)	0 (0.0)	15 (100)
Norfloxacin	12 (80.0)	0 (0.0)	12 (80.0)	3 (20.0)
Ofloxacin	13 (86.7)	0 (0.0)	13 (89.7)	2 (13.3)
Amikacin	15 (100)	0 (0.0)	15 (100)	0 (0.0)
Gentamicin	15 (100)	0 (0.0)	15 (100)	0 (0.0)
Tobramycin	15 (100)	0 (0.0)	15 (100)	0 (0.0)
Imipenem	15 (100)	0 (0.0)	15 (100)	0 (0.0)
Nitrofurantoin	10 (66.7)	1 (6.7)	11 (73.4)	4 (26.6)
Cotrimoxazole	8 (53.3)	0 (0.0)	8 (53.3)	7 (46.7)

Results are expressed as a percentage of 15 *S.saprophyticus* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

4.3.10. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Staphylococcus aureus were fully susceptible to ceftriaxone, ciprofloxacin, ofloxacin, amikacin, gentamicin, tobramycin, imipenem and nitrofurantoin, followed by cefotaxime 85.7%, cephalexin (85.7%), norfloxacin 85.7%, ceftazidime 71.4%, cotrimoxazole 71.4%, amoxicillin 57.1%, while it were showed less sensitivity against ampicillin 42.9% and nalidixic acid 28.6% (Table 19; Figure 12 and 13).

Table 19. Antibiotic susceptibility pattern of *Staphylococcus aureus.*

Antibiotic	Sensitive No (%)	Moderately Sensitive No (%)	Total Sensitive (%)	Total Resistant No (%)
Amoxicillin	4 (57.1)	0 (0.0)	4 (57.1)	3 (42.9)
Ampicillin	3 (42.9)	0 (0.0)	3 (42.9)	4 (57.1)
Cefotaxime	5 (71.4)	1 (14.3)	6 (85.7)	1 (14.3)
Ceftazidime	5 (71.4	0 (0.0)	5 (71.4)	2 (28.6)
Ceftriaxone	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Cephalexin	6 (85.7)	0 (0.0)	6 (85.7)	1 (14.3)
Ciprofloxacin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Nalidixic acid	2 (28.6)	0 (0.0)	2 (28.6)	5 (71.4)
Norfloxacin	6 (85.7)	0 (0.0)	6 (85.7)	1 (14.3)
Ofloxacin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Amikacin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Gentamicin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Tobramycin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Imipenem	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Nitrofurantoin	7 (100)	0 (0.0)	7 (100)	0 (0.0)
Cotrimoxazole	4 (57.1)	1 (14.3)	5 (71.4)	2 (28.6)

Results are expressed as a percentage of 7 *S.aureus* isolates susceptible, moderately susceptible and resistant, respectively, for each antimicrobial.

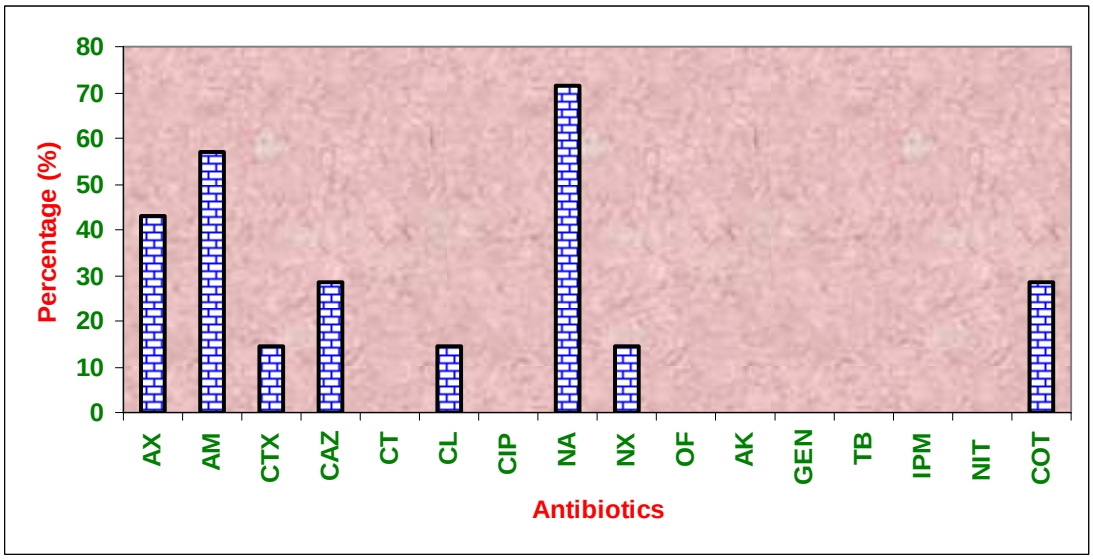


Figure 22. Antibiotic resistance of *S. aureus* from patients with UTIs.

4.3.11. Antibiotic resistance patterns of all Bacterial isolates from UTIs

All the bacterial isolates from urinary tract infection patients were highly susceptible to amikacin 90.4%, imipenem 88.6%, tobramycin 82.5%, gentamicin/nitrofurantoin 72.8%, ciprofloxacin 70.9%, ofloxacin 65.8%, norfloxacin 63.3%, cefotaxime 62.5%, ceftriaxone 55%, ceftazidime 53.7%, nalidixic acid 24.9%, cotrimoxazole 24.4%, cephalexin 20.4%, ampicillin 13.2%, and amoxicillin 12.7%. High level of resistance was seen to ampicilin, amoxicillin, cephalexin and nalidixic acid. Most of the isolates were sensitive to amikacin, imipenem, tobramycin, gentamicin, nitrofurantoin, ciprofloxacin and ofloxacin. Amikacin was found to be very effective against all the isolates. (Table 20 and Figure 23).

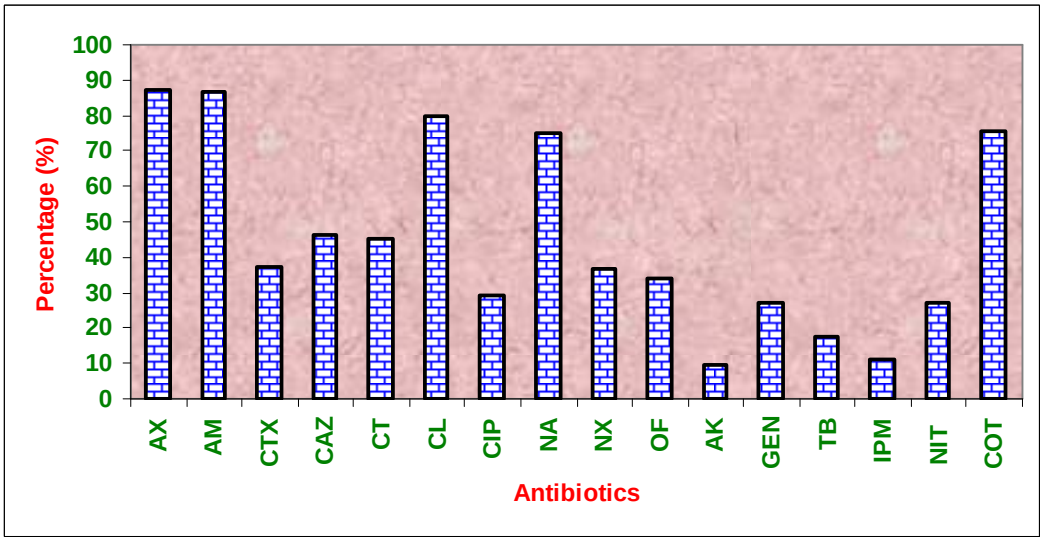


Figure 23. Antibiotic resistant of all bacterial strains isolated from UTIs.

4.3.12. Aminoglycoside resistance in bacterial isolates from UTIs

Three aminoglycoside antibiotics (µg/disc), amikacin, gentamicin and tobramycin were moderately resistant to all bacteria used, in ranges, 11.8% to 37.5% of 355 strains of *E. coli*, 13.9% to 19.4% of 72 strains of *K. pneumoniae* 11.8% to 30.9% of 68 strains of *Pseudomonas* spp. 0% to 9.1% of 44 strains of *Proteus* spp. 0% to 1% of 10 strains of *Enterobacter* spp. 0% of 27 strains of CoNS 0% of 24 strains of *Enterococcus* spp. 0% of 15 strains of *S.saprophyticus* and 0% of seven strains of *S. aureus.* Among these three antibiotics, gentamicin was recorded to be more resistant to these pathogens (Table 20).

Table 20. Antibiotic resistance pattern of bacteria isolated from urine sample (n=628).

Antibiotics	*E. coli* No. (%)	*Klebsiella* No. (%)	*Pseudomonas* No. (%)	*Proteus* No. (%)	*Enterobacter* No. (%)	CoNS No. (%)	*Enterococcus* No. (%)	*S. saprophyticus* No. (%)	*S.aureus* No. (%)	Total No. (%)
AX	326 (91.8)	64 (88.9)	68 (100)	35 (79.5)	7 (70)	13 (48.1)	12 (50)	15 (100)	3 (42.9)	**543 (87.3)**
AM	315 (88.7)	60 (83.3)	68 (100)	35 (79.5)	9 (90)	23 (85.2)	11 (45.8)	15 (100)	4 (57.1)	**540 (86.8)**
CTX	149 (42.0)	24 (33.3)	18 (26.5)	8 (18.2)	3 (30)	17 (63.0)	4 (16.7)	9 (60.0)	1 (14.3)	**233 (37.5)**
CAZ	161 (45.3)	33 (45.8)	27(39.7)	20 (45.5)	5 (50)	17 (63.0)	14 (58.3)	8 (53.3)	2 (28.6)	**287 (46.1)**
CT	188 (53.0)	24 (33.3)	42 (61.8)	7 (15.9)	2 (20)	8 (29.6)	0 (0.0)	9 (60.0)	0 (0.0)	**280 (45.0)**
CL	314 (88.5)	50 (69.4)	56 (82.4)	34 (77.3)	6 (60)	12 (44.4)	11 (45.8)	11 (73.3)	1 (14.3)	**495 (79.6)**
CIP	110 (31.0)	17 (23.6)	42 (61.8)	5 (11.4)	1 (10)	0 (0.0)	4 (16.7)	2 (13.3)	0 (0.0)	**181 (29.1)**
NA	274 (77.2)	40 (55.6)	63 (92.6)	15 (34.1)	4 (40)	27 (100)	24 (100)	15 (100)	5 (71.4)	**467 (75.1)**
NX	115 (32.4)	18 (25.0)	55 (80.9)	11 (25.0)	3 (30)	11 (40.7)	11 (45.8)	3 (20.0)	1 (14.3)	**228 (36.7)**
OF	126 (35.5)	15 (20.8)	43 (63.2)	9 (20.5)	2 (20)	10 (37.0)	6 (25.0)	2 (13.3)	0 (0.0)	**213 (34.2)**
AK	42 (11.8)	10 (13.9)	8 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**60 (9.60)**
GEN	133 (37.5)	14 (19.4)	21 (30.9)	0 (0.0)	1 (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**169 (27.2)**
TB	82 (23.1)	14 (19.4)	9 (13.2)	4 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**109 (17.5)**
IPM	23 (6.5)	10 (13.9)	28 (41.2)	5 (11.4)	0 (0.0)	1 (3.7)	4 (16.7)	0 (0.0)	0 (0.0)	**71 (11.4)**
NIT	46 (13.0)	36 (50.0)	48 (70.6)	24 (54.5)	6 (60)	0 (0.0)	5 (20.8)	4 (26.6)	0 (0.0)	**169 (27.2)**
COT	271 (76.3)	51 (70.8)	68 (100)	35 (79.5)	5 (50)	17 (63.0)	14 (58.3)	7 (46.7)	2 (28.6)	**470 (75.6)**
Total	**355 (54.6)**	**72 (11.2)**	**68 (10.5)**	**44 (6.8)**	**10 (1.5)**	**27 (4.1)**	**24 (3.7)**	**15 (2.3)**	**7 (1.1)**	**622 (95.7)**

4.3.13. Beta-lactam group of antibiotics resistance in bacterial isolates from UTIs

The percentages of resistance patterns of both Gram negative bacilli and Gram positive cocci with two antibiotics of the beta-lactam group **(**ampicillin, amoxicillin) are detailed (Table 20); resistance patterns were in ranges: 88.7% to 91.8% of 355 strains of *E. coli*, 83.3% to 88.9% of 72 strains of *K. pneumoniae* 100% of 68 strains of *Pseudomonas* spp. 79.5% of 4 strains of *Proteus* spp. 70% to 90% of 10 strains of *Enterobacter* spp. 48.1% to 85.2% of 27 strains of CoNS 45.8% to 50% of 24 strains of *Enterococcus* spp. 100% of 15 strains of *S.saprophyticus* and 42.9% to 57.1% of 7 strains of *S. aureus.* All these two antibiotics were almost equally resistant to the isolated UTI bacteria.

Imipenem is an intravenous β-lactam antibiotic Imipenem belongs to the subgroup of carbapenems. The percentages of resistance patterns of both Gram positive and Gram negative bacteria with these antibiotics are detailed (Table 20); resistance patterns were in ranges: 6.5% of 355 strains of *E. coli*, 13.9% of 72 strains of *Klesiella* spp. 41.2% of 68 strains of *Pseudomonas* spp. 11.4% of 4 strains of *Proteus* spp. 3.7% of 27 strains of CoNS 16.7% of 24 strains of *Enterococcus* spp. 0% of 10 strains of *Enterobacter* spp. 15 strains of *S. saprophyticus* and 7 strains of *S. aureus.*

Further, resistance-percent values of UTI bacteria to cephalosporin antibiotics (Cefotaxime, ceftazidime, ceftriaxone and cephalexin) were in ranges, 42% to 88.5% of 355 strains of *E.coli*, 33.3% to 69.4% of 72 strains of *K. pneumoniae* 26.5% to 82.4% of strains of *Pseudomonas* spp. 15.9% to 77.3% of 4 strains of *Proteus* spp. 20% to 60% of 10 strains of *Enterobacter* spp. 29.6% to 63% of 27 strains of CoNS 16.7% to 58.3% of 24 strains of *Enterococcus* spp. 53.3% to 73.3% of 15 strains of *S. saprophyticus* 4.3% to 28.6% of 7 strains of *S. aureus.* All these four antibiotics were moderately resistant to the isolated UTI pathogens. These

antibiotics were resistant to UTI pathogens in the order: cephalexin >ceftazidime>cefotaxime>ceftriaxone (Table 20).

4.3.14. Fluoroquinolone resistance in bacterial isolates from UTIs

Similarly, resistance-percent values of UTI bacteria to antibiotics of the fluoroquinolone group (ciprofloxacin, nalidixic acid, norfloxacin and ofloxacin) were in ranges; 31% to 77.2%of 355 strains of *E. coli*, 20.8% to 55.6% of 72 strains of *K. pneumoniae* 61.8% of 92.6 strains of *Pseudomonas* spp. 11.4% to 34.1 of 4 strains of *Proteus* spp. 10% to 40% of 10 strains of *Enterobacter* spp. 0% to 100% of 27 strains of CoNS 16.7% to 100% of 24 strains of *Enterococcus* spp. 13.3% to 100% of 15 strains of *S.saprophyticus* and 0% to 71.4% of 7 strains of *S. aureus* (Table 20). These antibiotics were resistant to UTI pathogens in the order: nalidixic acid> norfloxacin>ofloxacin>ciprofloxacin. Among these four antibiotics, nalidixic acid was recorded to be more resistant to these pathogens.

4.3.15. Two stand-alone antibiotics resistance in bacteria from UTIs

The detailed antibiograms of two stand-alone antibiotics, cotrimoxazole and nitrofurantoin were recorded. Nitrofurantoin was found resistance for 13% of 355 strains of *E. coli*, 50% of 72 strains of *K. pneumoniae* 70.6% of 68 strains of *Pseudomonas* spp. 54.5% of 4 strains of *Proteus* spp. 60% of 10 strains of *Enterobacter* spp. 0% of 27 strains of CoNS 20.8% of 24 strains of *Enterococcus* spp. 26.6% of 15 strains of *S.saprophyticus* and 0% of 7 strains of *S. aureus*. Surprisingly, resistance-percent values of UTI bacteria to antibiotic of the cotrimoxazole were 76.3% of 355 strains of *E. coli*, 70.8% of 72 strains of *K. pneumoniae*, 100% of 68 strains of *P. aeruginosa*. 79.5% of 4 strains of *Proteus* spp. 50% of 10 strains of *Enterobacter* spp. 63% of 27 strains of CoNS 58.3% of 24 strains of *Enterococcus* spp. 46.7% of 15 strains of *S. saprophyticus* and 28.6% of 7 strains of *S. aureus* (Table 20).

4.4. ISOLATION AND CHARACTERISATION OF THE PATHOGENIC *CANDIDA* SPECIES

Candida species were isolated from twenty two numbers of patients**.** The species were identified according to morphology and color of colonies on CHROMagar *Candida*. The color of colonies on CHROMagar *Candida* was similar as given by the manufacturer, i.e. green colonies of *C.albicans,* steel blue colonies of *C.tropicalis* accompanied by purple pigmentation which diffuses into surrounding agar by growth, and large, fuzzy, rose colored colonies with white edges of *C.krusei,* the smooth white to light pink colonies of C. *glabrata* which later became pink (Table 21). The prevalence candiduria in critically ill patients in the study was 3.4%. Out of the total 22 strains isolated, 4(18.2%) and 18(81.8%) *candida* strains were isolated from male and female patients respectively. *C. albicans* (45.5%) was the highest occurring pathogens isolated, followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%), and *C. krusei* (13.6%) (Table 21 and 22; Figure 24).

Table 21. Characteristics of *Candida* species on Chromagar *Candida* media.

Species	**Colony characteristics on CHROMagar *Candida***	**Total number. of isolates**
C. albicans	Apple green colonies; consistent	10 (45.5%)
C. glabrata	White large glossy pale pink to violet colonies	5 (22.7%)
C. tropicalis	Dull blue, to purple color that diffused into surrounding agar with pale pink edges	4 (18.2%)
C. krusei	Large, flat, spreading, pale pink colonies with matt surfaces	3 (13.6%)

Table 22. Distribution of isolated *Candida* species (n=22).

