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DETERMINATION OF ANTIBIOTIC RESISTANT
GRAM NEGATIVE URINARY PATHOGENS IN
PEDIATRIC PATIENT AT KANTI CHILDREN
HOSPITAL



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A Dissertation

BY

SAPHALA DHITAL

Presented to the Central Department of Microbiology
Tribhuvan University in Partial Fulfillment of the
Requirement for the Award of Degree of
Master of Science in Microbiology

2021

CENTRAL DEPARTMENT OF MICROBIOLOGY
TRIBHUVAN UNIVERSITY
KIRTIPUR, KATHMANDU

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TRIBHUVAN UNIVERSITY
KIRTIPUR, KATHMANDU**

2001

RECOMMENDATION

This is to certify that the dissertation entitled "**Determination of Antibiotic Resistant Gram Negative Urinary Pathogens in Paediatric Patient**" at Kanti Children Hospital submitted by **Saphala Dhital** for the partial fulfillment of M.Sc. Degree in Microbiology is based on the results of experiments carried out by her under our supervision. The results for the present work have not been submitted for any other degree to the best of our knowledge.



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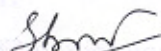
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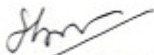
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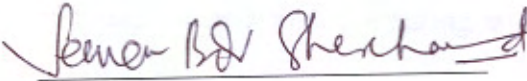
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ABSTRACT

90 bacteria were isolated from 346 children visiting at Kanti Children's Hospital, of age 2 days to 14 years in six months time period (from June-November). The gram negative bacteria were the commonest isolates (98%) and among them *E. coli* was most prominent 78% followed by *Klebsiella pneumoniae* (9%), *Proteus mirabilis* (2%), *Pseudomonas aeruginosa* (2%), *Citrobacter spp* (2%) and *Enterobacter spp* (1%). In infancy and early childhood rate of UTI is higher in male (33.3 per 100 cases) than in female (31.9 per 100 cases). The female: male ratio of UTI was 1.1: 1. The incidence of UTI in girls was found proportional with their age. High prevalence of UTI was found during rainy season.

88 gram negative bacteria isolated were tested against different antibiotics by disc diffusion method. Overall 80% of the gram negative bacteria were resistant to ampicillin, 72% were resistant to cephalexin and nalidixic acid, 70% resistant to cotrimoxazole and 54% to chloramphenicol. Norfloxacin was most active quinolone; 73% of the isolates were susceptible to this agent while resistance to amikacin was 29 percent. Overall resistance to ciprofloxacin, nitrofurantoin and gentamycin was 32%. The drug of choice for some of the gram negative bacteria were also tested.

The drug of choice for *E coli* infection was nitrofurantoin (77% sensitive) and 67% of it were sensitive to amikacin, gentamycin and norfloxacin. 88% of *E coli* were resistant to ampicillin, 72% resistant to nalidixic acid, 71% to cotrimoxazole and 61% to chloramphenicol. Overall 34% of *E coli* were resistant to ciprofloxacin. 100% sensitive antibiotics against *Klebsiella spp* were amikacin, norfloxacin and ciprofloxacin. 83% of these bacteria were resistant to cephalexin, 62% resistant to nalidixic acid, 40% to gentamycin and 33% to nitrofurantoin & chloramphenicol. For *Proteus spp.* amikacin/gentamycin (aminoglycosides) and norfloxacin were 100% susceptible. 50% resistance to ciprofloxacin, chloramphenicol, cephalexin and cotrimoxazole. 50% isolation of *Pseudomonas aeruginosa* was resistant to ciprofloxacin and 25% to norfloxacin. All the isolates were sensitive to tetracyclin, gentamycin, chloramphenicol and amikacin. All of the *Citrobacter spp* were sensitive to norfloxacin, ciprofloxacin, cotrimoxazole gentamycin and cephalexin. 50% of the isolates were resistant to chloramphenicol and nalidixic acid. 100% of the *Enterobacter spp* were resistant to cotrimoxazole, chloramphenicol, nalidixic acid and 50% resistant to nitrofurantoin. Ciprofloxacin/norfloxacin (quinolones) were most active; since 100% of the isolates were sensitive to these agents.

These in vitro results should be taken into account before initiating empirical therapy, broad-spectrum antibiotics should not be used if the isolate is susceptible to older drugs in order to prevent the increase in resistance.

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LIST OF ABBREVIATIONS

1.1 INTRODUCTION

CFU	-	Colony forming Unit
CVI-Complex	-	Crystal Violet Iodine Complex
DMSA	-	Dimercaptosuccinic acid
DNA	-	Deoxyribonucleic Acid
DTPA	-	Dietilenotriamine Penta-acetic Acid
ESBL	-	Extended Spectrum β -lactamase
IM	-	Intramuscular
IV	-	Intravenous
IVU	-	Intravenous Urography
MA	-	MacConkey Agar
MDR	-	Multi drug Resistant
MHA	-	Mueller-Hinton Agar
MRSA	-	Methicillin Resistant <i>Staphylococcus aureus</i>
MSA	-	Mannitol Salt Agar
NA	-	Nutrient Agar
NaCl	-	Sodium Chloride
NCCLS	-	National Committee for Clinical Laboratory Standard
OPD	-	Out Patient Department
PABA	-	Para Amino Benzoic Acid
PUV	-	Posterior Urethral Valves
RTF	-	Resistance transfer Factor
SSA	-	Salmonella- Shigella Agar
TMP/SMX	-	Trimethoprim/Sulphamethoxazole
UTI	-	Urinary Tract Infection
VRE	-	Vancomycin Resistant <i>Enterococci</i>
VUR	-	Vesicoureteral Reflux
WHO	-	World Health Organization

CHAPTER - ONE

1.1 INTRODUCTION

UTI is very common infection, approximately 8 to 10 million people (in world) seek professional medical assistance annually for this disease (Urology Forum, 2000). Most infections are not serious and can be treated with antibiotics. However, if left untreated, some infections can result in kidney damage and even death. Samsygina, G. A. *et al.*, (2000) states that second most frequent cases requiring hospitalization and antibiotic therapy was UTI (26.39%) after respiratory tract infections (54.09%). Urinary tract infection is the second most common infection after the respiratory tract infection for bacterial infection (Leigh, 1990).

Nepal being a developing country it has about 61.4% people illiterate who are not aware of health matters and don't have idea of hygienic living habit, so are always in the threat of infection caused by different types of organisms. According to the annual report of CWIN (2000), child population covers 52% of the total population where 49% is female and 51% is male population. 56% children in Nepal are suffered from malnutrition and 206 children die every day due to different reasons. The ratio of children per doctor is 1,44,000 children per doctor. 0.42% (88,037) of total population (21,126,638) was suffered from UTI based on the report (2053/54) published by Department of Health Services. The geographical distribution of UTI among Nepalese population is 0.57% (9,155) in mountain 0.47% (44,314) in hill and 0.35% (34,568) in Terai of total population of respective region. The strong reasons for this, are illiteracy, lack of hygienic and sanitary knowledge, lack of nutrition, underlying disease, superstition and mythology.

Urinary tract infection (UTI) is characterized by the multiplication rate of microorganisms (mainly bacteria) in any segment of the urinary tract. It encompasses a wide variety of clinical entities whose common denominator is microbial invasion of any tissue of the tract extending from the renal cortex to the urethral meatus. Infection may be expressed predominantly at a single site kidney (pyelonephritis), bladder (cystitis), prostate (prostatitis), urethra (urethritis) etc.

UTIs importance is related to many aspects (Antelo *et al.*, 2000):

Frequency of occurrence in pediatrics (corresponds to 5% of ambulatory complaints). UTI is the most common serious bacterial illness among febrile infants and young children. Risk

in the first 11 years of life for boys and girls are 1% and 3% respectively. About 40% of these children will have recurrent infections.