Species	No. of organisms	Male	Female
C. albicans	10 (45.5%)	2	8
C. glabrata	5 (22.7%)	1	4
C. tropicalis	4 (18.2%)	0	4
C. krusei	3 (13.6%)	1	2
Total	22 (100%)	4 (18.2%)	18 (81.8%)

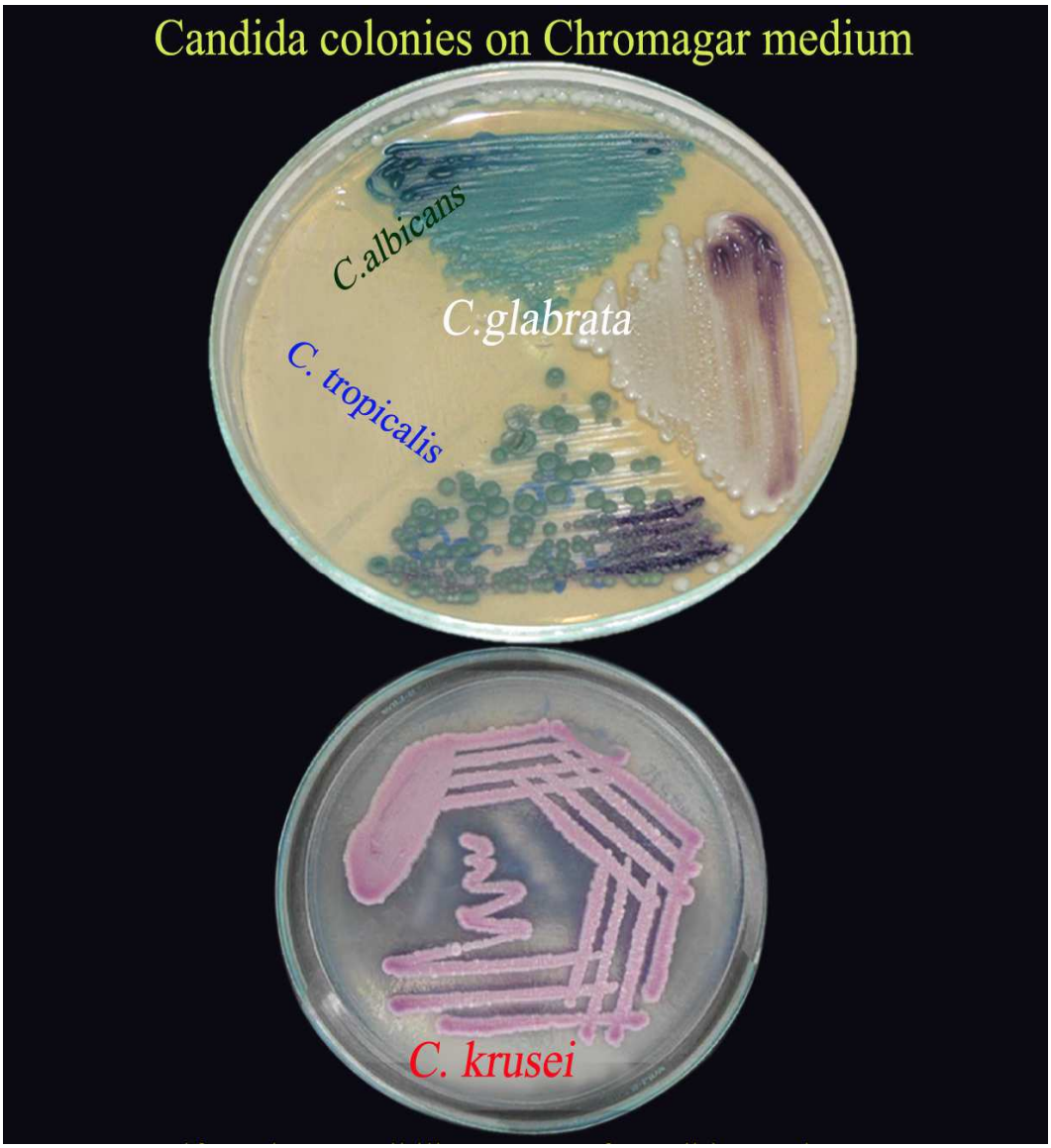


Figure 24. Characteristics of *Candida* species on Chromagar *Candida* media.

Table 23. Identification of isolated *Candida* species by Direct wet mount and Germ tube methods.

Sl. No.	Samples No	Sex of patient	Direct wet mount examination	Chromagar	Germ tube
1	210	Female	Yeast like structure	*C. tropicalis*	Not formed
2	314	Female	Yeast like structure	*C. albicans*	Formed
3	349	Female	Yeast like structure	*C. albicans*	Formed
4	620	Female	Yeast like structure	*C. albicans*	Formed
5	624	Female	Yeast like structure	*C. albicans*	Formed
6	675	Female	Yeast like structure	*C. albicans*	Formed
7	728	Female	Yeast like structure	*C. albicans*	Formed
8	734	Female	Yeast like structure	*C. krusei*	Not formed
9	788	Female	Yeast like structure	*C. krusei*	Not formed
10	842	Female	Yeast like structure	*C. albicans*	Formed
11	951	Female	Yeast like structure	*C. albicans*	Formed
12	954	Female	Yeast like structure	*C. krusei*	Not formed
13	1266	Male	Yeast like structure	*C. albicans*	Formed
14	1288	Male	Yeast like structure	*C. glabrata*	-
15	1290	Male	Yeast like structure	*C. tropicalis*	Not formed
16	1350	Female	Yeast like structure	*C. glabrata*	-
17	1401	Female	Yeast like structure	*C. glabrata*	-
18	1602	Female	Yeast like structure	*C. glabrata*	-
19	1655	Female	Yeast like structure	*C. tropicalis*	Not formed
20	1656	Female	Yeast like structure	*C. glabrata*	-
21	1806	Male	Yeast like structure	*C. albicans*	Formed
22	1995	Female	Yeast like structure	*C. tropicalis*	Not formed

4.5. ANTIFUNGAL SUSCEPTIBILITY PATTERN OF *CANDIDA* SPECIES CAUSING UTIs

The antifungal sensitivity tests carried out using commercially available antifungal disc that 10 (100%) strains of *C. albicans* were sensitive to amphotericin B and itraconazole, while 7 (70%) strains of *C. albicans* were resistant to fluconazole and 2 (20) to Ketoconazole. Of the *C. glabrata* strains, all (100%) strains were sensitive to Itraconazole, 4 (80%) were sensitive to amphotericin B and itraconazole, whereas 3 strains (60%) were resistant to Ketoconazole. Among the *C. tropicalis strains*, four (100%) were found sensitive to amphotericin B and Itraconazole. While all four strains (100%) were found resistant fluconazole and 2 stains resistant (20%) to Ketoconazole. *C. krusei*, 3 (100%) strains were found sensitive to amphotericin B and 100 % resistant to Fluconazole and Ketoconazole, followed by 1 (33.3%) to Itraconazole antifungal agents. Amphotericin B and itraconazole 21 (95.5%) was found to be the most effective antifungal agent (Table 24; Figure 25 and 26).

Table 24. Antifungal susceptibility pattern of *Candida* species causing UTIs.

***Candida* Species**	**Amphotericin B** (20µg)		**Fluconazole** (25µg)		**Itraconazole** (50µg)		**Ketoconazole** (10µg)	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**
C. albicans	10 (100%)	0 (0.0%)	3 (30%)	7 (70%)	10 (100%)	0 (0.0%)	2 (20%)	8 (80%)
C. glabrata	4 (80%)	1 (20%)	1 (20%)	4 (80%)	5 (100%)	0 (0.0%)	2 (40%)	3 (60%)
C. tropicalis	4 (100%	0 (0.0%)	0 (0.0%)	4 (100%)	4 (100%)	0 (0.0%)	0 (0.0%)	4 (100%)
C. krusei	3 (100%)	0 (0.0%)	0 (0.0%)	3 (100%)	2 (66.7%)	1 (33.3%)	0 (0.0%)	3 (100%)
Total	21 (95.5%)	1 (4.5%)	4 (18.2%)	18 (81.8%)	21 (95.5%)	1 (4.5%)	4 (18.2%)	18 (81.8%)

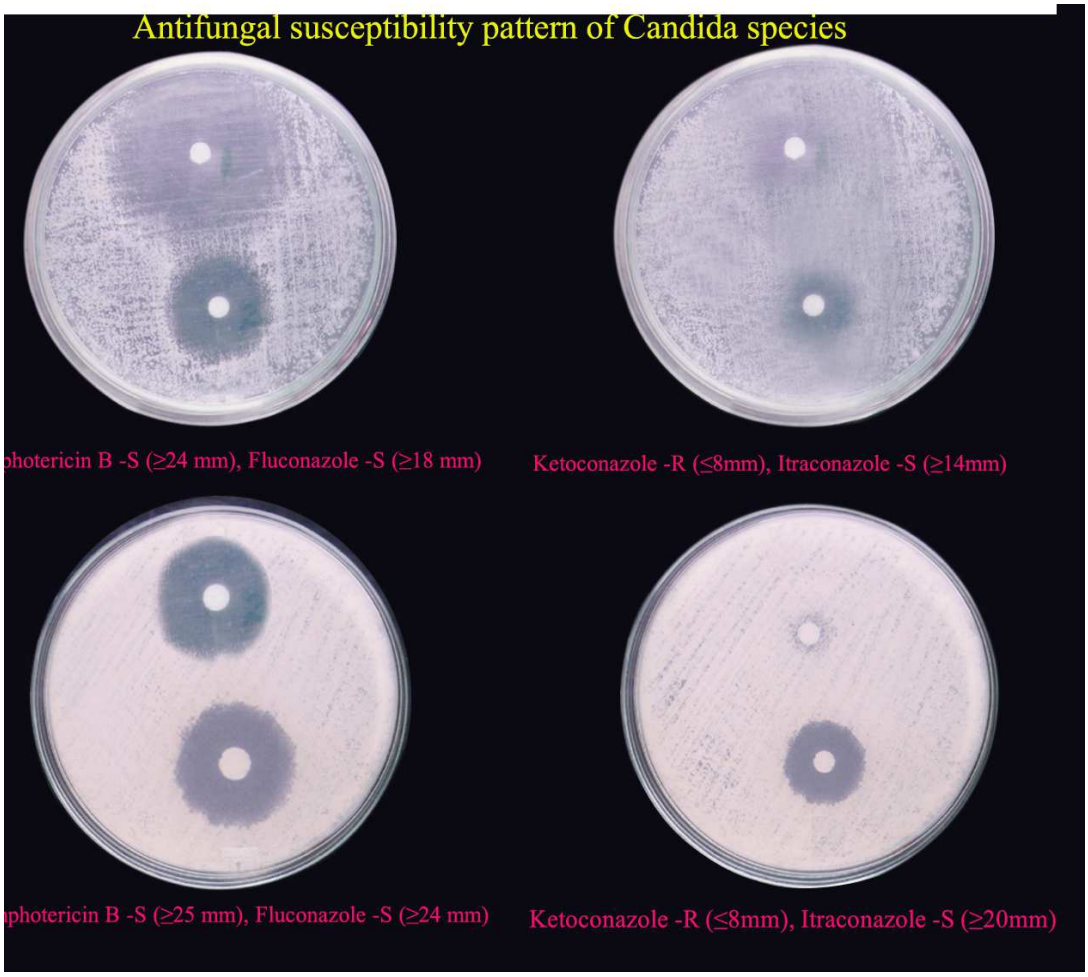


Figure 25. Antifungal susceptibility pattern of *Candida* species.

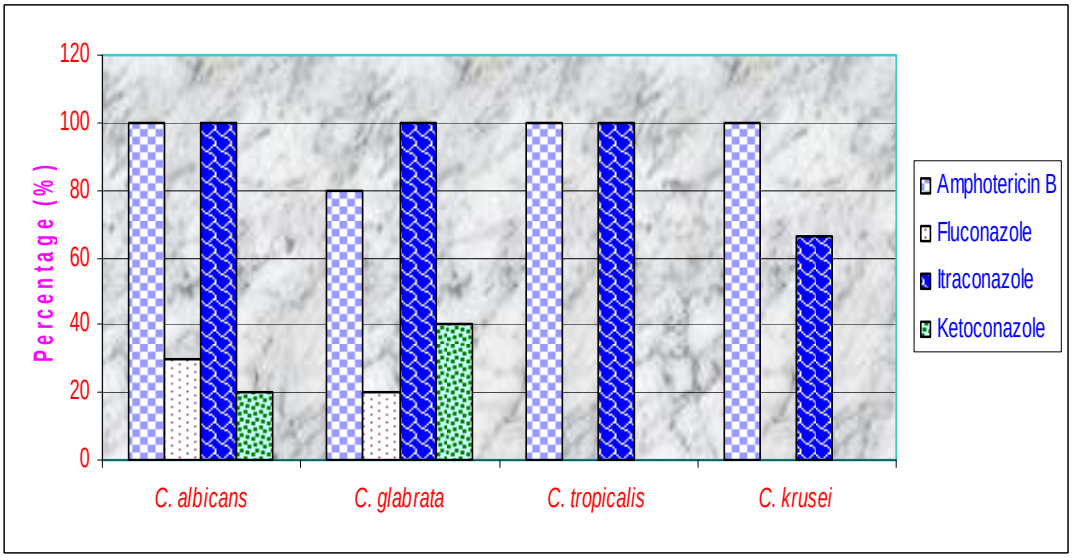


Figure 26. Antifungal susceptibility of *Candida* species isolated from candiduria

4.6. PLASMID ANALYSIS-MOLECULAR CHARACTERISATION OF ESBL PRODUCING *E.COLI*

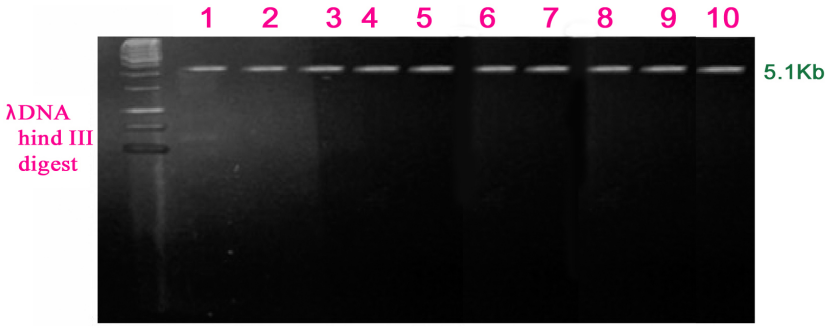
4.6.1. ESBL Positive *E.coli*

Totally 100 MDR *E. coli* isolate were isolated from different source of urine specimens. Ten isolates were confirmed positive for ESBL production. Third generation cephalosporins showed 37% to 45% resistance. For these isolates gentamicin showed 27.2% resistance, whereas amphicillin and amoxicillin showed 86% to 87% resistance. Imipenem and amikacin depicted 88.6% and 90.4% sensitive respectively. Using the DDST method, ESBL was confirmed in 10 isolates (10%) of which two from cystitis samples (20%), four from pyelonephritis samples (40%) and the rest were normal urine samples (40%).

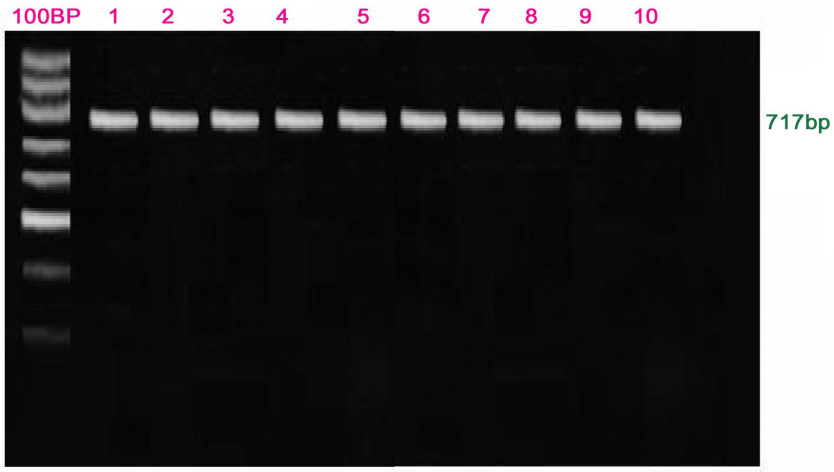
Figure 27. Double disc synergy test.(Augmentin Disc (20 g of amoxicillin + 10 g of clavulanic acid) with 30 g of cefotaxime).

4.6.2. PCR and RFLP analysis

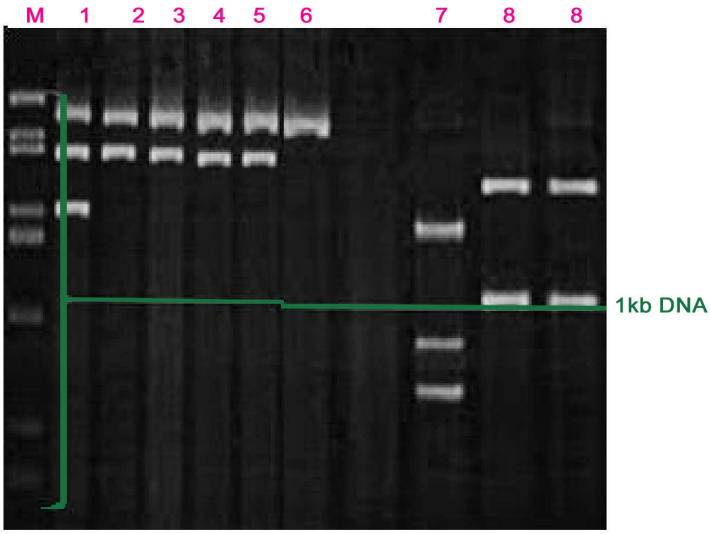
Confirmed ESBL samples were checked for the presence of TEM gene by PCR amplification and it was found to be positive. PCR products of molecular size 717 bps showed that it was TEM gene and it resulted in 10% of isolates contained bla_{TEM} genes (Figure 26). The results showed that 10% of isolates were positive for ESBL both by phenotypic and genotypic methods. Further Restriction fragment length polymorphism (RFLP) was carried out for genomic DNA with *Xba I* restriction enzyme to check for the polymorphism (Figure 26). RFLP analysis showed eight different banding patterns for the ten samples among which four samples showed identical banding pattern and other two samples also had the similar banding pattern, indicating two different source of origin. Other samples had different patterns indicating that they are not from same samples. Thus ten samples showed eight different banding patterns concludes that the samples are not from same origin.



Genomic DNA of ESBL producing E.coli samples



PCR products showing Tem gene of 717bp molecular weight



Digested DNA of E. coli samples

Figure 28. Molecular Characteristics of ESBL producing MDR *E.coli.*

5. DISCUSSION

This study shows the distribution of microbial species isolated from patients with UTI at a Gangasaras diagnostic laboratory center in Pattukkottai, Tamilnadu, India, and their susceptibility pattern to antimicrobial agents. Furthermore, in this investigation described the relationship between sex, age and isolated bacterial agents of UTI.

Out of the 2400 processed samples, six fifty (27.1%) midstream urine samples yielded significant microbial growth. One thousand fifty (72.9%) samples recorded no bacterial and fungal growth after 24 and 48 hours incubation at 37ºC respectively. The reason for this may be due to the fact that subjects (from whom negative samples were obtained), may have been on antibiotic therapy before reporting to the hospital and laboratory. The antibiotics may have inhibited bacterial growth (Okonofua and Okonofua, 1989; Parveen *et al.,* 2011; Sharma *et al.,* 2013). The use of midstream urine was aimed at reducing and eliminating the influence of normal flora and other contaminants on expected results.

Almost all samples showed pus cells. Therefore, presence of pus cells may not be a reliable indicator of UTI. In this study, the samples showing bacteria in Gram's stained smear also grew bacteria in culture in significant numbers (10^5 CFU/ml). However, 31.5% of the samples negative for bacteria in Grams stained smears, also showed growth in cultures. In a few of the studies microscopic bacteriuria was detected in Gram's stained, uncentrifuged urine in over 90% of UTIs with colony counts of 10^5 CFU/ml or more (Jenkins *et al.,* 1986).

In the present study, UTI prevalence rate of 27.1% was obtained. This was consistent with the reports of similar UTI studies by other workers

(Ebie *et al.,* 2001; Oladeinde *et al.,* 2011) who recorded 35.3% and 39.7% respectively. Other similar studies recorded much lower prevalence rates of 26.7% and 22.3% (Omonigho *et al.,* 2001). It was on record that much higher prevalence rates have been reported by some authors (Jellheden *et al.,* 1996; Onifade *et al.,* 2005; Obiogbolu *et al.,* 2009; Shirishkumar *et al.,* 2012) stating prevalence rates of 46.5%, 54.0%, 58.0%, 66.0% and 71.6% respectively.

In the present study found that girls in the 0-10 years of age group had 15.5% UTI. Generally UTIs are infrequent among girls aged 2-10 years, but some experience multiple repeated episodes of recurrent cystitis or pyelonephritis (Koff *et al.,* 1998). The present finding on UTIs among girls of 0-10 years was quite high (24.7%). The presence of bacteriuria in childhood defines a population at higher risk for the development of bacteriuria in adulthood (Sobel and Kaye, 2005).

In this study, age group of 20-30 years had got high prevalence of UTI, a total of 22.4% of patients were found to have significant bacteriuria. In the present study female of childbearing age group (20-40 years) had 27% growth positive cases. This result suggests that sexually active and women of childbearing age were more susceptible to UTI. Previous study done by Rajbhandari and Shrestha (2002); Sharma and Paul (2012) also found the similar results. The increased risk of UTIs in sexually active women has been attributed to mechanical effect of introducing uropathogens into the bladder (Schneider and Riley, 1996; Hooton, 2000). In the present investigation, this fact was supported where the rate of growth positivity was found to be 69.8% (454/650) in females and 30.2% (196/650) in males. This higher growth positivity seen in females was found to be statistically significant ($p<0.05$) and is attributed to their anatomical structure (short urethra and proximity to anal orifice) leading to easy access for coliform bacilli. This result confirms and expands the previous findings

(Strom *et al.,* 1987; Abu Shaqra, 2000; Rajbhandari and Shrestha, 2002; Khadri and Alzohairy, 2009).

The overall incidence of UTI observed among males in the present study was 30.2%. Observed data in this study also showed a high rate of UTI in male children of 0-10 years of age (9.2%). Generally infections were reported to be rare in boys except in association with anatomic or functional abnormalities in the first year of life (Koff *et al.,* 1998). In the present work, most of these boys were being investigated for febrile illness.

Generally low prevalence of UTI was observed among men. This was attributed to greater distance between the anus and urethra, the drier environment surrounding the urethral meatus, the greater length of male urethra and the antibiotic activity of the prostatic fluid (Lipsky, 1989). UTI may be present even in the absence of these factors. In the present study, observed that more than 70% infection was seen among men of below 40 years of age. Among males, highest growth positivity (9.6%) was found among age group of 30-40 years. The similar study done by Jha and Bapat (2005) also showed that the highest percentage of infected male was found in age group 31-40 years.

Results obtained in this study also showed that Gram negative bacilli constituted 85.4%% (of which Enterobacteriaceae made up 74.6%) while Gram positive cocci which constituted 11.2% of which the most prevalent and second most prevalent bacterial agents of UTI in the study area were *Escherichia coli* (54.6%) and *K. pneumoniae* (11.2%) respectively. This was similar to the report of Oluremi *et al.* (2011) which stated 85.0% of Gram negative bacilli (made of 66.7% Enterobacteriaceae) and 15.0% Gram positive cocci of which *Escherichia coli* and *K. pneumoniae* were the most and second most occurring bacterial agents respectively. The present report also agrees with reports of other studies (Grunerberg, 1980; Orret and Shurland, 1998; Daza *et al.,* 2001; Dimitrov *et al.,* 2004; Inabo and

Obanibi, 2006; Abubakar, 2009; Omigie, 2009; Nwadioha *et al.,* 2010; Manikandan and Amsath, 2013) but differs from the reports of Ehinmidu (2003) and Aboderin *et al.* (2009) which recorded *Pseudomonas aeruginosa* and *K. pneumoniae* respectively as the predominant bacteria.

The high occurrence of Enterobacteriaceae in this work (74.6%) and some of the isolated pathogens show that a high percentage of urinary tract infection may be due to faecal contamination arising from poor hygiene. This is so because, these organisms occur in the perineum of the large intestine as commensals (Anyamene *et al.,* 2002; Moore *et al.,* 2002; Behzadi *et al.,* 2008). Also, commensals of the intestine are more involved in UTIs because of the anatomy proximity to the genitourinary area (Obiogbolu, 2004).

In this study *E. coli* was the predominant uropathogen (38.9%) causing UTI. This was comparable to a retrospective study done at Bombay by Sonavane *et al.* (2008) in which *E. coli* was isolated from 41.31% of UTI cases. The pattern of isolation of organism in this study was similar to the results from various regions of India (Tankhiwale *et al.,* 2004; Kothari and Sagar, 2008; Taneja *et al.,* 2010; Agarwal *et al.,* 2012; Patel *et al.,* 2012; Gautam *et al.,* 2013) and other countries (Mordi and Erah, 2006, Obiogbolu *et al.,* 2009; Amin *et al.,* 2011; Oladeinde *et al.,* 2011; Oluremi *et al.,* 2011; Yakubu *et al.,* 2012; Ghazi *et al.,* 2013), which indicate that *Escherichia coli* is the commonest pathogen isolated in patients with UTIs.

The second major uropathogen isolated in the present study was *K. pneumoniae* (11.2%), which was similar to the result of 14% (Nimri and Batchoun, 2010) and 14.9% (Humayun and Iqbal, 2012). In majority of other Indian studies showed (Sonavane *et al.,* 2008; Taneja *et al.,* 2010; Manikandan and Amsath 2013) *K. pneumoniae* was the second major uropathogens.

The occurrence of *Pseudomonas aeruginosa* (10.5%) in this study was not in agreement the much higher incidence rates recorded by other authors in similar UTI investigations (Shigemura *et al.,* 2005; Tambekar *et al.,* 2006; Kolawole, 2009; Omigie *et al.,* 2009; Oluremi *et al.,* 2011). The incidence of *Pseudomonas aeruginosa* 10.5%, which was almost similar to 9.8% (Deshpande *et al.,* 2011). *Pseudomonas* spp. was an opportunistic pathogen causing infections mainly in debilitated or immunocompromised patients. Hospital acquired UTIs are common due to *Pseudomonas* spp. (Tambekar *et al*., 2006; Forbes *et al.,* 2007).

Proteus species (6.8%), which was higher than 5.5% (Baral *et al.,* 2012) and 4.2% (Ahmad *et al.,* 2011). *Enterobacter* species 1.5%, which was lower than 5.5% (Kothari and Sagar, 2008). *Citrobacter* species 0.6%, which was lower than 4.5% and 5% (Inyama *et al.,* 2011). *Acinetobacter* species 0.3%, which is very lower than 4.2% (Humayun and Iqbal, 2012). *Staphylococcus aureus* 1.1 % and *Enterococcus faecalis* 3.7% were almost similar finding (2.9%) reported by Jung *et al.* (2011). In this study also similar to Akortha and Ibadin (2008) and Mansour *et al.* (2009). A large number of microorganisms were isolated from female patients, especially *E.coli*. The frequency of UTI is greater in women as compared to men. This might be owing to anatomic and physical factors (Aiyegoro *et al.,* 2007).

In the present study, Chromagar was also used for identification of uropathogens. Six hundred fifty numbers of uropathogens were tested by inoculation on Chromagar media. In agreement with other studies on chromogenic media, the data proved Chromagar as an excellent medium for the isolation of uropathogens (Leela rani *et al.,* 2012).

Isolation of pathogens from any diseased human body site alone may be of no use without a corresponding prescription of appropriate antibiotic therapy. Besides, the need for constant monitoring of

susceptibility of specific pathogens in different populations to commonly used antimicrobial agents has been suggested (Shirishkumar *et al.,* 2012). For this purpose, antibiotic sensitivity test was done on all isolates by the disc diffusion technique using a commercially available disc. The antibiotics disc used were amoxicillin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, cephalexin, ciprofloxacin, nalidixic acid, norfloxacin, ofloxacin, amikacin, gentamicin, tobramycin, imipenem, nitrofurantoin and cotrimoxazole.

In the present study *E.coli* were the predominant uropathogens being responsible for 54.6% of community acquired UTIs in these area, and no change in its prevalence among all community acquired uropathogens were observed over the 18 months study period. Antibiotic resistance was a major clinical problem in treating infections caused by these microorganisms. In the study, the highest percentage of resistance was noted against amoxicillin (91.8%) followed by ampicillin (88.7%), cephalexin (88.5%), nalidixic acid (77.2%), cotrimoxazole (76.3%). The resistance to amoxycillin varies from place to place. While Kausar *et al*. (2009*)* from Hubli reported 91.5% resistance, Taneja *et al.* (2010) from Chandigarh observed 32.7% resistance. This was previously reported in other hospital as well as in other institutions in the various countries (Akujobi, 2005; Daoud *et al.,* 2006). Previously reported studies showed 60% of cotrimoxazole resistance and more recently 25% from Pakisthan Medical University (Khan and Ahmed, 2001). In the present study, *E.coli* showed 31% and 32.4% resistance to ciprofloxacin and norfloxacin respectively. In this finding was at par with the observation made by Kausar *et al*. (2009) from Hubli (83% and 85% respectively), and much higher to the report of Arjunan *et al.* (2010) from Tirunelvelli (22.3%).

Third generation cephalosporins were the other commonly used antibiotics in the treatment of UTI. In the present study *E, coli* exhibited

42% resistance to cefotaxime and 53% resistance to ceftrioxone. Cefotaxime resistance was similar to reports by Kausar *et al.* (2009*)* (59.5%) and ceftrioxone resistance was between the findings of Biswas *et al.* (2003) (22.6%) and Sonavane *et al.* (2008) (74.9%). Therefore, it appears that cephalosporins were still useful in the treatment of UTIs due to *E. coli* in this area.

E. coli showed a low resistance to nitrofurantoin (13%) in the present study. This is similar to the reports of Kauser *et al.* (2008) (15%) and Biswas *et al.* (2003) (9.3%). The reason for low resistance may be that nitrofurantoin was less frequently prescribed because of the requirement of sixth hourly dosage and gastrointestinal disturbances following oral administration. Nitrofurontoin resistance in western countries was as low as 0.4 % in the USA (Karlowsky *et al.*, 2002) to 5.7% in the Spain (Garcia *et al.,* 2007).

The resistance exhibited by *E.coli* to aminoglycosides varies. In this study, observed 37.5% resistance to gentamicin and 11.8% resistance to amikacin. Various other studies reported 5% resistance in non ESBL producing *E.coli* (Mahesh *et al.,* 2008) to 72.6% resistance in ESBL producing *E. coli* to gentamicin and 5% to 36.6% (Sonavane *et al.,* 2008) to amikacin. Gentamicin and tobramicin resistance has also increased as compared to other studies (Farooqi *et al.,* 2000), but amikacin has remained sensitive so far. Moreover, the result in this study of gentamicin and amikacin sensitivity against UTIs isolates was similarly to studies found in Karbala (Ali, 2011) and Lafi (Lafi *et al.,* 2012). The higher susceptibility of isolates to other antibiotics such as amikacin, gentamicin and ofloxacin expected, as this has been reported previously (Mbata, 2007). As a whole *E.coli* in the study area appeared to be still resistance to ofloxacin (35.5%), ciprofloxacin (31%), norfloxacin (32.4%) and nitrofurantoin (13%), which are the common antibiotics prescribed for

UTI. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country (Sharma *et al.,* 2005).

In most Indian studies *K. pneumoniae* occupy second place among uropathogens. However, in the present study also *K. pneumoniae* were the second (11.2%) common uropathoges. The highest percentage of resistance was noted against amoxicillin (88.9%) followed by ampicillin (83.3%), cotrimoxazole (70.8%), cephalexin (69.4%) and nalidixic acid (50.6%). This was previously reported in other hospital as well as in other institutions in the various countries (Akujobi, 2005; Daoud *et al.*, 2006).

In the present study, *K. pneumoniae* exhibited higher resistance to cephalosporins (ceftriaxone/cefotaxime, 33.3% and ceftazidime 45.8%) than other Indian studies (Khan and Zaman, 2006; Gupta *et al.,* 2007; Hasan *et al.,* 2007; Sonavane *et al.,* 2008; Bhargavi *et al.,* 2010). The other drugs for which *K. pneumoniae* showed higher resistance was amoxyclillin (88.9%), ampicillin (83.3%), cotrimoxazole (70.8%) nalidixic acid (50.6%), nitrofurantoin (50.0%) and norfloxacin (25.0%). Among these nalidixic acid is not commonly prescribed and norfloxacin is active against Gram negative rods. Amoxicillin resistance (88.9%) observed in the present study was lower than the reports of Sonavane *et al.,* 2008 (97.7%). The resistance pattern of *K. pneumoniae* to norfloxacin, nitrofurantoin and nalidixic acid in other studies showed wide variation. The resistance of *K. pneumoniae* to cotrimaxazole (70.8%) was high, compared to most Indian studies (Biswas *et al.*, 2006; Khan and Zaman, 2006; Hasan *et al*, 2007; Sonavane *et al.*, 2008). Only Arjunan *et al.,* 2010 from Tirunelvelli reported cotrimaxazole resistance (14.3%) that was lower than what has been observed in this study. Cotrimaxazole was a broad spectrum antibiotic widely used in UTI treatment. In this study revealed high resistance to aminoglycosides which was lower than most Indian studies. Among ciprofloaxacin resistance were moderate (23.6%),

amikacin (13.9%) and gentamycin (19.4) which was lower resistance to *Klebsiella* isolates. As a whole *K. pneumoniae* in this study area appeared to be still susceptible to ofloxacin (79.2%), ciprofloxacin (76.4%), norfloxacin (75%) and nitrofurantoin (50%), which are the common antibiotics prescribed for UTI.

Pseudomonas aeruginosa was the third major pathogen (10.5%) isolated from UTI in this study. All the isolates of *Pseudomonas* (100%) were resistant to ampicillin, amoxicillin and cotrimoxazole, while the highest sensitivity rate of 88.2% was recorded with amikacin (88.2%), tobramycin (86.8%), cefotaxime (73.5%), gentamicin (69.1%), ceftazidime (60.3%) and imipenem (58.8%). These findings compare favorably with that of Goniugur *et al.* (2003) in Turkey on 249 isolates of *Pseudomonas* in a teaching hospital where 100% resistance to ampicillin and amoxicillin was recorded.

In this study, observed 70.6% resistance to nitrofurantoin and 61.8% resistance to ciprofloxacin. Overview of literature shows that *Pseudomonas* exhibits up to 100% resistance to nitrofurantoin (Akram *et al.,* 2007). Ciprofloxacin resistance varies over 20% (Gupta *et al.,* 2007) to 100% (Bhargavi *et al.,* 2010). In this study revealed moderate aminoglycoside resistance 30.9% to gentamicin and 11.8% resistance to amikacin. Previous report shows resistance to gentamicin ranged from 80% (Bhargavi *et al.,* 2010) to 100% (Gupta *et al.,* 2007). Resistance to amikacin ranged from 33% (Akram *et al.,* 2007) to 60 (Gupta *et al.,* 2007). In this study revealed higher resistance to third generation cephalosporins like ceftriaxone (61.8%) and ceftazidime (39.7%). This was similar to earlier reports by Taneja *et al.,* 2006 (64.7%); Akram *et al.,* 2007 (67%) and Hasan *et al.,* 2007 (72.2%).

However, the isolates of *Pseudomonas* spp. tested were only low resistance to cefotaxime (26.5%). High resistance (68.7%) to cefotaxime

was observed in earlier studies (Hasan *et al.,* 2005). In the present study 100% of *Pseudomonas* spp. were resistance to amoxycillin. It is interesting to note that *Pseudomonas* spp. exhibited low resistance (13.2%) to tobramycin and (11.8%) to amikacin (11.8%) in this study. In a similar study carried out in Turkey (Goniugur *et al.,* 2003), resistance of *Pseudomonas* spp. to gentamicin and tobramycin was found to be 70%, while studies carried out in Spain (Bouza *et al.,* 1999) and Italy (Bonfiglio *et al.,* 1998) showed similar figures. Findings from America (Ronald and Jones, 2001) showed that, 16.3% of *Pseudomonas* isolates was resistant to fluoroquinolones and over 80% of the isolates was resistant to cotrimoxazole, erythromycin, and ampicillin in several studies (Jones *et al.,* 1998; Jones, 1999).

In the present study *Proteus* spp. were isolated in 6.8% of cases. This finding correlated with Lavanya and Jogalakshmi (2002), (2.3%) and Priyadarsini *et al.,* (2004) (4.17%). However, Kriplani *et al.,* (1993), Gratacos Eduard *et al.,* (1994) and Hagay Zion *et al*., (1996) found *Proteus* as the most common organism isolated (8.82%, 18.8% and 16% respectively). Isolates of *Proteus*, in this study were mostly found in catheter-associated urinary tract infections. This was supported by other literature (Cheesbrough, 2000) suggesting that urinary tract infections caused by *Proteus* are associated with hospital-acquired infections, often following catheterisation or gynecological surgery. In contrast to the previous studies done in Addis Ababa and Gondar (Moges *et al*., 2002) the most frequently isolated species from non-catheterised patients was *K. pneumoniae* followed by *E. coli.*

Proteus isolates exhibited highest susceptibility against amikacin 100%, gentamicin 100%, tobramycin 90.9%, ciprofloxacin 88.7%, imipenem 88.6%, ceftriaxone 84.1%, cefotaxime 81.8%, ofloxacin 79.5% and norfloxacin 75%. Other agents showed less sensitivity to nalidixic acid

65.9%, ceftazidime 54.6%, nitrofurantoin 45.5%, ampicillin 24%, cephalexin 22.7%, amoxicillin 20.5% and cotrimaxazole 20.5% (Table 14). This also corresponds to other previous studies (Kadri *et al.*, 2002; Moges *et al.*, 2002; Wazait *et al.*, 2003).

This study shows a very low distribution of *Enterobacter* species (1.5%) in Pattukkottai area hospitals. The antibiogram of *Enterobacter* showed the organism to be more susceptible to most antibiotics, except cephalexin, nitrofurantoin, amoxicillin and ampicillin. However, the high susceptibility to amikacin, tobramycin, imipenem, ciprofloxacin, gentamicin, ceftriaxone and ofloxacin are an indication that the drug could be the drug of choice for any *Enterobacter* urinary tract infection. They were moderately susceptible to cefotaxime, norfloxacin and nalidixic acid. There was high resistance to the recommended first-line antibiotics cotrimoxazole and ampicillin. The observed resistance was agrees with literature reports, which claimed that *Enterobacter* species were reisistant to most antibiotics. There are reports (Thiolas *et al.*, 2005) on increasing resistance of *Enterobacter* species to penicillins groups of antibiotics, all generations of cephalosporins and their emergence in clinical specimens.

The epidemiology of *Enterobacter* infection as seen from this study appear to place children at relatively higher risk of infection, followed by adult females, with adult males least predisposed to infection. Children have been known to be most susceptible to any infection as a result of habits, exposure and lower immunity. *Enterobacter* infection is largely associated with decreased immunity. Those heavily affected are the immunocompromised as well as factors such as increased length of stay in the hospital; increased length of stay in the intensive unit; those on urinary catheter and long-term antibiotic administration (Lin *et al.,* 2003).

In this study, *Citrobacter* infections were more among elderly people; this is because of the fact that these groups constitute large

proportion of hospital populations and reduced immunity in these people to fight against infection in general. Similar results were seen in the study conducted by Samonis *et al*. (2009).

Coagulase negative *Staphylococcus* were the most common cause of UTI among Gram positive bacteria emphasising to this fact that these pathogens were still the most important causes of UTI worldwide (Ranjbar *et al.,* 2009).