- If not treated properly, UTI can result in renal scarring and even insufficiency.
- 24% of children with renal scarring due to infection caused by reflux will present arterial hypertension in the future.

Many times UTI is not isolated fact; it is a manifestation of an underlying condition (vesicoureteral reflux, neurogenic bladder, vesical instability etc.)

The members of Enterobacteriaceae and other gram negative bacteria commonly cause UTI in children. There are various organisms for the cause of UTI which are *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus spp.*, *Citrobacter spp.*, *Enterobacter spp.*, *Streptococcus faecalis*, *Staphylococcus spp.*, *Lactobacillus spp.* etc. However bacteria are most commonly responsible, although, fungi and viruses may also occasionally produce UTI. Coliform group of bacteria especially *E.coli* has been found responsible for the 80% cases of UTI (Antelo *et al.*, 2000).

Sharma (1983) performed a study on "Urinary Infection" of one hundred children of age group of 4 days to 14 years. The higher percentages of isolate were *E. coli* (48%) followed by *Klebsiella* (19%), *Proteus* (16%), *Streptococcus faecalis* (13%) and *Citrobacter* (4%). He also showed Ampicillin resistance was present in more than 93% cases with *E. coli*. Cotrimoxazole showed resistance next to ampicillin for rest of all. According to (Pediatric Database, 1994) percentage of pathogenesis in UTI was found *E. coli* (72%), *Klebsiella - Enterobacter* (16.5%), *Proteus* (5%), *Staphylococcus* (5%), others i.e. *Pseudomonas spp.* (1.5%). Similarly Gautam *et al.*, (1998) isolated, 57% *E. coli* followed by *Klebsiella spp.* 24% and *Proteus spp.* 10%.

Among the isolates from UTI specimen reported by Manandhar *et al.*, (1996) in Teaching Hospital, 86% organism were gram negative and only 14% were gram positive. Among gram negative bacteria *E.coli* was 53.3% followed by *Klebsiella spp.* 16%, *Citrobacter spp.* and *Proteus spp.* etc. In vitro susceptibility test of the urinary tract pathogens done by Gautam *et al.* (1998), showed the highest sensitivity to Nitrofurantoin (88%) followed by Ciprofloxacin (81%), Nalidixic acid (69%) and Chloramphenicol (60%); Cotrimoxazole and Amoxicillin were least effective. Dhakal *et al.* (1999), reported Nitrofurantoin (84.21%) was the most effective antibiotic followed by Norfloxacin (42.11%), Cotrimoxazole (36.84%), Nalidixic acid (28.94%) and Ampicillin (10.52%) against urinary pathogens.

Hospitalized patients who become infected with multidrug resistant (MDR) bacteria can face severe, even fatal complications. Besides their own serious condition, however, they also become reservoir for the spread of such infections and a medium for the mutation of new antibiotic resistant strains. Bacterial resistance to drug therapy was first discovered in the 1940s, following the introduction of penicillin. However more types of bacteria have demonstrated resistance, and at an increasing swift rate to newer and more powerful antibiotics. Medical care costs associated with treating infections in human due to antibiotic resistant microorganisms are estimated to be over \$ 100 million annually in the US (Low DE *et al.*, 1988).

Continued surveillance, infections control procedures and improved antibiotic usage are critical to prevent the re-emergence of infectious diseases as a major cause of morbidity and mortality in developing country like Nepal as well as developed country. Overuse and misuse of newer broad-spectrum antimicrobial agents has accelerated the problem (Tenover FC *et al.*, 1996). For the reduction of antibiotic resistance, the medical establishment, regulatory committees, infectious disease specialists and community physicians need to come together to provide leadership for promoting proper use of antibiotics. Without aggressive collaboration, we may be faced with a public health crisis and return to the pre-antibiotic era.

1.2 AIMS AND OBJECTIVES OF THE STUDY

- a. To compare the relationship of bacteriuria with the patients presenting clinically UTI.
- b. Isolation and identification of organisms from urine specimens of children of different age groups.
- c. To determine the antibiotic test profile of the bacteria isolated from those samples and to determine drug of choice for some of gram negative isolates.
- d. To describe the factors which contribute to the development of antibiotic resistance and the clinical significance of the emergence of resistance.
- e. To describe the mechanisms of antibiotic resistance, and provide examples of bacteria and antibiotics demonstrating these mechanisms.

CHAPTER - TWO

LITERATURE REVIEW

2.1 DEFINITION OF URINARY TRACT INFECTION

Urinary tract infection can be defined as a spectrum of disease involving microbial invasion of any of the genito-urinary tissues extending from the renal cortex to the urethral meatus (Sharma, 1983 & Singh *et al.*, 1991). The urinary tract consists of the kidneys, ureters, the bladder and the urethra (Bailey & Scott, 1990). Urinary tract infection is one of the most common bacterial infections in infancy and childhood (Ring E & Zobel *et al.*, 1988). Infection of adjacent structures such as prostate and epididymis are also included in the definition (Bailey & Scott, 1990). The severity of the infection depends on the virulence of the infecting strain and responsiveness of the infecting host. The severe infection are limited to the urinary tract, while the more severe infection involves tissues out side the urinary tract due to gram negative sepsis and death (Svandborg C, 1987).

Bacterial infection of the urinary tract affect the patient of all age, groups and of both sexes, and it may vary in severity from an unsuspected infection to the condition of severe systemic diseases.

Bacterial UTI in neonates and children can be life threatening and can cause severe renal disease of adulthood. So early detection and treatment is extremely important. UTI may be uncomplicated caused by *Escherichia coli* or may be complicated because of:

- (a) Residential inflammation caused by bacteria.
- (b) Obstruction due to stone or tumor.

Infection of urinary tract in children is a common clinical problem. The only problem for a patient or clinician is that no or very few symptoms appear in the early stages of infections/ diseases. The occurrence of bacteria i.e. the growth of single species of bacteria within the urinary tract is the common denominator of these disorder (Svandborg C, 1987). UTI is confirmed by finding appreciable number of pathogenic bacteria in the bladder urine (White RHR *et al.*, 1997).

Patient with true UTI and whose urine may yield fewer numbers of bacteria than the classical 10^5 CFU/ml include infants and children (Bailey & Scotts, 1990).

Patient with true UTI and whose urine may yield fewer numbers of bacteria than the classical 10^5 CFU/ml include infants and children (Bailey & Scott, 1990).

Sydney M Finegold & Ellen Jo Baron (1990) in their book Diagnostic microbiology stated that although the urethra hosts a resident microflora that colonizes its transitional epithelium, all areas of the urinary tract above the urethra in a healthy human are sterile.

To distinguish contaminating organisms from etiologically important organism, only quantitative urine examination can yield meaningful results. The following steps are essential in proper urine examination.

- a) Proper collection of specimen
- b) Microscopic examination
- c) Urine culture

The usual procedure of urine culture is to spread 0.001 ml of undiluted urine, 0.01 ml of 1:100 dilution, and 0.1 ml of 1:10,000 dilution on blood agar plates or to incorporate these amounts into agar pour plates for quantitative culture.

Kass provided systematic statistical analysis of urine bacterial count in order to establish reliable criteria for separating contamination from true infection. The most widely used cultural method is the viable colony count which relies on the fact that the bacteria incubated in the bladder urine at body temperature will have multiplied many times in 2-3 hours, whereas contaminant will not. A significant colony count is 10^5 CFU/ml and can be regarded as unimportant if there is mixed growth and may require repetition of associated with a single pathogen.