In the present study CoNS constituted 4.1% of the strains isolated. This confirmed with the finding of Biswas *et al.* (2003). CoNS exhibited 63% of isolates was resistance to cotrimoxazole. However Sonavane *et al.* (2008) reported 100% resistance of CoNS to cotrimoxazole. In this study reported no resistance to nitrofurantoin. The reason may be low exposure of CoNS to nitrofurantoin due to limited prescription of the drug in UTI. Earlier studies from different parts of India documented 9.6% (Biswas *et al.,* 2003) to 25% (Sonavane *et al.,* 2008) resistance of CoNS to nitrofurantoin. In the present study, showed higher rate of resistance of CoNS to fluoroquinolones (norfloxacin 40.7%), than the earlier reports (25% to 57.3%) (Hasan *et al.,* 2007; Sonavane *et al.,* 2008). The sensitive pattern of CoNS to aminoglycosides was 100% sensitive (gentamicin and amikacin), in this study, which is almost similar to previous study by Biswas *et al.* (2003) (gentamicin 79.8% and amikacin 62.8%). Amikacin is preferred to gentamicin in UTI because of its wider spectrum of activity. High resistance to cephalaxin (44.4%) and moderate resistance to amoxicillin (48.1%) were recorded in this study. Sonavane *et al. (*2008) reported 50% resistance to both these antibiotics.

Enterococcus species constituted 3.7% of the uropathogens in this study, which often occurred after instrumentation of the patient's urinary tract. This was comparable with the report of Taneja *et al.* (2010), (8.7%) from Chandigarh during 2009. *Enterococcus* spp. was the second

commonest Gram Positive organism isolated from UTI in the present study.

In the present study, observed that 23.08% of the *enterococci* were resistant to nitrofurantoin. The resistance pattern of *enterococci* to nitrofurantoin ranged from 0.78% in the study by Miskeen *et al.* (2002) to 58% reported by Sonavane *et al.* (2008). Both reports are from Mumbai. The ampicillin resistance of *enterococci* observed in the present study (45.8%) was comparable to the study by Miskeen *et al.* (2002) (23.13%) from Mumbai. The nalidixic acid resistance (100%) was observed in this study. Very high nalidixic acid resistant *enterococci* were observed in UTI by Sonavane *et al.* (2008) (98.2%) from Mumbai and Bhargavi *et al.* (2010) (88.9%) from Vijayawada. These finding shows that nalidixic acid was no use in the treatment of enterococcal UTI in these area.

The low incidence (1.1%) of *S.aureus* recorded in this study. It agrees with the results of a similar work previously carried out by Akortha and Ibadin (2008). Urinary tract is supposed to be sterile, but the fact that this study shows high incidence of *S. aureus* in women than men could be due to the proximity between the genital tracts and the urethra/anus, which perhaps facilitate auto transmission as earlier suggested by Audu and Kudi (2004). In general, as high as incidence of *S. aureus* was observed among the ages of 21 to 30 years. It can be speculates that this is the sexually active and also the child bearing age group. The study therefore supports the report of Akortha and Ibadin (2008), that there is high rate of bacterial infection among sexually active women of childbearing age.

In this study it was found that *S. aureus* showed highest sensitivity (100%) to ceftriaxone, ciprofloxacin, ofloxacin, amikacin, gentamicin, tobramycin, imipenem and nitrofurantoin, moderate susceptibility (85.7%) to cefotaxime, cephalexin, norfloxacin, respectively, followed by sensitive to ceftazidime (71.4%), cotrimoxazole (71.4%), amoxicillin (57.1%), while

the highest resistance to ampicillin 42.9% and nalidixic acid 28.6% was also observed. Sensitivity patterns of *S. aureus* to antibiotics recorded by other work showed similarity except in few cases. In the study carried out by Olowu and Oyetunyi (2003) showed *S.aureus* is being 60% sensitive to gentamycin and resistant to naladixic acid cotrimoxazole. Akortha and Ibadin (2008) documented *S. aureus* strains were sensitive to augmentin (83%), ofloxacin (75.9%), nitrofurantion (63.5%) and gentamicin (50.2%). *S. aureus* was found to be highly resistant to naladixic acid (79.3%) and contrimoxazole (87.3%).

The low incidence (2.3%) of *Staphylococcus saprophyticus* recorded in this study. Honeymoon women were showing higher rate of UTI than other women (80%). It agrees with the results of a similar work previously carried out by Nada Khazel *et al.* (2013), honeymoon women were showing higher rate of UTI than other women in (87 from 95) (91.5 %) and (19 from 35) (54.3%) respectively. In this study, *Staphylococcus saprophyticus* isolates, exhibited high resistance to amoxicillin, ampicillin and nalidixic acid and 100% susceptibility to amikacin, gentamicin, tobramycin and imipenem, while it was high sensitive to ofloxacin 89.7%, ciprofloxacin 86.7%, norfloxacin 80% and nitrofurantoin 73.4%. It agrees with the results of a similar work previously carried out by Khoshbakht *et al.* (2013). Gram positive cocci play a lesser role in UTIs. However, *Staphylococcs saprophyticus,* a novobiocin-resistant, coagulase-negative species, accounts for 10–15% of acute symptomatic UTIs in young females (Walter and Braunwald, 2001).

In this study, 3.4% of culture from sampled patients yielded different species of *Candida*. A total of twenty two *Candida* isolates from urine clinical specimens were included in this study, of which *C. albicans* showed the highest number of isolates (45.5%), followed by *C. glabrata* (22.7%), *C. tropicalis* (18.2%) and *C. krusei* (13.6%) respectively. According to Patel *et al.* (2012), *Candida* species is the seventh most

common nosocomial hospital wise pathogen, which caused 25% of all the urinary tract infections. Other studies have documented that hospitalised patients are relatively susceptible to candiduria (Kobayashi *et al.,* 2004; Sellami *et al.,* 2006). Several reports show that the frequency of candiduria in women is more than men (Achkar and Fries, 2010). In this study observed that the female: male percentage in this study is 82:18, contrary to the male predominance reported in the study by Paul *et al.* (2007). In this study candiduria were also more prevalent in age range 31-60 years (72.7%) followed by; 9.1% < 30 years and >61-80 years.

C. albicans had remained the major agents of candiduria until recently (Weinberger *et al.,* 2003) however; several reports show that non-albicans species, especially *C.tropicalis* and *C. glabrata* now predominate in many regions (Lagrotteria *et al.,* 2007). Non-*albicans* species accounted for 64.4% and 71% of isolates in Kobayashi *et al.* (2005) and Paul *et al.* (2007) reports, respectively. Although the majority of candiduria in the present study were caused by *C. albicans* (45.5%), non-*albicans* species, especially *C. glabrata* (22.7%) was emerging as a nosocomial infection. Similar reports (Zarei *et al.,* 2012) from Iran showed the most common isolates were *C. albicans* (53.3%), followed by *C. glabrata* (24.4%), *C. tropicalis* (3.7%), *C. krusei* (2.2%), and *Geotrichum* spp. (0.7%). Several reports from Iran showed the *Candida* colonisation in the urinary tract (Behzadi *et al.,* 2010 and Mardani *et al.* (2008) reported a case of *Candida cystitis* in a patient with diabetes mellitus.

There are a few reports about in vitro assessment of antifungal activity candiduria agents. In a study conducted by Manzano *et al.* (2008) several species of *Candida*, isolated from candiduria were tested against some antifungal drugs. They have shown that amphotericine B, and ketoconazole have less activity against *C. glabrata* isolates, whereas fluconazole presented higher activity (Manzano *et al.,* 2008). In addition, Chen *et al.* (2008) reported that all candiduria isolates were susceptible to fluconazole, and amphotericin. In the present study shows that all isolates

of *C. albicans*, *C. krusei* and *C. glabrata* were resistant to fluconazole, with the exception of three isolates of *C. glabrata* that were sensitive to fluconazole. UTIs due to *C. glabrata* have recently increased and these infections are usually resistant to fluconazole (Yang *et al.*, 2003).

The present study clearly shows that there was a high prevalence of ESBL *E.coli* in urinary isolates and its resistance to commonly used antibiotics is found in various hospitals, Pattukkottai area, India. The prevalence of *E. coli* infection was almost 10%. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different hospitals. Previous studies from India have reported ESBL production varying from 28% to 84% (Narayanaswamy and Mallika, 2011). It may be noted that urinary tract infection is the most commonly encountered infection found in women in many parts of the world. Reports of high incidence of community acquired urinary tract infections are available from Asia, Pacific, Denmark, Japan, India, Russia and the USA (Rumana *et al.*, 2012). Further the incidence of urinary tract infections by ESBL producing *E. coli* was found highest in India (60%) followed by Hongkong (48%) and Singapore (33%) (Hseuh and Hoban *et al.*, 2011).

A point of interest noted in the present study was that all the selected strains were at least resistant to 3-5 antibiotics which include the first and second generation of cephalosporins. The resistance was very high to amoxicillin (91.7%), ampicillin (88.7%) and cephalexin (88.573%). cefotaxime (42%), ceftazidime (45.3%) and ceftriaxone (53.0%) which is comparable with the studies of Padmini and Appalraju, 2004. Almost all the ESBL-positive isolates were found to be resistant to amoxicillin and ampicillin and sensitive to imipenem, which again advocates the usage of carbapenem antibiotics as the therapeutic alternative to β-lactam antibiotics. The total resistance of these antibiotics might be due to continuous use of it for many years and may also be due to empirical treatment.

This study demonstrates the presence of ESBL mediated resistance of *E.coli* from urine specimens collected from urinary tract infection patients. The prevalence of ESBLs is 10%. This study, aimed to detect and to do the molecular characterization of the types of ESBL genes in isolates of *E coli*. In addition to DDST, PCR amplification assay for the detection of the ESBL genes in clinical isolates of *E coli* was used in this study because this assay has been shown to have the advantage of rapidly screening large numbers of clinical isolates (Woodford *et al.*, 2006) and the DNA was used for further molecular epidemiological characterisation (Bradford, 2001).

The prevalence of ESBLs among the clinical isolates varies greatly world-wide and in geographical areas and it was rapidly changing over time. In the west, the ESBL production in Enterobacteriaceae varies from 5 to 52 per cent and in other Asian countries, it varies from 10 to 46.5% (Dhillon and Clark, 2012). Other studies from India have reported a high prevalence of the ESBL production, which ranges from 41.0 to 63.6 per cent in *E. coli (*Goyal *et al.*, 2009).

Hence the detection of ESBL is necessary to formulate the treatment policy (David *et al.*, 2005). There have been reports of considerable geographical differences in ESBLs worldwide and within countries (Coque *et al.*, 2008). The hospital to hospital variability that occurs possibly reflects the direct proportionality between use and misuse of antibiotics (Deepti and Deepthi, 2010). In India, prevalence rate of ESBLs varies from 28 to 84%. While earlier reports from North India have documented the prevalence of ESBLs ranging from 55 to 69%, a recent study from North India reported an alarming 64.8% of ESBL producing isolates. In Southern India, 21.6% ESBL producing bacteria have been documented in a study from Karnataka and another study from Coimbatore reported about 40% ESBL producers (Al-Agamy *et al.*, 2009). In this study reported that the prevalence of ESBL in Pattukkottai area in Tamilnadu is 10%, which was

similar to that of the other study from Chennai found ESBL mediated resistance to 3GCs in 6.6% of the isolates recovered (Tanhkiwale *et al*., 2004).

The population of India of over one billion represents a potentially vast reservoir of antimicrobial resistance genes including those ESBLs (Tsering *et al*., 2009). ESBLs are undergoing continuous mutations, causing the development of new enzymes showing expanded substrate profiles. At present, there are more than 300 different ESBL variants and these have been clustered into nine different structural and evolutionary families based on amino acid sequence (Muzaheed *et al*., 2008). In the present study, ESBL positive isolates screened by Double disc synergy test were also screened by PCR technique. All isolates were found to contain TEM gene.

6. SUMMARY AND CONCLUSION

A total of 2400 midstream urine samples were processed from patients having clinically suspected Urinary Tract Infection (UTI) attending various hospitals in Pattukkottai area from January 2012 to June 2013. Out of the 2400 specimens, 650 (27.1%) were culture positive and 1750 (72.9%) specimens were negative. The most common isolates in this study have been the Gram negative bacilli which accounts for 85.4% of the total positive isolates. Major Gram negative isolates were E.coli (54.6%); *K. pneumoniae* (11.2%) was the second major isolates and *Pseudomonas* spp., (10.5%) was the third major isolates. In Gram positive isolates; CoNS, *Enterococcus* and *Staphylococcus saprophyticus* were the least dominant uropathogen causing UTI strains.

Overall incidence of UTI in the present study was higher in females (69.8%) than in males (30.2%). There is significant difference of positive growth between male and female patients ($P<0.05$) and this study shows the significant association between infection among female and male patients ($p<0.05$). In the present finding on UTI among girls of 0-10 years were quite high (24.7%). High rate of UTI in male children of 0-10 years of age (9.2%). Higher rate of UTI was seen in females in age groups from 30 years and above. More than 70% infection was seen among men of below 40 years of age. Pyuria and Gram's stain microscopy were found to be of low sensitivity in detecting UTIs.

Sensitivity of *E.coli* to imipenem, amikacin, nitrofurantoin, tobramycin and ciprofloxacin were more than other antibiotics. The lowest

percentage of sensitivity was manifested against amoxicillin and ampicillin. Sensitivity of *K. pneumoniae* to amikacin, imipenem followed by gentamicin and tobramycin were more than other antibiotics. The lowest percentage of sensitivity was observed against amoxicillin followed by ampicillin, cotrimoxazole and cephalexin. *Pseudomonas* spp. were more sensitive to amikacin, tobramycin, cefotaxime, gentamicin and ceftazidime than to any other antimicrobial with high resistance to nalidixic acid; 100 % resistance to amoxicillin, ampicillin and cotrimoxazole. *Proteus* isolates exhibited highest sensitivity against amikacin and gentamicin.

Coagulase negative *Staphylococcus* was more sensitive to ciprofloxacin, amikacin, gentamicin, tobramycin and nitrofurantoin. *Enterococci* were sensitive to ceftriaxone, amikacin, gentamicin, tobramycin, cefotaxime, ciprofloxacin and nitrofurantoin. *S.saprophyticus* was 100% sensitive to amikacin, gentamicin, tobramycin, imipenem, while it was highest sensitive to ofloxacin, ciprofloxacin, norfloxacin and nitrofurantoin.

Candida albicans was observed in 3.4% cases of UTI. A total of twenty two numbers of *Candida* species were isolated, of which *C. albicans* showed the highest number of isolates.

The multidrug resistant ESBL producing *E.coli* isolates were present in these area hospitals with a prevalence of 10%. There were two predominant genes TEM and SHV detected among MDR *E.coli* uropathogenic strains circulating in these area hospitals.

Conclusion

This study concludes that understanding the effect of the different factors on community acquired urinary tract infections and Gram negative bacilli were responsible for urinary tract infections in patients will aid the proper management of this disease.

The present study also showed a high incidence of resistance to most antimicrobial agents tested. Particularly, increasing amoxacillin, ampicillin and fluoroquinolone resistance in pathogens isolated from patients is frustrating. When antibiotics treatment is needed, the susceptibility, potential availability, simplicity of use, safety and low cost of the antimicrobial agent has to be taken into account.

Nitrofurantoin resistance is low and has significantly decreased and therefore should now be considered the mainstay of empiric treatment of uncomplicated UTIs in females. Gentamicin resistance remains low but susceptibility trends should be monitored considering the reliance placed on it as part of the empiric treatment of severe sepsis (including urosepsis) in the Pattukkottai area hospital.

This study showed that antibiotic like imipenem, amikacin, nitrofurantoin and gentamicin which display high resistance in other regions across India is still very effective in our environment while conversely an increased resistance to amoxycillin, ampicillin, cotrimoxazole and norfloxacin were observed. Antimicrobial resistance pattern are constantly evolving in our region. There is a necessity for constant antimicrobial sensitivity surveillance and susceptibility testing to be conducted prior to antibiotics prescription. It was also observed that route of antibiotic could contribute in checking disperse of antibiotic

resistance. Plamid profile revealed that the antibiotic resistance in this geographical area is plasmid borne. Hence, our data will help clinician in this region provide safe and effective emperic therapies and could contribute to decrease in emergence of resistance.

The clinical laboratories should develop a habit of screening all uropathogens to determine the susceptibility and ESBL producers in order to advise the hospital the way forward to managing the UTI patients. The extended spectrum lactamase producers should not be treated with the third or fourth generation of cephalosporins, quinolones, fluoroquinolones and β lactams, but instead use other therapeutic alternatives such as amikacin, nitrofurantoin and gentamycin that are susceptible to ESBL producers.

REFERENCES

Aboderin, O.A., Abdu, A., Odetoyinbo, B.W. and Lamikanra, A., 2009. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. *Nat. Med. Assoc.,* 101:1268-1273.

Abrahamian, F.M., Krishnadasan, A., Mower, W.R., Moran, G.J. and Talan, D.A., 2013. Association of pyuria and clinical characteristics with the presence of urinary tract infection among patients with acute nephrolithiasis. *Ann. Emerg. Med.,* 62(5):526-533.

Abubakar, E.M., 2009. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the specialist hospital, Yola, Adamawa state, Nigeria. *J. Clin. Med. Res*., 1(1):1-8.

Abu Shaqra, Q., 2000. Occurrence and antibiotic sensitivity of Enterobacteriaceae isolated from a group of Jordanian patients with community acquired urinary tract infections. *Cytobios.*, 101:15-21.

Acar, J.F. and Goldstein, F.W., 1997. Trends in bacterial resistance to fluoroquinolones. *Clin. Infect. Dis.,* 24:67-73.

Acharya, A., Gautam, R. and Subedee, L., 2011. Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. *Nepal. Med. Coll. J.*, 13(1):30-33.

Acharya, V.N., 1992. Urinary tract infection- a dangerous and unrecognised forerunner of systemic sepsis. *J. Postgrad. Med.,* 38:52-54.

Achkar, J.M and Fries, B.C., 2010. *Candida* Infections of the genitourinary tract. *Clin. Microbiol. Rev.,* 23:253-273.

Adhikary, R and Joshi, S., 2011. Species distribution and antifungal susceptibility of Candidaemia at a multi super-speciality center in souther India. *Indian. J. Med. Microbiol.,* 29:309-311.

Agarwal, J., Srivastava, S. and Singh, M., 2012. Pathogenomics of uropathogenic *Escherichia coli*. *Indian. J. Med. Microbiol.,* 30:141-149.

Ahmad, S., 2012. Pattern of urinary tract infection in Kashmir and antimicrobial susceptibility. *Bangladesh. Med. Res. Counc. Bull.,* 38:79-83.

Ahmed, S.I., 2004. Urinogenital infections in the tropics. The Australasian college of tropical medicine. Chapter 8, parts A, page 8.1-8.46.

Ahmad, S., Shakooh, S., Salati, S.A. and Muniem, A., 2011. Prevalence of asymptomatic bacteriuria among pregnant women in Kashmir. *S.L.J.O.G.,* 33:158-162.

Aiyegoro, O.A., Igbinosa, O.O., Ogunmwonyi, I.N., Odjadjare, E.E., Igbinosa, E.O. and Okoh, I.A., 2007. Incidence of urinary tract infection among children and adolescents in life Nigeria. *Afr. J. Microbiol. Res.,* 1(2):13-19.

Akortha, E.E. and Ibadin, O.K., 2008. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection (UTI) in UBTH Benin City, Nigeria. *Afr. J. Biotechnol.,* 7(11):1637-1640.

Akram, M., Shahid, M. and Khan, AU., 2007. Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital, Aligarh, India. *Ann. Clin. Microbiol. Antimicrobial.,* 6(4):1-7.