2.2 ETIOLOGY

2.2.1 Gram - negative Bacteria

- *Escherichia coli* (80%) of cases)
- *Klebsiella pneumoniae*
- *Proteus mirabilis*
- *Enterobacter aerogenes*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Salmonella*

2.2.2 Gram - positive Bacteria

- *Staphylococcus epidermidis*
- *Enterococcus*
- *Staphylococcus aureus*
- *Staphylococcus saprophyticus*

2.2.3 Fungi Causing UTI

- *Candida albicans*
- *Torulopsis glabrata*
- *Gardenerella vaginalis*

2.2.4 Virus Causing UTI

Adenovirus type 11 and 12

The gram-negative bacteria are the most common pathogen and among them *E. coli* causes most of the acute UTIs. The other gram-negative bacteria (*Proteus*, *Pseudomonas* and *Klebsiella*) are probably related to chronic infections and/or repeated infections. Salmonella generally relates bacteriuria by *Salmonella* to sepsis.

The gram-positive bacteria are also cause of UTI. *Staphylococcus* coagulase-negative is detected as pathogens of urinary tract in young women who are sexually active and newborns. These microorganisms can reach the urinary tract by the haematogenic pathway.

Adenovirus type 11 and 12 are related to acute hemorrhagic cystitis. *Candida albicans* can cause UTI in patients manipulated by catheters or in immune deficiency (Antoniou *et al.*, 2000).

2.3 CLINICAL FEATURES (Source: Pediatric Database, wavesite 1994)

2.3.1 UTI

i. Neonates

- Nonspecific - loss of appetite, nausea/vomiting, diarrhea, seizures, jaundice, lethargy, irritability, sepsis with temperature instability.

ii. Toddlers

- More specific-frequency, fever, abdominal pain but also quite nonspecific.

iii. Children

- cystitis - dysuria, frequency, urgency
- pyelonephritis - fever, flank pain, malaise, chills

- clinically cannot differentiate between an upper UTI (pyelonephritis) and a lower UTI (cystitis)

2.3.2 Voiding Dysfunction

- urgency and urge incontinence
- day and night wetting
- daytime frequency and incontinence
- infrequent voiding
- urinary retention

2.3.3 Physical Findings

- meatal stenosis
- diminished anal sphincter tone
- fecal accumulation
- labial fusion
- examination of the lower spine:
 - sacral genesis
 - evidence for occult spinal dysraphism
 - hairy patch, sacral dimple or tract, lipoma, abnormal gluteal fold, bony irregularity.

2.3.4 UTI Complications

2.3.4.1 Vesicoureteral Reflux (20-35%)

- Features:
 - shrunken kidneys
 - hypertension
 - renal scarring
 - proteinuria
 - distal renal tubular acidosis (acidosis, enuresis, hyperkalemia)
- Majority of cases improve over time
- Close follow-up important even when reflux has been surgically corrected

2.3.4.2 Injuries associated with chronic reflux and infections

i. Renal

- calculi
- chronic pyelonephritis
- end stage renal disease

- hypertension (10-30%)
- pyelonephritic scars (10-20%)
 - tend to occur early
 - decreases in incidence with prophylactic therapy
 - scarred kidneys will tend to increase in size during adolescence

ii. Bladder

- trabeculations
- diverticula

2.3.4.3 Recurrence rate 50% with symptomatic UTI

2.3.4.4 Adverse reactions to long-term antibiotics

2.4 ENTEROBACTERIACEAE

Members of the family enterobacteriaceae are gram-negative non-spore forming bacilli that grow aerobically and anaerobically on ordinary laboratory media. They are oxidase negative and catalase positive, they ferment glucose and other carbohydrates in peptone water with the production of acid or acid and gas; reduced nitrates to nitrites and are either motile with peritrichous flagella or non motile. Treatment of nosocomial infections associated with any of the Enterobacteriaceae usually consists of a β -lactam or quinolones.

2.4.1 *E.coli*

German bacteriologist Theodar Escherich first isolated *E.coli*, a gram-negative rod in 1885. *E.coli* is the organism most commonly isolated from all types of urinary tract infections and it has prevailing role in extraintestinal infections (Orskov, 1981; Sussman, 1985).

The species *E.coli* are purely commensal as well as possessing of combination of virulence determinants that enable them to act as specific pathogen of gut and extraintestinal site especially urinary tract. They are haemolytic on blood agar and grow over a wide range of temperature (15-45°C).

Most strains of *E.coli* can grow on simple laboratory media containing glucose as sole source of carbon. They ferment D-mannitol and D-mannose, and do not ferment meso-inositol. The optimal growth temperature is 36-37°C. They ferment lactose forming small pink colonies in MacConkey agar and forms yellow colonies on xylose lysine deoxycholate (XLD) agar 80% of the strains are motile.

2.4.2 *Klebsiella*

Klebsiella are non-motile members of Enterobacteriaceae and are usually capsulated and do not produce H₂S in TSI agar or liquefied gelatine. Most strains are lactose fermenter and forms mucoid pink colonies on MacConkey agar. They are opportunistic pathogens in the human body and are associated with both endemic and epidemic infections in hospitals. *Klebsiella pneumoniae* are resistant to ampicillin and carbenicillin.

2.4.3 *Proteus*

Proteus *sps* are also a commonest cause of urinary tract infections. Members of genus *Proteus* are motile, lactose non-fermenting, phenylalanine deaminase and methyl red positive. They hydrolyse urea rapidly. The swarming growth with their characteristic 'fishy' smell may cover most or the entire surface in nutrient agar. *Proteus* *sps* are generally resistant to nitrofurantoin and tetracycline (Miles and Amyes SGB).

2.4.4 *Citrobacter*

Citrobacter *sps* are motile, gas producing and citrate utilizing. They are lactose fermenter and ferment arabinose, mannose, mannitol, sorbitol and trehalose. They are methyl red positive and voges-Proskauer negative, common clinical isolates produce H₂S. They are resistant to ampicillin and carbenicillin.

2.4.5 *Enterobacter*

Enterobacter are usually motile. Common clinical isolates produce gas and ferment lactose, voges-Proskauer positive and urease variable. They are resistant to ampicillin and cephalexin although newer cephalosporins are more susceptible.

2.4.6 *Pseudomonas*

Pseudomonas are aerobic gram-negative motile bacilli, oxidase and catalase positive. They produce H₂S in TSI. *Pseudomonas aeruginosa* can grow at 42°C.

2.4.7 *Enterococci (Streptococcus faecalis)*

Enterococci are the bowel flora of most humans and are able to grow in urine. They form small-pigmented pink colonies in MacConkey agar. *Enterococci* possess group D teichoic acid antigen, are able to grow in 6.5% sodium chloride and in 40% bile, and exhibit resistance to some beta lactam agents such as penicillin.

2.5 SEXES AND FREQUENCY

In childhood small girls are more prone to infection than boys so, UTI is more frequent in girls (from 4 girls: 1 boy to 20 girls: 1 boy), but in the first year of life it is more frequent in males. UTIs occur more often in women than men at least partially because of the short female urethra and its proximity to the anus. In addition, uncircumcised boys are more likely to get an infection than circumcised boys because bacteria survive in warm, moist areas like the area under the foreskin.

2.6 CLASSIFICATION OF UTI

2.6.1 Asymptomatic Bacteriuria

UTI may be asymptomatic at any age.. Upto 2-3 years of pre-school and schoolgirls may be symptom free with their first UTI. In girls with asymptomatic bacteriuria *E.coli* is not able to attach the urinary tract and is sensitive to the high bactericidal capability of the plasma. Because of that, most of the studies suggest that asymptomatic bacteriuria may not be treated, since a therapy can lead to antibiotic resistance and reinfection.

2.6.2 Urethritis

- It is the infection of the urethra.

2.6.3 Cystitis (infection of the bladder)

Localisation of the site of infection may be very difficult, specially in young children clinical manifestations are:

- suprapubic pain
- dysuria
- frequency
- urgency
- enuresis

2.6.4 Pyelonephritis

Occur when bacteria ascends the ureters and infect the kidneys.

- < 2 years of age clinical manifestations are: Fever (we must ask for uroculture at this age in case of fever because in this group, most of the UTI corresponds to pyelonephritis)

- >2 years of age
- high fever
- toxicity
- flank pain
- costovertebral angle tenderness

Bacteria that enter the kidney through the bloodstream from an infected source in the body can infect from above the urinary tract, or the UTI can occur from below by bacteria entering the urethra and spreading upward. Infection from above is most often seen in newborn babies with a systemic infection known as sepsis. Infection from below is much more common and it is most often seen in small children and adults.

Table No. 1: Classification of UTI (Norrby SR, 1990).

	Groups	Definition
Classification by symptoms	Symptomatic	UTI symptoms during the preceding two weeks.
	Asymptomatic	No symptoms during the preceding two weeks.
Level	Lower (cystitis)	Bacteriuria limited to the bladder.
	Upper (Pyelonephritis)	Bacteriuria involving the kidney.
Complications	Uncomplicated	No identified anatomical defects, foreign bodies or tumours.
	Complicated	Identified anatomical defects, foreign bodies or tumours.
Recurrences	<u>Sporadic</u>	< 2 episodes of UTI in the preceding six months and < 3 episodes in the preceding year.
	<u>Recurrent</u>	> 2 episodes of UTI in the preceding six months or > 3 episodes in the preceding year.

2.7 PATHOGENESIS

Routes of infection : Ascending (via urethra), Haematogenous through blood and Lymphatic route (Sobel D Jack and Kaya Donald *et al.*, 1971). The ascendent pathway (via urethra) is the most important pathway at all ages, except in the newborn in which the hematogenic pathway is the main way.

The urinary tract is the most common site of nosocomial infection (Stamm, 1977) according for more than 40% of the total number reported by acute care hospitals and affecting an estimated 600,000 patient per year (National Nosocomial Infection Study Report, Atlanta, 1979).

Blood born infection can occur in neonates and some older children with skin infection (*Staphylococcus*) (Jack R Burke *et al.*, 1993). Bacterial adherence to the urothelial surface is an important first stage in the development of UTI. There are many factors related to the micro organisms and to the host in UTI pathogenesis:

2.7.1 Host-parasite Interactions and Genetic Influences

E.coli binds to glycolipid and glycoprotein receptor on the surfaces of uroepithelial cells via various adhesins, especially the genetically related P and F adhesins. The carbohydrate compositions of glycolipid such as ABO, P and Lewis antigens, are determinate by the synthetic activity of genetically controlled glycosyltransferases. Thus, inheritance of the gene that encodes the glycosyltransferases influences the cell surface glycolipid composition of tissues in which the gene is expressed.

In the Pediatric population, the P1 blood group phenotype has been associated with increased risk of recurrent pyelonephritis (Antelo *et al.*, 2000).

After *E.coli* adherence to epithelial cells, *E.coli* transfers toxins and endotoxins to the host, resulting in the effects below:

- Ureter peristalsis paralysation
- Consequent ureter dilatation
- Decrease in mitotic activity of the vesicoureteral junction cells.
- Inflammatory response: fever
- Reactive C protein
- Leucocytosis

A typical uropathogenic strain of *E.coli* posses P. fimbriae which seem to be the major virulence factor possessed by *E.coli* (Gupta *et al.*, 1993)

In addition to this the surface agglutination of the *E.coli* pathogen appear to be a key determinant of virulence permitting it to colonise the urinary tract. The strain of *E.coli* rich in K antigen are more likely to succeed in invading the kidney probably because of the inhibitory action of K antigen on phagocytosis and destruction by complement (Bailey R.R. *et al.*, 1996).

2.7.2 Predisposing Factors Related to the Host

- a. Vesicoureteral reflux : Increases the risk of pyelonephritis, occurring in 30-50% of the cases, principally in young children. In these cases *E.coli* does not need virulence factors to determine renal scarring (Antelo *et al.*, 2000). No correlation was found between renal parenchyma defects and clinical presentation of the pyelonephritis, type of the microorganism, presence of bacteraemia, or the number of recurrent infections. DMSA scintigraphy should not be performed routinely in every infant with UTI and should be reserved primarily for children with VUR grad 3 and above (Goldman M, Bistritzer T *et al.*, 2000)
- b. Obstruction : Leads to urinary stasis and facilitates bacterial adhesion (remember that urine is a good culture medium).
- c. Female short urethra : Facilitates the ascendent via of infection.
- d. Bladder disfunction : Vesical instability and neurogenic bladder may function as urinary tract obstruction.
- e. Intestinal constipation : Fecalomas may alter urinary voiding by compression, leading to stasis and infection.
- f. Colonization of glans and foreskin : In glans and foreskin *E.coli* the receptors density is increased.
- g. Instrumentation (catheterization) : Increases the risk of UTI.
- h. Previous antibiotic administration : Can lead to drug resistance and clonal expansion of resistant bacteria.

2.7.3 Protective Factors Related to Host

- a. Circumcision : There is a relationship of lack of circumcision to an increased risk of UTI that was first noted among infants (in boys, 1982). The risk increases in 2-5 fold in UTI

- of uncircumcised boys aged to 1 to 14 as compared with circumcised boys of the same age (Antelo *et al.*, 2000).
- b. Periodic and complete urinary voiding decreases risk of UTI because it prevents stasis.
 - c. Satisfactory hydric regiment increases frequency of micturition leading to urinary voiding.
 - d. Low pH: The acid vaginal pH is an important fact to the lack of colonization. Low pH has an inhibitory effect on *P. mirabilis* and *Pseudomonas aeruginosa*, may be it explains the high incidence of UTI caused by *E.coli*.
 - e. Inespecific mechanisms of mucosa mediated by electrostatic forces: Risk groups for bacteriuria or symptomatic UTIs with subsequent renal damage include:
 - i. Premature infants discharge from neonatal intensive care unit.
 - ii. Children with systemic or immunologic diseases.
 - iii. Children with urinary tract abnormalities, renal calculi, neurogenic bladder, voiding disfunction, constipation or a family history of UTI with abnormalities such as reflux.
 - iv. Girls younger than 5 years with a previous history of UTI.
 - v. Uncircumcised males: increased risk of UTI.

2.8 CLINICAL MANIFESTATION

UTI in children tends to manifest with different symptoms below 2 years of age group fever can corresponds to pyelonephritis and at this we must ask for uroculture. At this age other symptoms are failure to gain weight, prostration, gastrointestinal disfunction, vomiting, jaundice etc. Local symptoms are very rare bacteriuria may be the first sign of infection.

Above 2 years of age group pyelonephritis display symptoms such as high fever, toxicity, flank pain, costovertebral tenderness etc.

Cystitis: Localization of the infection may be very difficult, especially in young children. When children with cystitis may display localized symptoms such as frequency, enuresis, dysuria, suprapubic pain and urgency enuresis (Sobel D Jack & Kaya Donald *et al.*, 1971)

2.9 DIAGNOSIS

2.9.1 Microscopic Urinalysis

If a UTI is suspected, the urine is cultured to see what type of bacteria is growing and which antibiotics will be effective in killing it (called a sensitivity test).

It has poor predictability of abnormal urinalysis. It may be normal, so it is not used for UTI diagnosis neither treatment. In other cases it may be abnormal and does not correspond to an UTI (e.g.: pyuria can occur in glomerulonephritis without UTI).

2.9.2 Urinoculture

Gold standard for UTI diagnosis. Bacteria grown from an urinoculture may arise from:

- i. contamination outside of the urinary tract
- ii. colonization of the distal urethra (contamination from within the urinary tract)
- iii. asymptomatic colonization of the bladder urine
- iv. true urinary infection

Due to the factors above, we must be careful in collecting the urinary specimen. If the urinary specimens are obtained with use of a plastic bag attached to the perineum, even after extensive cleaning these specimens may more often reflect perineal and rectal flora and are mainly useful if they grow few or no organisms. Although midstream-voided specimens in older girls, circumcised boys, and older boys whose foreskin is easy retracted may be fairly reliable for culture, these same specimens obtained in young girls and uncircumcised boys may reflect periurethral and preputial skin colonisation.