Akujobi, C.N., 2005. Antimicrobial susceptibility pattern of *Klebsiella* species from Ebonyi state university teaching hospital Abakaliki, Nigeria. *Niger. J. Clin. Pract.,* 8(2):90-93.

Al-Agamy, M.H., A.M. Shibl. and Tawfik, A.F., 2009. Prevalence and molecular characterisation of extended spectrum beta lactamase producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Ann. Saudi Medi.,* 29(4):253-257.

Al-Badr, A. and Al-Shaikh, G., 2013. Recurrent urinary tract infections management in women. *Sultan. Qaboos. University. Med. J.,* 13(3):359-367.

Al-Haddad, A.M., 2005. Urinary tract infection among pregnant women in Al-Mukalla district, Yemen. *East. Mediterr. Health. J.,* 11(3):505-510.

Ali, M., 2011. Evaluation of antimicrobial susceptibility and rapid urine screening tests in asymptomatic urinary tract infection in pregnant women in Karbala. *Kerbala.J. Pharm. Sci.,* 2:22-34.

Al-Jasser, A. M., 2006. Extended spectrum β-lactamases (ESBLs): Global problem. *Kuwait Med. J.,* 38(3):171-185.

Allan, R., 2003. The Etiology of urinary tract infection: Tradition and Emerging pathogens. Dis Mon, 49:71-82.

Alonto, A.M., 2007. Urinary Tract Infections. In: Mahon, C.R, Lehman, D.C, Manuselis. G (Editors). Textbook of Diagnostic Microbiology. 3rd Edn. Saunders: St Louis, USA. pp. 1010-1029.

Amin, M., Mehdinejad, M. and Pourdangchi, Z., 2011. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur. J. Microbiol.,* 2(3): 118-123.

Anyamene, C.O., Stellamaris, N., Muoneke. and Umerie, G.N.C., 2002. Bacterial isolates associated with urinary tract infections in Akwa and environs. *J. Appl. Sci.,* 5(4): 3092-3098.

Arjunan, M., Ali, A., Al-Salamah. and Amuthan, M., 2010. Prevalence and antibiotic susceptibility of uropathogens in patients from a rural environment, Tamil Nadu. *American. J. Infect. Dis.,* 6(2): 29-33.

Astal, E. Z., 2005. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza Strip, Palestine. *J. Biomed. Biotechnol*., 3: 238-241.

Audu, B.M. and Kudi, A.A,, 2004. Microbial isolate and antibiogram from endocervical swabs of patients with pelvic inflammatory diseases. *J.Obst.Gyne.*, 24(161): 161-164.

Bahrani, M.F., Gunther IV, N. W., Donnenberg, M.S. and Mobley, H.L.T., 2002. In *Escherichia coli*: Virulence mechanisms of a versatile pathogen (ed. Science, E.).

Baral, P., Neupane, S., Marasini, B. P., Ghimire, K.R., Lekhak, B., Shrestha, B., 2012. High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. *BMC Research Notes.,* 5: 38:1-9.

Barron, E.J.O., Peterson, L.R. and Finegold S.M., 1994. Enterobacteriaceae. In: Bailey and Scotts-Diagnostic Microbiology, Mosby, Missuri, USA., pp: 62-385.

Bashir, MF., Qazi, J.I, Ahmad, N. and Riaz, S., 2008. Diversity of urinary tract pathogens and drug resistant isolates of *Escherichia Coli* in different age and gender groups of Pakistanis. *Trop.J. Pharm.Res.*, 7(3): 1025-1031.

Bauer, A.W., Kirby, W.M.M. Sherris, J.C. and Turck, M., 1966. Antibiotic susceptibility testing by a standardised single disc method. *Am. J. Clin. Path.*, 45: 493-496.

Beaglehole, R., Irwin, A. and Prentice, T., 2004. Changing history, The World Health Report., 157: 122.

Behzadi, P., Behzadi, E., Yazdanbod, H., Aghapour, R., Akbari C.M. and Salehian O.D., 2010. Urinary tract infections associated with *Candida albicans*. *Maedica* (*Buchar*).5: 277-279.

Behzadi, P. and Behzadi, E., 2008. The microbial agents of urinary tract infections at central laboratory of Dr Shariati hospital, Tehran, Iran. *Turk. Klin. Tip. Bilim.*, 28: 445-449.

Benedict, S. and Colagreco, J., 1994. Fungal infections associated with malignancies, treatments, and AIDS, Cancer nursing. 17(5):411-417.

Bergogne, B. E., Joly G.M.L and K.J. and Towner., 1996. *Acinetobacter*: Microbiology, Epidemiology, Infections, Management. 1st Edn., CRC Press, Boca Raton, pp: 272.

Betty, A.F., Daniel, F.S. and Alice, S.W., 1998. Diagnostic microbiology. 10th Ed. Moshy publishing.

Beyer, I., Mergan, A., Benoit, F., Theunissen, C. and Pepersack, T., 2001. Management of urinary tract infections in the elderly. *Z.Gerontol. Geriatr.*, 34:153-157.

Bhargavi, PS., Gopala R.T.V., Mukkanti, K., Dinesh K.B. and Krishna, TP., 2010. Increasing emergence of antibiotic resistance mainly in uropathogens: southeast part of India. *Int. J. Microbiol. Res.,* 2:1-6.

Biswas, D., Gupta, P., Prasad, R., Singh, V. and Arya, M *et al.*, 2006. Choice of antibiotic for empirical therapy of acute cystitis in a

setting of high antimicrobial resistance. *Indian. J. Med. Sci.* 60(2):53-58.

Bonacorsi., Lefebvre, S., Clermont, O., Houdouin, V., Bourrillon, A., Loirat, C., Aujard, Y. and Bingen, E., 2000. *Escherichia coli* strains causing urinary tract infection in uncircumcised infants resemble urosepsis-like adult strains. *The J. Urology*., 173: 195-197.

Bonfiglio, G., Carciotta, V., Russo, G., Stefani, S., Schito, G. C., Debbia, E. and Nicoletti, G., 1998. Antibiotic resistance in *Pseudomonas aeruginosa*: an Italian survey. *J. Antimicrob. Chemother.*, 41: 307-310.

Bonilla, A., R. and Muniz K. P., 2001. Antibiotic resistance: Causes and risk factor, mechanisms and alternatives. *Nova Science Publishers*., pp 79-95.

Bouza, E., Gercia-Garrote, F., Cercenado, E., Marin, M. and Diaz, M. S., 1999. *Pseudomonas aeruginosa*: a survey of resistance in 136 hospitals in Spain. *Antimicrob. Agents and Chemother.,* 43: 981-982.

Bradford, P. A., 2001. Extended-Spectrum β-Lactamases in the 21st Century: Characterisation, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev.,* 14(4): 933-951.

Bradshaw, CS., Tabrizi, S.N, Read, T.R, *et al*., 2006. Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital exposure. *J. Infect. Dis.*, 193(3):336-345.

Brammer, K.W., Farrow, P.R. and Faulkner, J.K., 1990. Pharmacokinetics and tissue penetration of fluconazole in humans. *Rev Infect Dis*., 12(3): 318-326.

Briggs, G.G, McKeown, A. and Bustillo, *et al.,* 2004. Urinary tract infections during pregnancy. *Ann Pharm.*, 38(10): 1692-1701.

Bukhary, ZA., 2008. Candiduria: a review of clinical significance and management. *Saudi J Kidney Dis Transpl*., 19(3): 350-360.

Bush, L. M., Fredy, C. R., Victorm, O. and Josephm, E., 2011. Cumulative clinical experience of levofloxacin in urinary tract infections. *Infection and Drug Resistance*., 4: 177–189.

Cabral, J.P.S, 2010. Water microbiology. Bacterial pathogens and water. *Int. J. Environ. Res.* Public Health 7: 3657-3703.

Cappuccino, G. J. and Sherman, N., 2001. iochemical activities of microorganisms. In: Microbiology: A Laboratory Manual, 6th Edn. Benjamin-cummings publishing company, USA., pp: 133-194.

Caramia, G, and Fanos, V., 2002. Asymptomatic bacteriuria. *Ped Med Chir.*, 24: 117-118.

Caroline, M. O., Francesm W. B. and Michael, M. J., 2000. Classification, identification and clinical significance of *Proteus, Providencia* and *Morganella. Clin. Microbiol. Rev.*, 13(4): 534-546.

Carrasco, L., Ramos, M., Galisteo, R., Pisa, D., Fresno, M. and Gonza´lez, M. E., 2005. Isolation of *Candida famata* from a patient with acute zonal occult outer retinopathy. *J. Clin. Microbiol.*, 43: 635-640.

Catell, W.R., 2005. Lower and upper urinary tract infections in adult. In: Davison AM, Cameron JS, Grunfeld JP, Ponticelli C, Ritz E *et al.*, (Editors). *Oxford Textbook of Clinical Nephrology*., 3rd Edn. Oxford University Press: London. pp. 1111-1129.

CDC., 2012. About antimicrobial resistance: A brief overview. Retrieved from http: //www.cdc.gov/drugresistance/about.Html. Centers for Disease Control and Prevention (CDC).

CLSI., 2004. Method for antifungal disc diffusion susceptibility testing of yeasts; approved guidelines. 24(15).

CLSI., 2009. Performance standards for antimicrobial disc susceptibility tests. 29(3).

Coates, A. R., Halls, G. and Hu, Y., 2011. Novel classes of antibiotics or more of the same. *British J. Pharm.,* 163(1): 184-194.

Cocchi, S., Grasselli, E., Gutacker, M., Benagli, C., Convert, M and Piffaretti, J., 2007. Distribution and characterisation of integrons in *Escherichia coli* strains of animal and human origin. *FEMS Immunology and Medical Microbiol.,* 50(1):126-132.

Coleman, D.C., Rinaldi, M.G, Haynes, K.A, Rex, J.H, Summerbell, R.C, Anaissie, E.J. and Sullivan, D.J., 1998. Importance of *Candida* species other than *Candida albicans* as opportunistic pathogens. *Med. Mycol*., 36:156-165.

Collee, J.G., Marr, W., Fraser, A.G., Marmion, B.P. and Simmons, A., 2007. Specimen collection, Culture cntainers and media. In: Mackie and Mac Carthey practical medical microbiology, 14th ed. Churchill Livingstone, New York, London, Tokyo.

Cooke, V. M., Miles, R. J., Price, R. G., Midgley, G., Khamri, W. and Richardson, AC., 2002. New chromogenic agar medium for the identification of *Candida* spp. *Appl. Environ. Microbiol.,* 68: 3622-3627.

Coque, T.M, Baquero F. and Canton, R., 2008. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 13.

Chaudary, U., Deep, A. and Chabbar, N., 2009. Rapid identification and antifungal susceptibility pattern of *Candida* isolates from critically ill patients with candiduria. *J. Infect. Dis. Antimicrob. Agents.,* 26:49-53.

Chaudhary, U. and Aggarwal, R., 2004. Extended spectrum-lactamases (ESBL)-an emerging threat to clinical therapeutics. *Indian. J. Med. Microbiol*., 22(2): 75-80.

Chaudhary, N. K. and Murthy, S. M., 2013. Extended expectrum betalactamases in uropathogen. *Asian J. Pharm. Clin. Res.,* 6(3): 207-210.

Cheesbrough, M., 2000. Biochemical test to identify bacteria. In: Laboratory practice in tropical countries, Cambridge University Press, UK., 2: 63-70.

Chen, S.C., Tong, Z.S., Lee, O.C., Halliday, C., Playford, E.G. and Widmer, F, *et al.*, 2008. Clinician response to *Candida* organisms in the urine of patients attending hospital. *Eur. J. Clin. Microbiol. Infect. Dis.*, 27(3): 201-208.

Dalen, D.M., Zvonar, R.K. and Jessamine, P.G., 2005. An evaluation of the management of asymptomatic catheter-associated bacteriuria and candiduria at The Ottawa Hospital. *Can. J. Infect. Dis. Med. Microbiol.,* 16(3): 166-170.

Denise, S.L., Huei, H. S., Selma L. B. Ragazzi, *et al.*, 2013. Community-acquired urinary tract infection: age and gender dependent etiology. *J. Bras.Nefrol.,* 35(2): 93-98.

Daoud, Z, C., Moubareck, N., Hakime. and Doucet, P., 2006. Extended spectrum β-lactamase producing Enterobacteriaceae in Lebanese ICU patients: epidemiology and patterns of resistance. *J. Gen. App. Microbiol.,* 52(3): 169-178.

Das, N. and Borthaku, A.K., 2012. Antibiotic coresistance among extended-spectrum beta lactamase-producing urinary isolates in a tertiary medical center: A prospective study Year. *Chron Young Sci.*, 3(1):53-56.

Das, R., Virginia, T.E.P., Peter, H., Van, N. and Manisha, J.M., 2009. Antimicrobial susceptibility of bacteria isolated from urine samples obtained from nursing home residents. *Infect. Control. Hosp. Epidemiol*., 30(11):1116-1119.

David, L., Paterson and Robert, A. B., 2005. Extended-Spectrum - Lactamases: a clinical update, *Clin. Microbiol. Rev.*, 18(4): 657-686.

Davies, J., 2007. Microbes have the last word. A drastic re-evaluation of antimicrobial treatment is needed to overcome the threat of antibiotic-resistant bacteria. EMBO reports., 8:616-621.

Davies, J. and Davies, D., 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mole. Bio. Rev.*, 74(3):417-433.

Daza, R., Gutierrez, J. and Piedrola, G., 2001. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. *Intern. J. Antimicrob. Agent.*, 18:211-215.

Deepti, R. and Deepthi, N., 2010. Symposium on infectious agents in a multidrug resistant globe: Extended-spectrum ß-lactamases in Gram negative bacteria. *J. Glob. Infect. Dis.*, 2(3):263-274.

Deshpande, K.D., Pichare, A.P., Suryawanshi, N.M. and Davan, MS., 2011. Antibiogram of Gram negative uropathogens in hospitalised patients. *Inter. J. Curr. Trends Sci. Tech.*, 1(2):56-60.

Dhillon, R.H.P. and Clark, J., 2012. ESBLs: A Clear and present Danger. *Crit Care Res. Prac.* Pp:1-9.

Dimitrov, T. S., Udo, E. E., Emara, M., Awni, F. and Passadilla, R., 2004. Etiology and antibiotic susceptibility patterns of community-acquired urinary tract infections in a Kuwait hospital. *Med. Princ. Pract.*, 13:334-339.

Donders, G., 2010. Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: a review. *Obstet. Gynecol. Surv.,* 65: 462-473.

Doran, T.I., 1999. The role of *Citrobacter* in clinical disease of children: Review. *Clin. Infect. Dis.,* 28: 384-394.

Ducel, G., Fabrey, J. and Nicolle, L., 2002. Prevention of hospital-acquired infections- A practical guide. World Health Organisation.

Ebie, M. Y., Kandakai-Olukemi, Y. T., Ayanbadejo, J. and Tanyigna, K. B,, 2001. Urinary tract infections in a Nigerian Military Hospital. *Nig. J. Microbiol.,* 15(1): 31-37.

Eduard, G., Pere-Joan, T., Vila, J., Alonso, P.L. and Cararach, V., 1994. Screening and treatment of asymptomatic bacteriuria in pregnancy prevent pyelonephritis. *J. Infect. Dis.,* 169:1390-1392.

Ehinmidu, J. O., 2003. Antibiotic susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. *Trop. J. Pharm. Res.,* 2(2): 223-228.

Eisenstein, B. I. and Jones, G.W., 1988. The spectrum of infections and pathogenic mechanisms of *Escherichia coli*. *Adv. Intern. Med.,* 33:231-252.

Elder, J. S., 2004. Urinary tract infection. Behrman R.E, Kliegman R.M, Jenson H.B, editors. Nelson Textbook of Pediatrics. 17th ed. Philadephia: WB Saunders Company. Pp. 1621-1625.

El-Mosalamy, *et al.,* 2012. Role of chronic *E. coli* infection in the process of bladder cancer- an experimental study. *Infec. Agents. Can.,* 7:19.

Enan, G. and El-Sayed, M.A., 1996. Taxonomy, antimicrobial potential and industrial importance of lactic acid bacteria. *Int. J. Union Araf Biol.,* 3: 231-270.

Erdem, B., 1999. *Pseudomonas*. In: Ustacelebi S (ed.) Basic Clinical Microbiology. Ankara, Gunes Publication. pp: 551-558.

Farooqi, B.J, Shareeq, F. and Rizvi, Q.K, *et al.*, 2000. Changing pattern of antimicrobial susceptibility of organisms causing community acquired urinary tract infections. *J.Pak.Med.Assoc.*, 50:369-373.

Feglo, P.K. and Narkwa, P., 2012. Prevalence and antifungal susceptibility patterns of yeast isolates at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. *British Microbiol. Res. J.*, 2(1): 10-22.

Feld, L.G. and Mattoo, T.K., 2010. Urinary tract infections and vesicoureteral reflux in infants and children. *Pediatr Rev.*, 31(11):451-463.

Fihn, SD., 2003. Clinical practice: Acute uncomplicated urinary tract infection in women. *N. Engl. J. Med*., 349:259-266.

Finch, R.G., 2004. Antibiotic resistance: a view from the prescriber. *Nat. Rev. Microbiol.*, 2(12):989-994.

Fitz G.M.P. and Graziano, S., 2007. Anatomic and functional changes of the lower urinary tract during pregnancy. *Urol. Clin. North Am.*, 34(1):7-12.

Forbes, B.A, Sahm, DF. and Weissfeld, A.S, 2007. Infections of the urinary tract. In: *Bailey and Scotts Diagn. Microbiol.*, 12th Edn. Mosby Inc: USA. pp. 842-855.

Forbes, B.A., Saham, D.F. and Weissfeld, A.S., 2002. Diagnostic microbiology. 10th ed. Mosby, Inc.

Forouzan, M.Z.A. and Amir, B., 2013. Prevalence and antimicrobial susceptibility patterns of uropathogens among patients referring to valieasr laboratory in Najafabad, Isfahan, Iran. *Middle-East J. Sci. Res.*, 13(1): 85-90.

Fournier, P.E. and Richet, H., 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin.Infect. Dis.*, 42: 692-699.

Fouzia, K. and Rakhshanda, B., 2010. In vitro antifungal sensitivity of fluconazole, clotrimazole and nystatin against vaginal candidiasis in females of childbearing age. *J Ayub Aed Aoll Abbottabad*., 22(4): 197-200.

Foxman, B., 2010. The epidemiology of urinary tract infection. *Nat. Rev. Urol*., 7(12): 653-660.

Foxman, B. and Riley, L., 2001. Molecular epidemiology of infection. *Am. J. Epidemiol.*, 153(12): 1135-1141.

Foxman, B., Marsh, J., Gillespie, B., Rubin, N. and Koopman, J.S, *et al.*, 1997. Condom use and first time urinary tract infection. *Epidemiology*. 8: 637-641.

Foxman, B., 2003. Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Dis. Mon*., 49(2):53-70.

Franco, A., 2005. Recurrent urinary tract infections. *Best Pract. Res. Clin. Obst*., 19: 861-873.

Garcia, M.I.G., Munoz. J.L.B. and Garcia, J.A.R., 2007. Spanish cooperative group for the study of antimicrobial susceptibility of community uropathogens. In vitro susceptibility of community-acquired urinary tract pathogens to commonly used antimicrobial agents in Spain: a comparative multicenter study (2002- 2004). *J. Chemother.*, 19: 263-270.

Gautam, R., Acharya, A., Nepal, H.P., Rimal, B. and Shrestha, S., 2013. Urinary tract infections in diabetic patients attending outpatient departments at kasturba Medical college teaching hospital, Manipal, India. *World j. pharm. Pharm. Sci.*, 2(3): 1141-1146.

Gerand, J.T., Berdell, R., Funke. and Christinel, L.C., 2007. Microbiology. 9th ed. Published by Pearson Benjumin Cummings.

Ghazi, K., Sajjad, A. and Sadia, A., 2013. Frequency of uropathogens in different gender and age groups. *G.J.M.S.,* 11(1): 20-23.

Glew, H., Arthur, R.R and Perfect, J.R., 2005. Is it time to abandon the use of amphotericin B bladder irrigation. *Clin. Infect. Dis.,* 40:1465-1470.

Goniugur, U., Bakici, M. Z., Ozdemir, L., Akkurt, I., Icagasiogh, S. and Gultekin, F., 2003. Retrospective analysis of antibiotic susceptibility patterns of urinary isolates of *Pseudomonas aeruginosa* in a Turkish University Hospital. *Ann. Clin Microbiol. Antimicrob.,* 2: 2-5.

Govan, J.R.W., 1998. *Pseudomonads* and non-fermenters. In: Greenwood D, Slack RCB, Peutherer JF (eds.). Medical microbiology. Edinburgh, Churchill Livingstone. Pp. 284-289.

Goyal, A., Prasad, K.N, Prasad, A., Gupta, S., Ghoshal, U. and Ayyagari. A., 2009. Extended spectrum β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* and associated risk factors. *Indian J. Med. Res.,* 129:695-700.

Grabe, M., Bjerklund, J.T.E., Botto, H. and Wullt, B, *et al.,* 2008. Guidelines on Urological Infections. *Europian Association of Urology.,* pp. 8-111.

Graybill, J. R. L.W., Marshall, P., Charache, C.K., Wallace. and V.K., Melwin., 1973. Nosocomial pneumonia: A continuing major problem, *Am. Rev. Respir. Dis.,* 108:1130-1140.

Grunberg, R. N., 1980. Antibiotic sensitivities of urinary pathogens. *J. Clin. Pathol.,* 33: 853-856.

Gupta, N., Kundra, S., Sharma, A., Gautham, V. and Arora, D.R., 2007. Antimicrobial susceptibility of uropathogens in India. *J. Infect. Dis. Antimicrob. Agents.,* 24:13-18.

Gupta, K., 2001. Antimicrobial resistance among uropathogens that cause community acquired urinary tract infection in women: a nationwide analysis. *Clin. Infect. Dis.,* 33*:* 89-94.

Gupta, K., Hooton, T.M. and Naber, K.G, *et al.,* 2011. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis*., 52(5): 103-120.

Gupta, K., Hooton, T.M. and Stamm, W.E., 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Am. Intern. Med*., 135:41-50.

Gupta, K., Scholes, D. and Stamm, W.E, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. *J.A.M.A.,* 281: 736-738.

Gill, M.A and Schutze, G.E., 1999. *Citrobacter* urinary tract infection in children. *Pediatr. Infect. Dis. J.,* 18: 889-892.

Hagay, Z., Levy, R., Miskin, A, and Milman, D, *et al.,* 1996. Uriscreen, a rapid enzymatic urine screening test: Useful predictor of significant bacteriuria in pregnancy. *Obstet. Gynecol.* 87(3): 410-413.

Handley, M. A., Reingold, A. L., Shiboski, S. and Padian, N. S., 2002. Incidence of acute urinary tract infection in young women and use of male condoms with and without nonoxynol-9 spermicides, *Epidemiology.,* 13(4):431-436.

Hanif, S., 2006. Frequency and pattern of urinary complaints among pregnant women. *J. Coll. Physicians Surg. Pak*., 16(8): 514-517.

Harrington, R.D. and Hooton, T.M., 2000. Urinary tract infection risk factors and gender. *J. Gend. Specif. Med*., 3(8): 27-34.

Harrigan, W.F and M.E. McCance., 1966. Laboratory Method in Microbiology. Academic Press, London and New York.

Hasan, A.S., Nair, D., Kaur, J., Baweja, G. and Deb, M. *et al.*, 2007. Resistance patterns of urinary isolates in a tertiary Indian hospital. *J. Ayub. Med. Coll. Abbottabad*., 19(1): 39-41.

Hassan, M.I., Alkharsah, K.R., Alzahrani, A.J., Obeid, O.E., Khamis, A.H. and Diab, A., 2013. Detection of extended spectrum beta-lactamases-producing isolates and effect of AmpC overlapping. *Infect. Dev. Ctries.,* 7(8): 618-29.

Heilmann, C. and Peters, G., 2000. Biology and pathogenicity of *Staphylococcus epidermidis*. Gram positive pathogens. Washington, DC: ASM., pp. 442-449.

Hooton, *et al.*, 2010. Diagnosis, prevention, and treatment of catheter associated urinary tract infection in adults. International Clinical Practice Guidelines from the Infectious Diseases Society of America. Urinary Catheter Guidelines. CID. 50: 625-663.

Hooton, T.M, 2000. Urinary tract infections in adults. In: Johnson RJ, Feehally J (Editors). Comprehensive Clinical Nephrology. Mosby: London. pp. 561- 562.

Hooton, T.M, Hillier, S., Johnson, C., Roberts, P.L. and Stamm, W.E, 1991. *Escherichia coli* bacteriuria and contraceptive method. *Am. Med. Asso.,* 265: 64-69.

Hooton, T.M., Scholes, D. and Stapleton, A, *et al,* 2000. A prospective study of asymptomatic bacteriuria in sexually active young women. *N.E.J.M.,* 14: 992-997.

Hooton, T. M., 2000. Pathogenesis of urinary tract infections: an update. *J. Antimicrob. Chemother.,* 46(1):1-7.

Hooton, T. M., 2003. The current management strategies for community-acquired urinary tract infection. *Infect. Dis. Clin. North Am.,* 17: 303-332.

Hooton, T. M. and Stamm, W. E., 1997. Diagnosis and treatment of uncomplicated urinary tract infection. *Infect. Dis. Clin. North Am.,* 11: 551-581.

Horvath, L.L., Hospenthal, D.R., Murray, C.K. and Dooley, D.P., 2003. Direct isolation of *Candida* spp. from blood cultures on the chromogenic medium CHROMagar *Candida*. *J. Clin. Microbiol.,* 41: 2629-2632.

Hsueh, P.R, Hoban, D.J and Carmeli, Y, *et al.,* 2011. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. *J. Infect*., 63(2): 114-123.

Hugbo, P.G. and Olurinola, P.F., 1992. Resistance of *Pseudomonas aeruginosa* to antimicrobial agents: Implications in medicine and pharmacy. *Nigerian J. Pharmaceut. Sci.,* 4: 1-10.

Humayun, T. and Iqbal, A., 2012. The culture and sensitivity pattern of urinary tract infections in females of reproductive age group. *Ann. Pak. Inst. Med. Sci.* 8(1): 19-22.

Inabo, H. I. and Obanibi, H. B. T, 2006. Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics. *Afri.J. Biotech.,* 5(5): 487-489.

Inyama, H.K., Revathi, G., Musandu, J. and Odero, T., 2011. The incidence of nosocomial urinary tract infections: kenyatta national hospital - intensive care unit. *B.I.RJ.*, 2:12-21.

Ipe, D.S., Sundac, L., Benjamin, W.H., Jr, Moore, K.H. and Ulett, G.C., 2013. Asymptomatic bacteriuria: prevalence rates of causal microorganisms, etiology of infection in different patient populations and recent advances in molecular detection. *FEMS Microbiol. Lett*. 346(1): 1-10.

Ishihara, S., Yokoi, M., Ito, S., Kobayashi. and Deguchi, T., 2001. Pathologic significance of *Staphylococcus saprophyticus* in complicated urinary tract infections. *Urology*., 57: 17-20.

Jacoby, G., Walsh, K. and Walker, V., 2006. Identification of Extended-Spectrum, AmpC, and CarIbapenem- Hydrolysing ß-Lactamases in *Escherichia coli* and *Klebsiella pneumoniae* by Disc Tests. *J. Clin Microbiol.,* 44: 1971-1976.

Jacqueline, M., Achkar. and Bettina, C., 2010. Candidal infections of the genitourinary tract. *Clin. Microbiol. Rev.*, 23(2):253-273.

Jain, M., Dogra, V., Mishra, B., Thakur, A., Loomba, P.S. and Bhargava, A., 2011. Candiduria in catheterised intensive care unit patients: Emerging microbiological trends. *Indian Pathol. Microbiol.,* 54: 552-555.

Jain, N., Kohli, R., Cook, E., Gialanella, P., Chang, T. and Fries, B.C., 2007. Biofilm formation by and antifungal susceptibility of *Candida* isolates from urine. *Appl. Environ. Microbiol.,* 73: 1697-1703.

Jankovic, S and Radosavljevic, V., 2007. Risk factors of bladder cancer. Tumori. 93:4-12.

Jellheden, B., Norrby, R. S. and Sandberg, T., 1996. Symptomatic urinary Tract Infection in women in Primary Healthcare. Bacteriological,

Clinical and Diagnostic aspect in relation to host response to infection. *Scand. J. Pry. Health Care.,* 14(2): 122-128.

Jenkins, R.D., Fenn, J.P and Matson, J.M., 1986. Review of urine microscopy for bacteriuria. *J.A.M.A.*, 255: 3397-3403.

Jennifer, P., Cyril, R., Piyumi, P., Nimesha, G. and Renuka, J., 2012. Asymptomatic bacteriuria in pregnancy: prevalence, risk factors and causative organisms. *Sri Lankan J. Infect.Dis.,* 2(1): 41-45.

Jepson, R. G. and Craig, J. C., 2008. Cranberries for preventing urinary tract infections. Cochrane Database Syst. Rev. 23(1): 1321.

Jha, N. and Bapat, S.K., 2005. A study of sensitivity and resistance of pathogenic micro-organisms causing UTI in Kathmandu valley. *K.U.M.J.,* 3: 123-129.

John, F. and Fisher., 2011. *Candida* urinary tract infections: epidemiology, pathogenesis, diagnosis, and treatment: Executive summary. 52(6): 429- 432.

Johnson, J.R., 2008. Urinary tract infection. Screening: Screen asymptomatic bacteriuria only in selected high risk patients. American college of physicians, Pier. pp 5-7.

Jones, R. N., 1999. Contemporary antimicrobial susceptibility patterns of bacterial pathogens commonly associated with febrile patients with neutropenia. *Clin. Infect. Dis.,* 29: 495-502.

Jones, R. N., Ballow, C. H. and Biedenbach, D. J, *et al.,* 1998. Antimicrobial activity of quinupristin-dalfopristin (RP59500, Synercid) tested against over 28,000 recent clinical isolates from 200 medical centres in the United States and Canada. *Diag. Microbiol. Infect. Dis.* 31: 437- 451.

Jones, R. N., Kugler, K. C., Pfaller, M. A. and Winokur, P. L., 1999. Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY Antimicrobial Surveillance Program, *Diagn. Microbiol. Infect. Dis.*, 35:55-63.

Joshi, M.C., Shalini, Rashid, M.K. and Joshi, H.S., 2011. Study of antibiotic sensitivity pattern in urinary tract infection at a tertiary hospital. *N.J.I.R.M.*, 2(3):43-46.

Joumana, N. and George, F., 2000. Recent developments in β lactamases and extended spectrum β lactamases. *B.M.J.*, 327:1209-1213.

Judith, A.O.D., Steven, P.G. and Elius, A., 2002. Selecting drug regimens for urinary tract infections: current recommendations. *Infect Med.*, 19(1):14-22.

Jung, Y.S., Shin, H.S. and Rim, H., 2011. The influence of chronic renal failure on the spectrum and antimicrobial susceptibility of uropathogens in community-acquired acute pyelonephritis presenting as a positive urine culture. BMC Infectious Diseases., 11(102):1471-2334.

Kader, A.A and Nasimuzzaman, M., 2001. Antimicrobial resistance patterns of Gram negative bacteria isolated from urine cultures in Almana general hospital. *Annals of Saudi Medicine.,* 21(2): 110-112.

Kadri, S.M, Gash, B. and Rukhsana, A., 2002. Antibiotic sensitivity and resistance profile of the microorganisms responsible for urinary tract infection observed in Kashmir, India. *J. Indian Med. Assoc.,* 100: 656-60.

Khadri, H and Alzohairy., 2009. High prevalence of multi-drug resistance (MDR) and extended spectrum beta lactamase (ESBL) producing

bacteria among community-acquired urinary tract infections (CAUTI) *J. bacteriol. Res.,* 1(9):105-110.

Kahlmeter, G., 2003. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECOSENS Study. *Int. J. Antimicrob. Agents.,* 22(2):49-52.

Kalra, O.P and Raizada, A., 2009. Approach to a patient with urosepsis. *J. Glob. Infect. Dis*., 1(1): 57-63.

Kammire, L., 2013. Prevention of urinary tract infections in the outpatient and inpatient settings. USA, National Institute of Environmental Health Sciences. Pp: 1-20.

Karlowsky, J.A., Kelly, L.J., Thornsberry, C., Jones, M.E. and Sahm, D.F., 2002. Trends in antimicrobial resistance among urinary tract infection isolates of *Escherichia coli* from female outpatients in the United States. *Antimicrob. Agents Chemother.,* 46: 2540-2545.

Kashef, N., Esmaeeli, D.G. and Shahbazi, S., 2010. Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. *J. Infect. Dev. Ctries.,* 4(4): 202-206.

Kass, E. H. and Finland., M, 2002. Asymptomatic infections of the urinary tract. *J. Urol.*, 168: 420-424.

Katouli, M., 2010. Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iranian J. Microbiol*., 2(2): 59-72.

Kauffman, C.A., 2005. Candiduria. *Clin. Infect. Dis.,* 41(6): 5371-5376.

Kausar, Y., Chunchanur, S.K., Nadagir, S.D., Halesh, L.H and. Chandrasekhar, MR., 2009. Virulence factors, serotypes and antimicrobial susceptibility pattern of *Escherichia coli* in urinary tract infections. *Al-Ameen J. Med. Sci.,* 2(1): 41-51.

Kaye, K., Howard, S., Schrader, M., Lata, V., Youlin Qi., Paola, C., Samore, M., Greg, A., Rasheed, J. and Tenover F., 2004. Variety of ß-Lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the Northeastern United States. *Antimicrob. Agents Chemother.,* 48:1520-1525.

Kayo, O., Katsumi, S., Hiroyuki., Masato, F. and Soichi, A., 2013. *Candida* urinary tract infection and *Candida* species susceptibilities to antifungal agents. *The J. Antibiotics.,* 66:651-654.

Kennedy, R. P., Plorde, J. J. and Petersdorf, R. G., 1965. Studies on the epidemiology of *Escherichia coli* infections. Iv. Evidence for a nosocomial flora. *J. Clin. Invest.,* 44: 193-201.

Kobayashi, C.C., de Fernandes, O.F, Miranda, K.C., de Sousa, E.D. and Silva, M. R., 2004. Candiduria in hospital patients: A study prospective. *Mycopathologia.,* 158: 49-52.

Koff, S., Wagner, T. and Jayanthi, V., 1998. The relationship among dysfunctional elimination syndromes, primary vesicoureteral reflux and urinary tract infections in children. *J. Urol*., 160: 1019-1022.

Kolawale, AS., Kolawale, O.M., Kandaki-Olukemi, Y.T., Babatunde, S.K. and Kplawale, C.F., 2009. Prevalence of urinary tract infections among patients attending Dalhatu Araf specialist hospital, Lafia, Nasarawa state, Nigeria. *Int. J. Med. Sci.,* 1(5): 163-167.

Koneman, E.W., Allen, S.D and Janda, W.A., 1997. Guidelines for the collection transport, processing, analysis and reporting of cultures from specific specimen sources. Color atlas and text book of diagnostic, Microbiology, Lipoincott Raven, Washington. pp 121-162.

Kothari, A and Sagar, V., 2008. Antibiotic resistance in pathogens causing community acquired urinary tract infection in India: a multi-center study. *J. Infect. Developing Countries.,* 2(5): 354-358.

Krcmery, V and Barnes, AJ., 2002. Non-albicans *Candida spp.* causing fungaemia: pathogenicity and antifungal resistance. *J. Hosp. Infect*., 50: 243-260.

Kriplani, A., Bukshee, K. and Ratan, A., 1993. Asymptomatic bacteriuria in pregnant Indian patients at All India Institute of Medical Sciences, New Delhi and treatment with single dose antimicrobial therapy. *J Obst Gyn India.,* 43: 489-491.

Kristina, B., Charles, M. and Gerard. R., 1999. Renal candidiasis in neonates with candiduria. *Pediatr. Infect. Dis. J*., 18(11):959-963.

Kumari, N., Rai, A., Jaiswal, C.P., Xess, A, and Shahi, SK., 2001. Coagulase negative *Staphylococci* as causative agents of urinary tract infections prevalence and resistance status in IGIMS, Patna. *Indian J. Pathol. Mic*robiol., 44: 415–419.

Kunin, CM., 1987. In: Detection, prevention and management of urinary tract infections. 4th Edn. Lea and Febiger: Philadelphia, USA.

Kutlay, S., Kutlay, B., Karaahmetoglu, O. and Erkaya, S., 2003. Prevalence, detection and treatment of asymptomatic bacteriuria in a Turkish obstetric population. *J. Reprod. Med.*, 48(8): 627-630.

Khan, A.U and Zaman, MS., 2006. Multiple drug resistance patterns in urinary tract infection patients in Aligarh. *Biomedical Research.*, 17(3): 179-181.

Khan, S.W and Ahmed, A., 2001. Uropathogens and their susceptibility pattem: a retrospective analysis. *J.Pak.Med.Assoc.*, 5(1): 98-100.

Khoshbakht, R., Ayub, S., Hesamadd, SA. and Hal, K., 2013. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Karaj, Iran. *Jundishapur J. Microbiol.,* 6(1): 86-90.

Lafi, S., Alkarboly, A. and Lafi, M., 2012. Bacterial urinary tract infection in adults, Hit District Anbar Governorate, west of Iraq. *Egypt. Acad. J. Biolog. Sci*., 4(1):21-26.

Lagrotteria, D., Rotstein, C. and Lee, C.H., 2007. Treatment of candiduria with micafungin: A case series. *Can. J. Infect. Dis. Med. Microbiol.,* 18: 149-150.

Lane, D.R and Takhar, S.S., 2011. Diagnosis and management of urinary tract infection and pyelonephritis. *Emerg. Med. Clin. North Am.,* 29: 539-552.

Lavanya, S.V and Jogalakshmi, D., 2002. Asymptomatic bacteriuria in antenatal women. *Indian J. Med. Microbiol*., 20(2): 105-106.

Laverdiere, M., Labba, A.C., Restieri, C., Rotstein, C., Heyland, D. and Madger, S, *et al.,* 2007. Susceptibility patterns of *Candida* species recovered from Canadian intensive care units. *J. Crit. Care*., 22(3): 245-250.

Leela Rani, Venkata Bharat Kumar Pinnelli, Hemavathi, Sandhya Belwadi and Rajendran, 2012. Utility of HiChrome urinary tract infection (UTI) agar medium for identification of uropathogens: A comparative study with other conventional media. *Intl. J. Chem. Pharm. Res.*, 1(4): 95-105.