When cultures are difficult to interpret and there is a doubt that the child is actually experiencing infections, reliable specimens taken by suprapubic or urethral catheterization when the child is symptomatic are needed to clarify the situation.

UTI present if:

- * 100,000 colonies/cc (F) - bag/clean-catch
- * 10,000 colonies/cc (M) - bag/clean-catch
- * 1,000 colonies/cc - catheterized, suprapubic

(Source: Pediatric Database, website, 1994)

2.9.2.1 Results of urinoculture

Table No. 2 :

Specimens	CFU/ml*	Probability of UTI
Midstream-voided specimen	1 sample $>10^5$	80%
	3 sample $>10^5$	95%
Suprapubic aspiration	Any number of colonies	
Catheterized specimen	1 sample $>10^4$	99%

* CFU - Colonies-forming unit

2.9.2.2 Urinoculture errors

Table No 3 :

<p>False - Positive</p> <ul style="list-style-type: none"> - inadequate urine collection - procedure delay - vaginal/foreskin contamination
<p>False-negative</p> <ul style="list-style-type: none"> - Ph < 5 - Urinary dilution (decreases bacterial concentration) - bacteriostatic agents used in genitalia assepsis - antibiotic use

(Source: Antelo *et al.*, 2000)

An urine culture should be obtained one week after therapy has been initiated, and at this time the urine should be sterile.

2.10 IMAGING STUDIES

Imaging studies are part of standard care after diagnosis of UTI in young children.

Indications for radiologic evaluation of a child with UTI are:

- i. Pyelonephritis
- ii. First UTI in a boy (of any age)
- iii. First UTI in a girl younger than 3 years of age.
- iv. Second UTI in a girl 3 years of age or older.
- v. first UTI in a child (of any age) with a family history of UTIs, abnormalities of the urinary tract, an abnormal voiding pattern, hypertension or poor growth.

The aim of these imaging studies is detect urinary tract morphologic/functional alterations and children at risk of renal damage.

Young child and UTI duration are important factors related to the renal damage extension. The pyelonephritic scarring can result from a UTI, mainly at the first two years of age.

Urinary tract abnormalities can be found, mainly vesicoureteral reflux, alteration that is highly associated with UTI. Vesicoureteral reflux can be tenuous (I and II levels), moderate (III level) or severe (IV and V levels). V level is characterized by pyelotubular (intrarenal) reflux that damages renal parenchima, leading to progressive and irreversible deterioration. It is important to observe that not always there is a relationship between reflux level and renal damage (pyelonephritic scarring).

Which study we might ask?

- a. Intravenous urography (IVU)
 - good for obstruction detection
 - good for pyelonephritic scarring (does not detect recent scars)
- b. Voiding cystourethrogram
 - excellent for vesicoureteral diagnosis
 - excellent for intrabladder obstructions
 - identify vesical capacity and its wall morphology
- c. Renal and bladder ultrasonogram

Incidence for vesicoureteral reflux (VUR) was significantly higher in infants with repeated pathological findings on ponography. Ultrasonography is of definite value in infants with UTI, but cannot replace radiological work up (Riccabona M, Ring E *et al.*, 1991).

- good resolution for liquid collections (abcess, cyst)
- good resolution for calculi (<0.5 cm)
- low specificity and sensibility in acute pyelonephritis and pyelonephritic scarring detection (Source: Antelo *et al.*, 2000).

d. Urodynamic Evaluation

It's indicated in patients with micturition alteration (urinary incontinence, intravesical obstruction, neurogenic bladder), which can lead to UTI because, occurs an inadequate voiding bladder and urine stasis. Besides bladder disfunction causes vesical pression elevation, consequently increases vascular permeability and facilitates macromolecules passage from the lumen to the vesical wall.

e. Dimercaptosuccinic acid nuclear scan (DMSA)

Best method for acute pyelonephritis diagnose and possible evaluation of pyelonephritic scarring. Radiopharmac low-captation indicates proximal tubular disfunction and/or blood flux obstruction.

- alteration in this exam means renal involvement, which can be transitory (acute pyelonephritis) or permanent (pyelonephritic scarring)

f. Renal Dynamic study using DTPA (dietilenotriamine penta-acetic acid)

Evaluates renal function (statics and dynamic) through concentration and excretion capacity of each kidney.

2.11 SOURCES OF ORGANISMS CAUSING URINARY TRACT INFECTION

It is now firmly established that most infections are caused by the organisms belonging to enterobacteriaceae family derived from the patient's own bowel (Horkners JL, Anderson, *et al.*, 1975). Again in most cases the offending bacteria comes from the faecal flora and ascending from the perineum through the urethra into the bladder. Urine may possibly reflux from the urethra into the bladder carrying with it enterobactericea that colonize and cause urinary tract infection. In the neonate, however the urinary tract infection may becomes colonized

is an associated factor in some cases in children, perhaps by encouraging bacterial invasion of the bladder by external trauma to the urethra. Catheterization of the ureter is also recognised as the major risk factor for urinary tract infection in children (Craylon A. Fargason).

Most of the UTI were due to the re-infection, not persistence of the pathogen within the urinary tract and suggested that the colony flora was the reservoir for these re-infecting strains (Russo, Thomson & Ann Stepton). Prolong use of antibiotics in children will alter the intestinal flora and the development of resistant strains in the bowel will led to recurrent UTI.

2.12 RISK FACTORS

UTIs may occur in infants who are born with abnormalities of the urinary tract, such as posterior urethral valves or vesicoureteral reflux. These conditions sometimes require surgery. Additional studies have shown that women with specific blood types are more prone to UTIs.

Another common source of infection is catheters or tubes placed in the bladder. Catheters are normally used when people cannot void, are unconscious or are critically ill.

People with certain medical conditions are more likely to get a UTI than others. Any abnormality in the urinary tract e.g. kidney stone, urinary stasis or enlargement of the prostate raise the risk of infection.

Sexually active teenagers and women are more prone to UTI.

Patient with diabetes have a higher risk of infection because of changes in the immune system secondary to the high sugar concentration.

Children with urinary tract infections are at risk of renal scarring which may lead to impaired renal function and hypertension. According to study done by Panaretto K; Craig J *et al.*, (1999) the independent risk factors for recurrent UTI identified on age of less than 6 months at the index UTI and grade 3-5 VUR. The recurrence rate was 12%, multiple recurrences was 34%. Urinary tract infection was significantly associated with bilateral and intrarenal reflux.

2.13 EPIDEMIOLOGY

a. Incidence:

- Females : 3.0 - 28/1000

- Males : 1.5-7/1000
- Most common disorder of the kidneys and urinary tract in childhood.

b Risk Factors

i. Sex

- Female > male. (2:1)

ii. Anatomical defects

- Obstructive lesions
 - Posterior urethral valves (PUV)
 - urolithiasis
- iii. Functional voiding disturbances**
- Uncontrolled bladder contractions (maturational defect)
 - neurogenic bladder
 - constipation (with secondary bladder dysfunction)

iv. Infants

- Male > Female
- uncircumcised under 3 months of age

v. Residual urine

- "holding-on" behaviour
- Female > Male
- squatter, squirmer, dancer, starrer

2.14 PREDISPOSING FACTORS FOR UTI

The prevalence of UTI during childhood is less common than the later adult life which is related to age and gender (Gautam K. *et al.*, 1998). The majority of urinary tract infections in children are confined clinically to the bladder and the signs and symptoms are often non-specific (Fowler, 1990). During the neonatal period, infection is 3 to 5 times greater in males than in females (Bergstrom *et al.*, 1972; Drew and Acton, 1976). The prevalence of bacteriuria in males declines after 3 months of age (Ginsberg and McCracken, 1982). The prevalence of bacteriuria

declines after 3 months of age (Ginsberg and McCracken, 1982). The prevalence of bacteriuria in females is relatively constant from birth to puberty (DeLuca, Fisher and Swenson, 1963; Dickinson, 1979; Meadow, White & Johnson, 1986).