Leflon, G.V., Spelddooren, V., Heym, B. and Nicolas, C.M., 2000. Epidemiological survey of Amoxicillin-clavunate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: new genetic features of bla-TEM genes. *Antimicrob. Agents Chemother*., 44: 2709-2714.

Lewis, D.A, Lindy, Y. E. G., Louis, A. H., Gloria, N. G. and Elsabe, J. E. K, *et al.,* 2013. Antimicrobial susceptibility of organisms causing community acquired urinary tract infections in Gauteng Province, South Africa. *S.A.M.J.,* 103(6): 377-381.

Lin, W.H, Wang, M.C., Tseng, C.C., Ko, W.C., Wu, A.B., Zheng, P.X. and Wu, J.J., 2010. Clinical and microbiological characteristics of *Klebsiella pneumoniae* isolate causing community acquired urinary tract infections. 38(6): 459-464.

Lipsky, B.A., 1989. Urinary tract infections in men. Epidemiology, pathophysiology, diagnosis and treatment. *Ann. Intern. Med.* 110:138-150.

Lipsky, BA., 1999. Prostatitis and urinary tract infection in men: what's new; what's true. *Am. J. Med.,* 106(3): 327-334.

Lisa, L.. Maragakis and Trish, MP., 2008. *Acinetobacter baumannii:* Epidemiology, Antimicrobial Resistance, and Treatment Options. *Clin. Infect. Dis. Eliopoulos.,* 46: 1254-1263.

Livermore, D. M and Woodford, N., 2004. Laboratory detection and reporting of bacteria with extended spectrum β-lactamases. Antibiotic resistance monitoring and reference laboratory, specialist and reference Microbiology division, Health Protection Agency - Colindale, London.

Macfaddin, J. F., 2000. Biochemical tests of medical bacteria. 3rd (ed.). Lippincott Williams and Wilkins, USA.

Magill, S.S and Hellinger, W., 2012. Prevalence of healthcare associated infections in acute care facilities. *Infect. Control. Hosp. Epidemiol.,* 33:283-291.

Mahesh, E., Ramesh, D., Indumathi, V.A., Punith, K. and Raj, K, *et al.*, 2010. Complicated urinary tract infection in a tertiary care center in south India. *Al-Ameen J. Med. Sci.,* 3(2): 120-127.

Mahmoud, A and Hanan, H. B., 2012. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council Countries. Antimicrobial Resistance and Infection Control, 1(26): 1-5.

Maki, D.G and Tambyah, P.A., 2001. Engineering out the risk of infection with urinary catheters. *Emer. Infect. Dis*., 7: 1-6.

Manchanda, V., Sing, NP., Goyal, R., Kumar, A. and Thukral, S.S., 2005. Phenotypic characteristics of clinical isolates of *Klebsiella pneumoniae* and evaluation of available phenotypic techniques for detection of extended spectrum β-lactamases. *Ind. J. Med. Res*., 122: 330-337.

Mandell, G.L., Bennett, J.E. and Dolin, R., 2005. Principles and practice of infectious diseases. Churchill Livingstone, pp. 881-882.

Manikandan, C and Amsath, A., 2013. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from urine samples. *Int.J.Curr.Microbiol.App.Sci.,* 2(8): 330-337.

Manikandan, C and Amsath, A., 2013. Incidence and distribution of uropathogens isolated from patients with urinary tract infections in Pattukkottai, Tamil Nadu, India. *Int. J. Pure Appl. Zool.,* 1(3): 213-222.

Mansour, A., Manijeh, M. and Zohreh, P., 2009. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur J. Microbiol.*, 2(3): 118-123.

Manzano, G. P., Hernandez, H. F., Zavala, V.N., Mendez, T.L.J, *et al.*, 2008. Candiduria in type 2 *diabetes mellitus* patients and its clinical

significance. *Candida* spp. antifungal susceptibility. *Rev. Med. Inst. Mex. Seguro. Soc*., 46(6): 603-610.

Manges, A.R., Johnson, J.R., Foxman, B., O"Bryan, T.T., Fullerton, K.E. and Riley, L.W., 2001. Widespread distribution of urinary tract infections caused by a muti-drug resistant *Escherichia Coli* clonal group. *N.Egl. J. Med.*, 345: 1007-1013.

Mardani, M., Shahzadi, M., Rakhshani, N., Rahnavardi, M., Rezvani, J., and Sharifinejad, A., 2008. Spontaneous perforation of urinary bladder secondary to *Candida* cystitis: acute abdomen of urologic origin. *Surg. Infect* (*Larchmt*), 9(5): 525-527.

Marques, L.P., Flores, J.T, Barros Junior, O.O., Rodrigues, G.B., Mourão, C. M . and Moreira, R.M., 2012. Epidemiological and clinical aspects of urinary tract infection in community-dwelling elderly women. *Braz. J. Infect. Dis.*, 16(5): 436-441.

Mathur, N.B., Khalib, A., Sarkar, R. and Puri, R. K., 1991. Mortality in neonatal septicaaemia with involvement of mother in management. *Indian J. Pediatri*., 28(2): 1259-1264.

Mazel, D and Davies, J., 1999. Antibiotic resistance in microbes. *Cellular and Molecular Life Sciences*, 56(9-10): 742-754

Mbata, T.I., 2007. Prevalence and antibiogram of urinary tract among prison inmates in Nigeria. *Int. J. Microbiol*., 3(2): 1-4.

Meurman, J.H., Siikala, E., Richardson, M. and Rautemaa, R., 2007. Non-*Candida albicans Candida* yeasts of the oral cavity: *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, (1): 719-731.

Miano, R., Germani, S. and Vespasiani, G., 2007. Stones and urinary tract infections. *Urol. Int.*, 79(1): 32-36.

Miskeen, P.A and Deodhar, L., 2002. Antimicrobial susceptibility pattern of *Enterococcus* species from urinary tract infections. *J. Assoc. Physicians India.,* 50: 378-381.

Moges, F., Mengistu, G, and Genetu, A., 2002. Multiple drug resistance in urinary pathogens at Gondar college of medical sciences hospital, Ethiopia. *East. Afri. Med. J.,* 79: 415-419.

Mohan, U., Jindal, N. and Aggarwal, P., 2002. Species distribution and antibiotic sensitivity pattern of Coagulase negative *Staphylococci* isolated from various clinical specimens. *Indian. J. Med. Microbiol.,* 20: 45-46.

Mohandas, V and Ballal, M., 2011. Distribution of *Candida* species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalised patients in southern India. *J. Glob. Infect. Dis*., 3(1): 4-8.

Mohsin, R and Siddiqui, K.M, 2010. Recurrent urinary tract infections in females. *J.P.M.A.,* 60(1):55-59.

Monali, P. M., Nagen, K. D. and Rabindra, N. P., 2013. Surveillance of multi-drug resistant uropathogenic bacteria in hospitalised patients in Indian. *Asian Pac. J. Trop. Biomed*., 3(4): 315-324.

Moore, K. N., Day, R. A. and Albers, M., (2002). Pathogenesis of urinary tract infections. A review. *J. Clin.Nurs.,* 11: 568-574.

Mordi, R.M and Erah, P.O., 2006. Susceptibility of common urinary tract isolates to the commonly used antibiotics in a tertiary hospital in Southern Nigeria. *Afr. J. Biotechnol.,* 5(11): 1067-1071.

Mucheye G, *et al.,* 2013. *Escherichia coli* isolated from patients suspected for urinary tract infections in Hawassa referral hospital, Southern Ethiopia: An institution based cross sectional study. *J.Microbiol.Res.,* 1(1): 9-15.

Muzaheed, Y. D., Jennifer, M., Adams, H. A., Endimiani, H. S. E., Subhaschandra M G. and David, L. P., 2008. High prevalence of CTX-M-15 producing *Klebsiella pneumoniae* among inpatients and outpatients with urinary tract infection in Southern *Ind. J. Antimicrob. Chemother.*, 61(6): 1393-1394.

Nada Khazal, K.H., Shaimaa, O. H. and Shatha Khazal, K. H., 2013. Bacteriological study of urinary tract infections with antibiotics susceptibility to bacterial isolates among honeymoon women in Al Qassim Hospital, Babylon Province, Iraq. *British Biotech. J.*, 3(3): 332-340.

Najar, M.S., Saldanha, C.L. and Banday, K.A., 2009. Approach to urinary tract infections. *Indian. J. Nephrol*., 19(4): 129-139.

Narayanaswamy, A. and Mallika M, 2011. Prevalence and susceptibility of extended spectrum beta-lactamases in urinary isolates of *Escherichia coli* in a Tertiary Care Hospital, Chennai, South India, *Int.J.Med.*, 6(1): 39-43.

Nayman, A S., Ozgunes, I., Ertem, O.T, Erben, N., Doyuk, K. E. and Tozun M, *et al.,* 2011. Evaluation of risk factors in patients with candiduria. *Microbiol. Bul*. 45(2): 318-324.

Nerurkar, A., Priti, S and Shanta, S. N., 2012. Bacterial pathogens in urinary tract infection and antibiotic susceptibility pattern. *JPBMS.*, 21(12): 1-3

Niall, F. D. and Hugh D. F., 2011. The pathogenesis of urinary tract infections, clinical management of complicated urinary tract infection. Pp. 101-120.

Nicolle, L.E, 2001. Urinary tract infections in the long term care facilities. *Infect Con. Hosp. Epidermiol*., 22: 167-175.

Nimri, L. and Batchoun, R., 2010. Community-acquired urinary tract infections In a rural area: Predominant uropathogens, and their antimicrobial resistance. *Web. Med. Cen. Microbial.,* 1(9): 1471-2334.

Nguyen S, 2011. Treatment of urinary tract infections in children. *US Pharm.,* 36(3): 2-7.

Nordamann P, G. Cuzon. and T. Naas., 2009. The real threat of *Klebsiella pneumonia* carbapenemase-producing bacteria. *Lancet Infec. Dis.,* 9 (4):228-236.

Norrby and Urinary tract infection group, 1987. Coordinated multicenter study of Norfloxacin vs Cotrimoxazole treatment in symptomatic urinary tract infection. *The J. Infect. Dis.*, 155(2): 170-177.

Nwadioha, S. I., Nwokedi, E. E., Jombo, G. T. A., Kashibu, E. and Alao, O. O., 2010. Antibiotics susceptibility pattern of uropathogenic bacterial isolates from community and hospital acquired urinary tract infections in a Nigerian Tertiary Hospital. *Internet J. Infect. Dis.*, 8(1): 1-8.

Obiogbolu, C. H., Okonkwo, I. O. and Anyamene, C. O, 2009. Incidence of urinary tract infections among pregnant women in Akwa metropolis, Southeastern Nigeria. *Sci. Res. Essays.,* 4: 820-824.

Odds, F. C and Bernaerts R., 1994. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J. Clin. Microbiol.,* 32:1923-1929.

Okonofua, E. E and Okonofua, B. N., 1989. Incidence and pattern of asymptomatic bacteriuria of pregnancy in Nigeria woman. *Nig. Med. Pract.,* 17: 354-358.

Okulicz, J. F., Rivard, R.G, Conger N.G, Nguyen, M. X. and Hospenthal D.R., 2008. Primary isolation of *Candida* species from urine specimens using chromogenic medium. *Mycoses*., 51: 141-146.

Oladeinde, B. H., Omoregie, R., Olley, M. and Anunibe, J. A., 2011. Urinary tract infection in a rural community of Nigeria. *North Amer. J. Med. Sci.*, 3(2): 75-77.

Olowu, W.A, Oyetunji, T.G., 2003. Nososcomial significant bacteriemia: prevalence and patterns of bacterial pathogens among children hospitalised for non-infecturing urinary tract disease. *W. Afr. J. Med*., 22(1): 72-74.

Oluremi, B. B., Idowu, A. O. and Olaniyi, J. F., 2011. Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a Teaching Hospital in Southwestern Nigeria. *Afri.J.Microbiol. Res.*, 5(22): 3658-3663.

Omigie, O., Okoror, L., Umolu, P and Ikuuh, G., 2009. Increasing resistance to quinolones: A four year prospective study of urinary infection pathogens. *Int. J. Gen. Med.*, 2: 171-175.

Omonigho, S. E., Obasi, E. E. and Akukalia, R. N., 2001. Invitro resistance of urinary isolates of *Escherichia coli* and *Klebsiella* spp to Nalidixic acid. *Nig. J. Microbiol.*, 15(1): 25-29.

Onifade, A. K., Omoya, F. O. and Adegunloye, D. V., 2005. Incidence and control of urinary tract infections among pregnant women attending antenatal clinics in government hospitals in Ondo state, Nigeria. *J.Food Agric. Environ.*, 3(1): 37-38.

Orenstein, R and Wong, E.S., 1999. Urinary tract infections in adults. *Am. Fam. Physician.*, 59(5): 1225-1237.

Orret, F. A and Shurland, S. M., 1998. The changing patterns of antimicrobial susceptibility of urinary pathogens in Trinidad. *Singapore Med. J.*, 39(6): 256-259.

Orret, F. A and Davis, G. K., 2006. A comparison of the antimicrobial susceptibility profile of urinary pathogens for the years 1999 and 2003. *West Indian Med. J.*, 55: 95-99.

Orskov, I and Orskov, F., 1985. *Escherichia coli* in extra-intestinal infections *J. Hyg*., 95: 551-575.

Otajevwo, F. D., 2013. Urinary tract infection among symptomatic outpatients visiting a tertiary hospital based in midwestern Nigeria. *Global J. Health Sci.,* 5(2): 187-199.

Padmini, S and Appalraju, B, 2004. Extended Spectrum beta Lactamases in urinary isolates of *E. coli* and *Klebsiella pneumoniae*-prevelance and susceptibility pattern in a Tertiary care hospital. *I.J.M.M*., 22(3): 172-174.

Palleroni, N. J., 2005. *Pseudomonas.* In: D. J. Brenner, N. R. Krieg, J. T. Staley, and G. M. Garrity (Eds.) Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer- Verlag. New York, NY. 2: 323-379.

Parveen, K., Momen, A., Begum, A.A. and Begum, M., 2011. Prevalence of urinary tract infection during pregnancy. *J. Dhaka National Med. Coll. Hos.* 17(02):8-12.

Pasin, E., Josephson, DY., Mitra, AP., Cote, R.J. and Stein, G.P, 2008. Superficial bladder cancer: An update on etiology molecular development classification and natural history. *Rev Urol.* 10(1): 31-43.

Patel, L.R., Pethani, J.D., Bhatia, P., Rathod, S.D. and Shah, P.D., 2012. Prevalence of *candida* infection and its antifungal susceptibility

pattern in tertiary care hospital, Ahmedabad, *National. J. Med. Res*., 2(4): 439-441.

Patel, H.P., 2006. The Abnormal Urinalysis. *Pediatr. Clin. N. Am.,* 53(3): 325-337.

Patel, S., Taviad, P.K.P., Sinha, M., Javadekar, T.B. and Chaudhari, V.P., 2012. Urinary tract infections among patients at G.G. Hospital and Medical College, Jamnagar. *Natl. J. Comm. Med.*, 3(1): 138-141.

Paterson A., 2004. Urinary tract infection: an update on imaging strategies. *Eur Radiol.,* 4: 89-100.

Parul, S., Rajni, S., Suman, R., Raman, S., Smita, S. and Deepali, P., 2008. Prevalence of extended spectrum beta lactamase and AmpC beta lactamase producers among *Escherichia coli* isolates in a tertiary care hospital in Jaipur. *Indian J.Pathol.Microbiol.,* 51(3): 367-369.

Paterson, D.L and Bonomo, R.A., 2005. Extended spectrum β-Lactamases: A clinical update. *Clin. Microbiol. Rev.*, 18(4): 657-686.

Paul, N., Mathai, E., Abraham, O.C., Michael, J.S. and Mathai, D., 2007. Factors associated with candiduria and related Mortality. *J. Infect.*, 55: 450-455.

Payam, B., Elham, B., Hodjjat, Y., Roghiyyeh, A., Mahboubeh, A. and Djaafar, S., 2010. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. *A. J. Clin.Med.*, 5(2): 111-115.

Perfecto, E.M and Gondek, K., 2002. *Escherichia coli* resistance in uncomplicated urinary tract infection: A model for determining when to change first-line empirical antibiotic choice. *Manag. Care. Interface*., 15: 35-42.

Pfaller, M., Ringenberg, D., Rames, I., Hageman, J. and Koontz, F., 1987. The usefulness of screening tests for pyuria in combination with culture in the diagnosis of urinary tract infection. *Diagn. Microbiol. Infect. Dis*., 6: 207-215.

Pfaller, M. A., 1996. Nosocomial candidiasis: emerging species, reservoirs, and modes of transmission. *Clin. Infect. Dis.*, 22: 489–494.

Pfaller, M. A., Houston, A. and Coffmann, S., 1996. Application of CHROMagar *Candida* for rapid screening of clinical specimens for *Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata*. *J.Clin. Microbiol.,* 34: 58-61.

Piette, A and Verschraegen, G., 2009. Role of Coagulase-negative *Staphylococci* in human disease. *Vet. Microbiol*., 134: 45-54.

Pollack, M., 2000. *Pseudomonas aeruginosa*. In principle and practice of infection Diseases. Edited by G. L.Mandell; J. Bennett and R, Dolin. Philadelphia: Churchill Livingstone. Pp. 2310-2335.

Poonam, U., Sharma and Ulka, B., 2013. Isolation and identification of bacteria causing urinary tract infections in pregnant women in vidarbha and their drug susceptibility patterns in them. *Int.J.Curr.Microbiol.App.Sci.,* 2(4): 97-103.

Prabhu, V and Selvaraj, P.R, 2012. Study on the prevalence of Urinary Tract Infection among the Paliyar Indian tribe. *I.J.C.R*., 4(1): 44-48.

Prakash, D and Saxena, R. S., 2013. Prevalence and distribution of Gram negative bacteria of Enterobacteriaceae causing Urinary tract infections among hospitalised patients. *J. Acad. Indus. Res.*, 1(11): 650-654.

Priyadarsini, I., Prabhakar., Mailapur, C., Rajesh K.R. and Revathy., 2004. Incidence of asymptomatic bacteriuria in antenatal women.

Proceedings of XXVIII National Congress Indian Association of Medical Microbiologists, Microcon. Lucknow, India. 107: 25-28.

Rahimkhani, M., Khavari D. H. and Sharifian, R., 2008. Asymptomatic bacteriuria and pyuria in pregnancy. *Acta. Medica. Iranica.,* 46(5): 410-112.

Rajbhandari, R and Shrestha, J., 2002. Bacteriological study of urinary tract infectionand its antibiotic sensitivity test (Hospital based study). *J. Nepal. Ass. Med. Lab. Sci.,* 4(4): 26-32.

Raka, L., Mulliqi, O.G., Berisha, L., Begolli, L., Omeragiq, S. and Parsons, L, *et al.,* 2004. Etiology and susceptibility of urinary tract isolates in Kosova. *Int. J. Antimicrob. Agents.,* 23(l1): 42-45.

Ramakrishnan, K and Scheid, D. C., 2005. Diagnosis and management of acute pyelonephritis in adults. *Am. Fam. Physician.,* 71(5): 933–942.

Ramzan, S., Bakhsh, M., Salam, A., Khanm G.M. and Ghulam, M., 2004. Risk factors in urinary tract infection, *Gomal j. med. Sci.,* 2(1): 1-4.

Ranjbar, R., Haghi-Ashtiani, M.T, Jonaidi, J. N. and Abedini, M., 2009. The prevalence and antimicrobial susceptibility of bacterial uropathogens isolated from pediatric patients. *Iranian J. Publ. Health.,* 38(2): 134-138.

Richard, C., Lindsay, E., Nicolle, A. M. and Thomas, M. H., 2006. Asymptomatic bacteriuria in adults. *Am. Fam. Physician.,* 74(6): 985-990.

Robert, O and Edwards, W., 1999. Urinary tract infections in adults, Virginia commonwealth University, Medical college of Virginia, Richmond, Virginia. *Am. Fam. Physician.,* 59(5): 1225-1234.

Robert, R., Muder., Carole, B., John, D., Marilyn, M. and Wagener, *et al.,* 2006. isolation of *Staphylococcus aureus* from the urinary tract:

Association of isolation with symptomatic urinary tract infection and subsequent *Staphylococcal* bacteremia. *Clin. Infect. Dis*., 42(1): 46-50.

Rodrigues, C., Joshi, P., Jani, S.H., Alphonse, M., Radhakrishnan, R. and Mehta, A., 2004. Detection of Beta- lactamases in nosocomial Gram negative clinical isolates. *Indian J. Med. Microbiol*., 22(4): 247-250.

Ronald, A., 2002. The etiology of urinary tract infection: Traditional and emerging pathogens. *Am. J. Med.,* 113(1): 14-19.

Ronald, A and Ludwig, E., 2001. Urinary tract infections in adults with diabetes, *Int. J. Antimicrob. Agents.,* 17(4): 287-292.

Ronald, N and Jones, M. D., 2001. Resistance patterns among nosocomial pathogens. Trends over the past few years. *Chest.*, 119:397-404.

Rosa, D., Jose, G. and Gonzalo, P., 2001. Antibiotic susceptibility of bacterial strains isolated from patients with community acquired urinary tract infections. *Inter. Antimicro. Agent.,* 18(3): 211-215.

Rumana, M., Al-Hasan Imam, K.M., Muhammad, A., Nishat, N., Sheikh, Z.R. and Chowdhury, A.K., 2012. Emergence of Multi-drug Resistant Extended Spectrum Beta Lactamase producing *E.coli* associated with Urinary tract infection in Bangladesh. *J. Basic. Clin. Pharm.,* 3(1):225-228.

Rupali, S.S., Geeta, V.K. and Seema, O., 2012. Characterisation and antimicrobial susceptibility pattern of clinical isolates of *Enterococci* at a tertiary care hospital in Mumbai, India. 5(2):85-88.

Rupp, M.E., Soper, D.E. and Archer, G.L., 1992. Colonisation of the female genital tract with *Staphylococcus saprophyticus*. *J. Clin. Microbial.,* 30(11): 2975–2979.

Saha, R., Dasm S., Kumar, A. and Kaur, I.R., 2008. Pattern of *Candida* isolates in hospitalised children. *Indian J. Pediatr.,* 75:858-860.

Sahm, D., Clyde, T., Mayfieldm D., Jonesm M. and Karlowskym J., 2001. Multidrug resistant urinary tract isolate of *Escherichia coli:* prevalence and patient demographics in the United States in 2000. *Antimicrob Agents Chemother.,* 45: 1402-1406.

Salyers, A., Gupta, A., Wang, Y., Riley, L., Remis, R., Helgerson, S., McGee H., Wells, J. and Davis, B., 2004. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends in Microbiol.,* 12: 412-416.

Sambrook, J., Fritsh, E.F. and Maniatis, T., 2001. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbour, NY: Cold Spring Horbour Laboratory.

Samonis, G., Karageorgopoulos, D.E, Kofteridis, D.P, Matthaiou, D.K, Sidiropoulou, V. and Maraki, S, *et al*., 2009. *Citrobacter* infections in a general hospital: Characteristics and outcomes. *Eur. J. Clin. Microbiol. Infect. Dis*., 28: 61-68.

Sandra M. J and Mark E. S., 2011. *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. www.landesbioscience.com. Virulence., 2(5): 1-6.

Sarathbabu, R., Ramani, T.V., Bhaskara rao, K. and Supriya P., 2012. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. IOSR. *J. Pharm. Biol. Sci.,* 1(2): 04-09.

Schaeffer, AT., Rajan, N., Cao, Q., Anderson, BE and Pruden, D.L, *et al.,* 2001. Host pathogenesis in urinary tract infections. *Int. J. Antimicrob. Agents*., 17: 245-251.

Schaeffer, JA., 1990. Infection of the urinary tract. In: Walsh, Retike and Vaghen Wein. Cambell‘s Urology, 7th ed. Philadelphia. 1: 534-611.

Schlager, T.A., 2001. Urinary tract infections in children younger than 5 years of age: epidemiology, diagnosis, treatment, outcomes and prevention. *Paediatr. Drugs.*, 3(3): 219-227.

Schneider, P.F and Riley, T.V., 1996. *Staphylococcus saprophyticus* urinary tract infections: Epidemiological data from Western Australia. *Eur. J. Epidemiol.,* 12: 51-54.

Schrier, RW., 2000. The patient with urinary tract infection. In: Redingtan J, Reller BL, editors. Manual of Nephrology. Lippincott Williams and Wilkins. pp. 91-113.

Sellami, A., Sellami, H., Makni, F., Bahloul, M., Cheikh R.F. and Bouaziz, M *et al.,* 2006. Candiduria in intensive care unit: significance and value of yeast numeration in urine. *Ann. Fr. Anesth. Reanim.,* 25(6): 584-588.

Sharma, K., 2008. Manual of Microbiology: Tools and Techniques, 2nd Ed. Ann Books Gopaljee Enterprises, Dehi, India.

Sharma, R., Sharma, C. L and Kapoor, B., 2005. Antibacterial resistance: current problems and possible solutions. *Indian J. Med. Sci.,* 59: 120-129.

Sharma, A.R., Bhatta, D.R., Shrestha, J. and Banjara, M.R., 2013. Antimicrobial susceptibility pattern of *Escherichia coli* isolated from uninary tract infected patients attending Bir hospital. *Nepal J. Sci. Technol.,* 14(1): 177-184.

Sharma, I and Paul, D., 2012. Prevalence of community acquired urinary tract infections in silchar medical college, Assam, India and its antimicrobial susceptibility profile. *Indian J. Med. Sci.,* 66: 273-279.

Shigemura, K., Tanaka, K., Okada, H., Nakano, Y., Kinoshita, S., Gotoh, A and Fujisawa, M., 2005. Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-year period (1983-2002) at a single institution in Japan. *Japanish J. Infect. Dis.*, 58: 303-308.

Shirishkumar, P., Pankajkumar, P. T., Mala, S., Javadekar, T. P. and Chaudhari, V. P., 2012. Urinary tract infections (UTI) among patients at G.G hospital and medical College, Jamnagar. *National J. Commu. Med.,* 3(1): 138-141.

Singh, D., Grewal, J. M. and Craig J., 2005. Circumcision for the prevention of urinary tract infection in boys: a systematic review of randomised trials and observational studies. *Arch. Dis. Child.,* 90: 853-858.

Sirot, D., Champs, C., Chanal, C., Labia, R., Darfeuille-Michaud, A., Perroux, R. and Sirot J., 1991. Translocation of antibiotic resistance determinants including an extended-spectrum beta-lactamase between conjugative plasmids of *Klebsiella pneumoniae* and *Escherichia coli. Antimicrob. Agents Chemother.*, 35: 1576-1581.

Smaill, F., 2001. Antibiotics for asymptomatic bacteriuria in pregnancy (Cochrane Review). In: The Cochrane Library Issue 2. Oxford: Update software.

Smith, P.W., Seip, C.W., Schaefer, S.C. and Bell D. C., 2000. Microbiologic survey of long-term care facilities. *Am. J. Infect. Control.,* 28:8-13.

Sobel, J.D and Kaye, D., 2005. Urinary Tract Infections. In: Mandell GL, Bennett JE, Dolin R (Editors). Mandell Douglass and Bennett's Principles and Practice of Infectious Diseases. 6th Ed. Elsevier: USA. pp. 875-905.

Sobel, J.D., Kaauffman, C.A., Mckinsey, D., Zervos, M., Vazquez, J.A, Karchmer, AW, *et al.,* 2000. Candiduria: A randomised, double-blind study of treatment with fluconazole and placebo. *Clin. Infect. Dis.*, 30: 19-24.

Sobel, J.D., 1991. Bacterial etiologic agents in the pathogenesis of urinary tract infection. *Med. Clin. North. Am.*, 75: 253-273.

Sonavane, A., Mathur, M., Turbadkar, D. and Baradkar, V., 2008. Antimicrobial susceptibility pattern in urinary bacterial isolates. *Bombay Hos. J.,* 50(2): 240-244.

Sood, S., Malhotra, M., Das, B.K. and Kapil, A., 2008. *Enterococal* infection and antimicrobial resistance. *Indian J. Med. Res.*, 128: 111-121.

Spach, D.H., Stapleton, A.E. and Stamm, W.E., 1992. Lack of circumcision increases the risk of urinary tract infection in young men. *J.A.M.A.*, 276: 679-681.

Stamm, W.E., 2008. Urinary tract infections, pyelonephritis and prostatitis. In: Fauici AS, Braunwald E, Kasper DL, Hauser SL, Longo DL *et al* (Editors). Harrison's Principles of Internal Medicine. 17th Edn. McGraw-Hill Medical: USA. pp. 1820-1827.

Stamm, W.E., Hooton, T.M, Johnson, J.R, Johnson, C. and Stapleton, A *et al.,* 1989. Urinary tract infections from pathogenesis to treatment. *J. Infect. Dis.*, 159: 400-406.

Stecher, B. and Hardt, W.D., 2008. The role of microbiota in infectious disease. *Trends Microbiol.,* 16(3): 107-114.

Stephan, D. F., 2003. Acute uncomplicated urinary tract infection in women. *N. Engl. J. Med.,* 349 (3): 259–266.

Strom, BL., Collins, M., West, SL., Kreisberg, J. and Weller, S., 1987. Sexual activity, contraceptive use and other risk factors for symptomatic and asymptomatic bacteriuria. A case control study. *Ann. Intern. Med.,* 107: 816-823.

Suman, K. M., Chiranjit, M., Suman, K. H., Tanmay, P., Pratip, K. K. and Keshab, C. M., 2013. Studies on drug sensitivity and bacterial prevalence of UTI in Tribal population of Paschim Medinipur, West Bengal, India. *Jundishapur J. Microbiol.,* 6(1): 42-46.

Susan, A., 2005. Diagnosis and management of uncomplicated urinary tract infections. American family physician. 72(3): 451-456.

Suwangool, P., 2012. Current management of urinary tract infections. *Bangkok Med. J.,* 3:101-106.

Talbot, G. H., Bradley, J., Edwards, J. E., Gilbert, D., Scheld, M. and Bartlett, J. G., 2006. Bad Bugs Need Drugs: An update on the development pipeline from the antimicrobial availability taskforce of the infectious diseases society of America. *Clin. Infect. Dis.,* 42(5): 657-668.

Tambekar, D.H., Dhanorkar, D.V., Gulhane, S.R., Khandelwal, V.K. and Dudhane, M.N., 2006. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. *African J. Biotech.,* 5(17): 1562-1565.

Taneja, N., Chatterjee, S.S., Singh, M., Singh, S. and Sharma, M., 2010. Pediatric urinary tract infections in a tertiary care center from north India. *Indian J. Med. Res.,* 131: 101-105.

Taneja, N., Rao, P., Arora, J. and Ashok, DA., 2008. Occurrence of ESBL and AmpC β-lactamases and susceptibility to newer antimicrobial agents in complicated UTI. *Indian J. Med. Res.,* 127: 85-88.

Taneja, N., Singh, G., Singh, M. and Sharma, M., 2011. Emergence of tigecycline and colistin resistant *Acinetobacter* baumanii in patients with complicated urinary tract infections in north India. *Indian J. Med. Res.*, 133:681-684.

Tankhiwale, S.S., Jalagaonkar, S.V., Ahmed, S. and Hassani, U., 2004. Evaluation of extended spectrum beta lactamase in urinary isolates. *Indian J. Med. Res.*, 120(6):553-556.

Todar, K., 2011. Bacterial resistance to antibiotics. Assessed from text book of bacteriology.net on 13th of September.

Topley and Wilson., 2006. Microbiology and Microbial infections: Medical Mycology, 10th Ed., Hodder Amold. Ch.15., pp 255.

Toya, SP., Schraufnagel, D.E. and Tzelepsis, G.E., 2007. Candiduria in intensive care units: in Association with heavy colonisation and candidemia. *J. Hosp. Infect.*, 66: 201-206.

Tsering, D.C., S. Das, L. Adhiakari, R. Pal. and Singh, T.S., 2009. Extended spectrum beta lactamase detection in Gram negative bacilli of nosocomial origin. *J. Global Infect. Dis*., 1(2): 87- 92.

Thiolas, A., Bollet, C., La S.B., Raoult, D. and Jean, M.P., 2005. Successive emergence of *Enterobacter aerogenes* strains resistant to imipenem and colistin in a patient. *Antimicro. Agent. Chemo.*, 49(4): 1354-1348.

Thomas, M., Hooton, Suzanne, F., Bradley, Diana, D. and Cardenas, *et al.*, 2010. Diagnosis, prevention, and treatment of catheter associated urinary tract infection in adults: Urinary catheter guidelines. *Clini. Infect. Dis.*, 50: 625-663.

Thomas, T., Yoshikawa, Suzanne, F. and Bradley., 2002. *Staphylococcus aureus* infections and antibiotic resistance in older adults. *Clin. Infect. Dis.* 34(2): 211-216.

Thomson, K., Nancy, E., Seong, G., Kim, H., Christian, H and Ellen, S., 2007. Extended-Spectrum-β-Lactamase detection tests for analysis of *Escherichia coli* and *Klebsiella* isolates with well-characterised β-Lactamases. *J. Clin. Microbiol.*, 45: 2380-2384.

Upadhyay, G., Geeta, S., Bishnu, P.U., Sony, S., Shamshul, A., Prakash, G. and Hari, P. D., 2013. Comparative evaluation of urine isolates among kidney transplanted and other UTI suspected patients visiting National Public Health Laboratory, (NPHL) Teku, Nepal. *I.J.B.A.R.* 4(6): 369-375.

Valerie, P., 2013. Saskatchewan Infection Prevention and Control Program (SIPCP), Guidelines for the prevention and treatment of urinary tract infections in continuing care settings. Pp 1-28.

Von, E.C., Proctor, R.A. and Peters, G., 2001. Coagulase-negative *Staphylococci.* Pathogens have major role in nosocomial infections. *Postgraduate Med*. 110(4): 63-76.

Wada, K.K.H. and Gan, T.K.R., 2012. Managements of urinary catheterisation and urinary tract infection in cancer patients. 39(2): 169-173.

Walter, E.S. and Braunwald, E., 2001. Urinary tract infection and pyelonephritis. Harrison's principles of internal medicine 15th ed. 2: 1620–1626.

Walter, E.S, Thomas, M.H. and James, R.J, 1989. Urinary tract infections: from pathogenesis to treatment. *J. Infect. Dis.*, 159: 400-406.

Wayne, P., 2008. Clinical Laboratory Standards Institute (CLSI) – Document. pp. 100-118.

Wazait, H.D, Patel, H.R., Kelsy, M., Miller, R.A. and Emberton, M., 2003. Catheter associated urinary tract infections: prevalence of

uropathogens and pattern of antimicrobial resistance in a UK hospital (1996-2001). *B.J.U. I.*, 91(9): 806-809.

Weinberger, M., Sweet, S., Leibovici, L., Pitlik, S.D. and Samra, Z., 2003. Correlation between candiduria and departmental antibiotic use. *J. Hosp. Infect.*, 53(3): 183-186.

WHO., 2012. Antimicrobial resistance: no action today, no cure tomorrow, World Health Day - 7 April.

Will, L., Dlawer, A.A. and Tim, B., 2005. Medical microbiology section F-clinical manifestations of infection. F10, Urinary tract infection. Published by Taylor and Francis group. Pp. 313-318.

Willinger, B., Hillowoth, C., Selitsch, B. and Manafi, M., 2001. Performance of *Candida* ID, a new chromogenic medium for presumptive identification of *Candida* species, in comparison to Chromagar *Candida*. *J. Clin. Microbiol.*, 39: 3793-3795.

Wilson, M.L. and Gaido, L., 2004. Laboratory diagnosis of urinary tract infections in adult patients. *Clin. Infect. Dis.*, 38: 1150-1158.

Winickoff, RN., Wilner, SI., Gal, G., Laage, T. and Barnet, G.O., 1989. Urine culture after treatment of uncomplicated cystitis in women. *South Med. J*., 74: 165-169.

Wing, D.A., Park, A.S, De Bugue, L. and Millae, L.K., 2000. Limited clinical utility of blood and urine cultures in the treatment of acute pyelonephritis during pregnancy. *Am. J. Obstet. Gynecol.*,182: 1437-1441.

Wolfgang, K.J., Willett, H.R., Amos, D.B. and Catherine, M., 1998. Oral Microbiology. In: Zinsser Microbiology, Joklik, W.K. (Ed.). 20th Edn. Appleton Lange, USA., pp: 682-693.

Woodford, N., Fagan, E.J. and Ellington, M.J., 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum-lactamases. *J. Antimicrob. Chemother.*, 57: 154-155.

Yakubu, B.N., Halima, I., Elijah, Y and Gabriel, O., 2012. Bacteriuria and antimicrobial susceptibility of *Escherichia coli* isolated from urine of asymptomatic university students in Keffi, Nigeria. *Jundishapur J. Microbiol.*, 5(1): 323-327.

Yang, Y.L, Cheng, H.H., Ho, Y.A., Hsiao, C.F. and Lo, H.J., 2003. Fluconazole resistance rate of *Candida* species from different regions and hospital types in Taiwan. *J. Microbiol. Immunol. Infect.*, 36: 187-191.

Yanga, Y.L., Wangb, A.H., Wangb, C.W., Chengb, W.T., Lic, S.Y and Lob, H.J., 2008. Susceptibilities to amphotericin B and fluconazole of *Candida* species in Taiwan surveillance of antimicrobial resistance of Yeasts. *Diagn. Microbiol. Infect. Dis.*, 61: 175-180.

Yoshikawa, T.T., Nicolle, L.E. and Norman, D.C., 1996. Management of complicated urinary tract infection in older patients. *J. Am. Geriatr. Soc.*, 44: 1235-1241.

Young, SP., 2012. Renal scar formation after urinary tract infection in children. *Korean J. Pediatr.*, 55(10): 367-370.

Yuksel, S., Ozturk, B., Kavaz, A., Ozcakar, Z.B., Acar, B., Guriz, H., Aysev, D., Ekim, M. and Yalcinkaya., 2006. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections. *Int. J. Antimicrob. Agents.*, 28: 413-416.

Zahra, S., Maryam, A, Zahra S., Ali, Z. M. and Ahmad, S., 2013. Candiduria in children and susceptibility patterns of recovered

Candida species to antifungal drugs in Ahvaz. *J. Nephro.*, 2(2): 122-128.

Zanel G.G., Harding, G.K.M. and Guay, D.R.P., 1990. Asymptomatic bacteriuria, which patients should be treated. *Arch. Intern. Med.*, 150: 1389-1396.

Zarei, MA, Zarrin M, Ghanatir, F. and Vazirianzadeh, B., 2012. Candiduria in hospitalised patients in teaching hospitals of Ahvaz. *Iranian J. Microbiol.*, 4 (4): 198-203.

Zhanel, G.G., Hisanaga, T.L., Laing, N.M. and Nichol, L.P *et al.*, 2005. Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int. J. Antimicrob. Agents*, 26: 380-388.