Residual urine due to incomplete bladder emptying serves as a source of infection because urine acts as a culture medium for bacterial growth. Foreign bodies also serve as a nidus for infection. Renal calculi and in dwelling bladder catheters are the foreign bodies implicated most frequently in UTI.

The renal urine contains sufficient amount of glucose, which help maximum growth for bacteria. (Foreland M, Thomas & N Shelokova *et al.*, 1977). The urinary pH is 6-7, which is appropriate for multiplication of pathogens (Winberg 1974 & Rantz 1962). The sufficient amount of amino acids in normal urine help in bacterial growth (Rzer Gabriel *et al.*, 1988).

2.15 MANAGEMENT OF UTI

2.15.1 Education

- * bubble baths, foreign bodies self-induced, tight jeans, wiping front to back.

2.15.2 Treat Underlying Disorders

- * Constipation- stimulate intestinal rhythm
- * Enuresis - treat "holding-on" behaviour with increased fluids and a voiding routine. (micturition with intervals of 3/3 hrs.)
- * Anatomical defects - surgery
- * VUR - medical or surgical therapy

Source: Pediatric Database (website, 1994)

2.16 ANTIBIOTIC THERAPY

2.16.1 Uncomplicated UTIs (oral treatment for 7-10 days)

- * **Nitrofurantoin:** 1 mg/kg/day, 3 dose a day.
- * Low resistance, best for treatment good tolerability.
- * Adverse effects gastric intolerance, transitory leukopenia

* **Nalidixic acid:** 30-50 mg/kg/day divided in 3 to 4 doses a day.

Adverse effects: hypersensitivity, blood dyscrasia.

* **Sulfamethoxazole - trimethoprim:** 6 mg/kg/day TMP and 40 mg/kg/day SMX divided in 2 doses a day.

Adverse effects-High level of treatment failure.

* **Ampicillin:** 100-200 mg/kg/day divided in 3 or 4 doses a day.

Adverse effects; diarrhoea - exanthema

* **Cephalosporins** (second-generation)

2.16.2 Pyelonephritis/sepsis/UTI resistant to oral treatment (IM or IV therapy)

* Aminoglycosides

* Cephalosporins (third-generation)

* Norfloxacin/Ciprofloxacin

2.16.3 Follow-up of a UTI and Prophylaxis

Follow-up of a UTI should be carefully organized, because infection tends to reoccur, often in asymptomatic form. Recurrence is most likely after the first 6-12 month after an infection.

i. After therapy is discontinued, urinoculture are indicated 1 week later, every month during the subsequent 3 months, every 3 months during the next 6 months, and then twice a year.

ii. Among patients with significant vesicoureteral reflux, recurrent infection is preventing by antibiotic therapy.

iii. In recurrent infection (at least 2 in 6 months) antibiotic prophylactic therapy are indicated.

Antibiotic prophylactic therapy

(1/4 of treatment dose-unique dose at night)

- * Nitrofurantoin: 1-1.5 mg/kg/day
- * Sulfamethoxazole: 10 mg/kg/day
- * Trimethoprim: 2 mg/kg/day

Newborn

- * Trimethoprim: 2 mg/kg/once a day
- * Cephalexin: 12.5 mg/kg/once a day

Source: Antelo *et al.*, (2000)

2.17 OPTIONS IN ANTIMICROBIAL MANAGEMENT OF URINARY TRACT INFECTION IN INFANTS AND CHILDREN

2.17.1 Antimicrobial Therapy of UTI in Infants Younger than 3 or 4 Months of Age

As with other infectious diseases in pediatric patients, management of UTI in newborn and young infants is considerably different from that of older infants and children.

UTI in infants younger than 3 or 4 months are best managed in the hospital. Approximately one-fifth of such infants have positive blood cultures, the incidence being largest in-neonates; as many as one-third have positive blood and urine cultures. (Ginsburg CM, McCracken GH *et al.*, 1982; Israeli V, Darabi A *et al.*, 1987). Initial therapy is provided with parenterally administered ampicillin and gentamycin or another of the aminoglycosides. If the infant has delayed improvement, of pyelonephritis is suspected clinically or radiographically or if there is obstruction of the urinary tract parenteral therapy should be continued for 7 days or longer (McCracken G. *et al.*, 1989).

Table No. 4

Antimicrobial therapy of urinary tract infections in infants younger than 3 to 4 months of age

Initial therapy.

Ampicillin, 75-100 mg/kg daily in 3 or 4 doses, iv or im, and gentamycin (a) 7.5 mg/kg daily in 3 doses iv or im.

Parenteral therapy is continued until there is evidence of clinical improvement and blood and urine cultures are stable.

Subsequent therapy

Amoxicillin, 50 mg/kg daily in 2 or 3 doses, po or Augmentin (a,b) 50 mg/kg daily in 2 or three doses, po, or cephalexin, 50 mg/kg daily in 2 or 3 doses, po or sulfasoxazole (c), 120 mg/kg daily in 3 or 4 doses.

Total Therapy

Therapy should be given for 10 days. In those with pyelonephritis, parenteral antimicrobial therapy should be given for the entire 10 days.

- a. Tobramycin, 4 to 6 mg/kg daily in 3 doses, or amikacin, 15 to 22.5 mg/kg daily in 3 doses is a suitable alternative.
 - b. Amoxicillin plus clavulate potassium.
 - c. A sulfonamide should not be administered to infants younger than 6 weeks or to infants with jaundice.
-

Source: McCracken G. *et al.*, 1989.

Selection of the most appropriate antibiotic is dependent on the susceptibility of the pathogen. Ampicillin resistant strains of *E.coli* will usually be susceptible to an orally administered cephalosporin or to amoxicillin plus clavulanate potassium. If a sulfonamide is to be administered sulfisoxazole or trisulfapyrimides are preferred.

2.17.2 Antimicrobial Therapy of UTI in Older Infants and Children

Most symptomatic children with pyelonephritis should be hospitalized and treated initially with parenterally administered antibiotics (Table no. 5). Once there is evidence of clinical improvement and urine and blood cultures are sterile, therapy can be given orally. The preferred drug for oral administration is trimethoprim/sulfamethoxazole (TMP/SMX) because of its excellent absorption and tissue penetration and because most urinary pathogens are highly susceptible to this combination of drugs. Exceptions include *Enterococci* and *Pseudomonas species* (McCracken G. *et al.*, 1989).

Children with upper UTI are best treated for a minimum of ten days; shorter courses of therapy can result in relapse of infection.

Table No. 5 : Antimicrobial therapy of urinary tract infections in older infants and children.

Pyelonephritis

Most children with clinical and/or radiologic evidence of pyelonephritis should be treated initially with parenterally administered antibiotics.

Ampicillin, 100 mg/kg daily in 3-4 doses, iv or im gentamycin, (a) 7.5 mg/kg. daily in 3 doses iv or im.

Ampicillin, (as above) and cefotaxime, 100±50 mg/kg daily in 3 doses. For oral therapy, TMP/SMX is preferred in a dosage of 6-8 mg of TMP/30-40 mg per kg in 2 doses.

Cystitis

Sulfisoxazole or trisulfapyrimidines, 120-150 mg per kg daily in 4 doses, po or amoxicillin, 50 mg/kg daily in 2 or 3 doses, po, or TMP/SMX (as above) or cephalexin, 50 mg/kg daily in 2 or 3 doses, po.

Duration of Therapy

Therapy is conventionally provided for 7-10 days. In children with uncomplicated UTI, therapy for 3-7 days is usually satisfactory.

Tobramycin or amikacin treatment is a suitable alternative.

Source: McCracken G *et al.*, 1989.

When it is likely that the patient has cystitis, orally administered sulfonamides, aminopenicillins or cephalosporins are satisfactory (Table no. 5). In general those antimicrobial agents do not need to be administered more often than two or three times daily. The duration of therapy for lower UTI may be as brief as one dose or several days or to can be for the conventional period of 7 to 10 days. Madrigal G. *et al.*, (1988) published results of short course therapy in 82 children who received treatment for 1 dose or 3 days and compared results with those children who received a conventional 7 - day course of antibiotic therapy (Table no. 6). The conclusion was that therapy for 3 or 7 days is satisfactory for most children with uncomplicated UTI. Shorter courses of treatment can result in higher rates of recurrent infection (Moffat M, Embree J *et al.*, 1988).

Table 6 : Duration of treatment and outcome from urinary tract infections in Children.

TMX/SMX ⁹ (4) Regimen	No. Studied	No. Cured after Treatment	No. With Recurrent UTI
A. 1 dose	42	39 (93) (b)	8 (20.5) (d)
B. 3 days	40	36 (90)	2 (5.6)
C. 7 days	50	50 (100)	4 (8)

a. TMP/SMX (6 mg of TMP -30 mg of SMX per day in two divided doses except for those receiving regimen A).

b. Numbers in parentheses, percentages.

c. A vs B or C, P=0.679

d. A vs B or C, P=0.033

Source: (Madrigal G. *et al.*, 1988)

Antibiotics with proved efficacy and safety in preventing UTI in infants and young children include sulfonamides (e.g. sulfisoxazole and the trisulfapyrimidines), nitrofurantoin, nalidixic acid and TMP/SMX. Because emergence of resistant strains of enterobacteriaceae occurs commonly during long term prophylaxis with the sulfonamides, these agents are not routinely recommended for that purpose (McCracken *et al.*, 1989).

Breakthrough urinary infections occur commonly in children who are receiving chemoprophylaxis. These infections are usually caused by *Enterococci*, *Proteus species* or *P. aeruginosa* although *Candida species* can also cause UTI in this setting. Ampicillin or amoxicillin therapy is effective for Enterococcal UTI and an aminoglycoside given intramuscularly once daily is usually curative for *Proteus* or *Pseudomonas* infection. A flouroquinolone could be used in the latter situation especially in adolescent girls. With *Candida* infection it usually suffices to stop antimicrobial prophylaxis until the infection clears (McCracken G. *et al.*, 1989).

2.18 DEVELOPMENT OF RESISTANCE TO ANTIBIOTICS

Antibiotic is a chemical substance that is produced from micro-organism, synthetically or semi-synthetically that inhibits the growth of other micro-organisms in low concentration and those antibiotics must have the following qualities.

- a. Antibiotics should have ability to destroy or inhibit the pathogens without damaging the host cell.
- b. They should not produce any adverse side effects in the host.
- c. They should have the ability to preserve the normal flora of the host during their use.
- d. They should be able to come in contact with pathogens by penetrating the cells and tissue of the host in effective concentration (Pelczar , 1986).

Drug resistance is one of the nature's never ending process whereby organisms develop a tolerance for new environmental conditions. It varies from individual to individual and also within the individual at different times (Power and Daginawala, 1982). The development of resistance to antibiotics may be inherent resistance or acquired resistance.

Antibiotic resistance is a serious and growing health problem, gaining national attention as resistance increases at an alarming rate in both hospital and physician practice settings (Low DE and Scheld WM *et al.*,1988).

Antibiotic resistance has become on increasingly pressing problem in the US (Doern G.V.*et al.*, 1995). Bacteria that consistently have been susceptible to antimicrobial agents for decades now have developed resistance not only to classic therapies but to newer agents as well. Other bacteria have developed resistance to recent antibiotics almost as soon as the drugs have been marketed. (Coronado BG, Edward JR *et al.*, 1995). In some cases, strains of bacteria, both

hospital and community acquired, that have developed resistance to numerous antibiotics have become so prominent that keeping patients with serious infections alive has become a difficult task, just as in the pre-antibiotic era (Cohen ML *et al.*, 1991; Greenwood D *et al.*, 1995).

2.18.1 Factors Contributing to the Development of Antibiotic Resistance

It has been suggested that factors contributing to bacterial resistance to antibiotics include the frequency of invasive medical procedures, a larger immunocompromised population, long term use of antibiotics, use of prophylactic antibiotics, societal changes (eg increased use of day - care centres and chronic care facilities), use of antibiotics in animal feeds and ease of worldwide travel. With the increased use of broad spectrum antibiotics (eg quinolones, third - generation cephalosporins) in the community and few new antibiotics currently in development, the significance of widespread resistance is further increased (Tomasz *et al.*, 1994; Cohen M *et al.*, 1998).

Three factors influence the evolution of resistant microorganisms (Cohen M *et al.*, 1991).

- a) Mutations in common bacterial genes
- b) Exchange of genetic material (eg. DNA)

Principally there are three distinct mechanisms allowing DNA transfer to take place.

- **Transformation:** by which one strain of double standard DNA molecules, extracted from the donor can be taken up by a recipient cells.
- **Trasduction:** by which the DNA is transferred by a bacteriophage from a donor to a recipient cell where it is then inserted into the cell's chromosomal DNA.
- **Conjugation:** by which single - stranded DNA is passed from donor to recipient cells through a pillus (Richmond, 1969). In bacteria such as *E. coli*, the donor carries a sex factor, which may not be incorporated into the major bacterial replicon i.e. the chromosome. This factor is referred as the factor F₁ Bacteria possessing such an F factor are male while without this factor is referred as female. In addition male cells can transfer the F factor to female cells producing not only a change in their sex but also promoting the transfer of chromosomal genes.

In bacteria "R" factors and self-replicating genetic elements conferring drug resistance to the host, possess episomic characteristics including autonomous replication, conjugal

transferability and stable resistance. Enterobacteriaceae plasmid bearing determinants for resistance require the co-operation of a resistance transfer factor (RTF) if transfer is to occur.

- c) Selective pressure caused by the widespread use of large quantities of antibiotics not just within the hospital environment, but in community, farm and aquaculture settings, is a driving force in the development of antibiotic resistance. (Greenwood D. *et al.*, 1995).

2.18.2 Societal Factors Contributing to Resistance

According to a report by American Medical Association, physicians often over-prescribe antibiotics because of patient's expectations, insufficient time to discuss with patient why an antibiotic is not needed, a concern that they may misdiagnose bacterial infections when an antibiotic is indicated (Schwart BH, Bell DM *et al.*, 1997).

Much of the increase in antibiotic resistance is a result of the use of antibiotics for viral infections, such as the common cold. Additionally, unlike years ago, physicians have less time to spend with patients in order to make an accurate diagnosis. In today's society, patients assume a more active role in their health care diagnosis and treatment. Many times patient often expect, sometimes even request specific antibiotic which may be stronger than necessary to treat their bacterial infection (Cohen M. *et al.*, 1998). These patient related factors can contribute to the development of resistant bacteria.

2.18.3 Mechanism of Resistance

Resistance to antibiotics may be intrinsic or acquired. Intrinsic resistance, which is always present, dictates the spectrum of activity of the antibacterial. For example, gram negative bacteria are intrinsically resistant to cloxacillin and vancomycin. Of increased clinical significance is acquired resistance, in which bacteria that were previously sensitive to antibiotics become resistant. Bacteria can acquire resistance through chromosomal mutations or acquisition of genetic material (e.g., Plasmids, transposons) which confers resistance to antibiotics. After an antibiotic penetrates the cell wall or membrane of the bacteria, it targets a specific bacterial enzyme (e.g. penicillin binding protein, DNA gyrase) or ribosome, thereby interfering with bacterial replication. The mechanisms of resistance to different antibiotics, therefore, include the following:

- decreased penetration through the bacterial cell membrane.
- enzymatic degradation or inactivation of the antibiotics.

- alteration of the target site and
- active efflux of the antibiotic out of the bacteria.

Within the gram-negative bacteria, the most common mechanism of resistance to β -lactam antibiotics is the production of β -lactamases. These enzymes inactivate β -lactam antibiotics by the hydrolysis of β -lactam ring and may be encoded by the bacterial chromosomes or by plasmids. Most or all bacteria possess chromosomally determined β -lactamases (Richmond and Sykes, 1973). In many cases these are however, some gram negative species including *Ps. aeruginosa*, *Enterob. cloacae*, *Citrobacter freundii*, *Serratia spp* and indole positive *Proteus* possess inducible group of β -lactamase (Sykes and Matthew, 1976). Ampicillin and most older cephalosporins are liable to these enzymes.

Plasmid or transposon determines the β -lactamases. The problem by this enzyme and subsequent concern with plasmid mediated β -lactamase has centered. Mostly on gram negative species over 30 plasmid mediated β -lactamases are recognized in gram negative rods. But few of them are common. These-type enzymes are the most important (Mederiros, 1984). In general the plasmid mediated β -lactamase of gram negative rods cause resistance to amino & carboxypenicillins and older cephalosporins, but not to temocillin, imipenem or most newer cephalosporins other than cefamandole and cefoperazone (Jacoby and Sutton, 1985). Tiwari *et al.*, (1998) observed, out of the total drug resistant *E coli*, 70.59% were β -lactamase positive and 8.10% *E coli* harbour the conjugative type of plasmid.

Some resistant mechanisms work by competitive antagonism. The sulfas are folic acid synthesis inhibitors. Those bacteria that are obligate folate synthesizers are inhibited because these are non-utilizable forms of folate that are synthesized when the sulfas are present some others are cell wall inhibitors. For example, penicillins and cephalosporins will cause defects in the formation of the cell walls.

Examples of different drugs and their resistance mechanisms are shown in following table.

Table No. 7 : Mechanisms of Bacterial Resistance to Antibiotics.

Antibiotics Class	Mechanisms	Examples
Penicillin and cephalosporins	Enzymatic degradation (beta-lactamases)	Beta-lactamase-producing <i>Haemophilus influenzae</i> , extended spectrum beta-lactamases (ESBL) produced in gram-negative bacilli.
	Alteration of penicillin-binding proteins	Penicillin-resistant <i>Streptococcus pneumoniae</i> , methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)
Carbapenems (e.g., imipenem, meropenem)	Enzymatic degradation Outer-membrane protein deletion	Resistance in <i>Pseudomonas aeruginosa</i> and <i>Bacteroides fragilis</i> .
Vancomycin	Inhibition of glycoprotein access	Vancomycin-resistant <i>Enterococci</i> (VRE)
Aminoglycosides (e.g., gentamicin, tobramycin, amikacin)	Inactivating enzymes Ribosomal alteration Decreased drug uptake	High-level aminoglycoside resistance in <i>Enterococci</i> , Enterobacteriaceae
Macrolides (e.g., erythromycin, clarithromycin, azithromycin)	Enzymatic degradation (methylating enzymes) Ribosomal alteration	<i>Streptococcus pneumoniae</i>
Quinolones (e.g., norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, trovafloxacin)	Alteration of DNA gyrase, Decreased drug permeability	<i>Pseudomonas</i> , Enterobacteriaceae
Tetracyclines (e.g., tetracycline, doxycycline, minocycline)	Active drug efflux Decreased permeability of cell surface	<i>Staphylococci</i> , <i>Streptococci</i>
Trimethoprim-Sulfamethoxazole	Increased production of dihydrofolate reductase, PABA or pteridine, production of drug-insensitive enzymatic pathways	<i>Shigella</i> , <i>Salmonella</i> , Enterobacteriaceae, <i>Staphylococci</i> , <i>Streptococci</i>

Source: Yamashita Sharon *et al.*, 1999.

2.18.4 UTI and Resistance

Uncomplicated urinary tract infections (UTIs) in the community are usually caused by gram negative bacteria the most common being *E.coli*. Over 30% of *E.coli* is now resistant to the traditional ampicillin or amoxicillin regimens (through production of beta-lactamases) and therefore these agents are no longer recommended as first-line therapy (Neu *et al.* 1992). Although newer agents such as the quinolones or higher generation (e.g., second, third) cephalosporins are effective, older drugs such as trimethoprim-sulfamethoxazole and nitrofurantoin remain active against the common urinary tract pathogens in Canada. In the U.S., rising rates of resistance to both trimethoprim-sulfamethoxazole and nitrofurantoin have been observed (Hooton T, Stamm W. *et al.*, 1997).

Penicillin resistant pneumococcus and other resistance have become a rapidly increasing problems. Ampicillin or vancomycin are the most commonly prescribed agents for enterococcal infection. As these antimicrobials are only bacteriostatic against enterococci, an aminoglycoside is often added for its synergistic effects. Resistance to ampicillin and the aminoglycosides has been increasing over the past several decades, with the first out-break of vancomycin-resistant enterococci being reported in the late 1980s. The mechanism of resistance to vancomycin appears to be an alteration in the site of action of vancomycin (the terminal peptide of one of the precursors of the bacterial cell wall). It is thought that the increased use of vancomycin of MRSA infections may have contributed to the development of VRE.

2.19 SOME OF THE COMMON ANTIBIOTICS

Tetracycline :

This group of tetracycline is chlortetracycline, tetracycline, oxytetracycline and doxycycline. The uses of tetracycline are numerous. They can be used in gram negative bacillary infections, for rickettsial infections. Tetracyclines are broad spectrum and bacteriostatic. Minor side effects include nausea, vomiting, epigastric disturbance and discoloration of teeth in the foetus and young children.

Macrolide Antibiotics :

They possess large lactone ring linked through glycosidic bonds with aminosugar. Common example is Erythromycin.

Erythromycin :

Derivatives do also come iv but the iv is extremely irritating. It's almost impractical to use this drug parenterally. There is no im erythromycin product available (Medical Library Records ,2000).

Polypeptide Antibiotics : They comprise a diverse group like bacitracin and Polymyxin.

Antimicrobial Agents : Sulfonamides, antitubercular, quinolones, nitrofuran..

Quinolones : ?**Nalidixic acid :**

It is a synthetic derivative of 1,8 naphthyridine and has been used successfully in clinical management of uncomplicated urinary tract infection caused by gram negative organisms. It inhibits the 'A' subunit of DNA gyrase and induces the formation of a relaxation complex analogue. Nalidixic acid inhibits the nicking and closing activity compared of DNA-gyrase. Nalidixic acid, the first quinolone antibiotic, has been used frequently in children with UTI without apparent adverse effect (Schaad VB *et al.*,1987). Until there is additional information the new fluoroquinolones should not be used routinely in children and adolescents.

Ciprofloxacin and Norfloxacin:

The new fluoroquinolones (e. g. ciprofloxacin and norfloxacin) are broad spectrum antibiotics that have been used effectively in adults with a variety of infectious diseases, including UTI. They have not been approved by the Food and Drug Administration because of concern about possible toxicity to cartilage of weight-bearing joints that had been observed in animals with incomplete skeletal growth (Schluter G. *et al.*, 1986).

Quinolones are derived from nalidixic acid basically but they have greater spectrum against gram negative aerobes and have fluorine substituted ring structure in fluoroquinolone. Some fluoroquinolones are norfloxacin, ciprofloxacin, ofloxacin. Fluoroquinolones are DNA gyrase inhibitors. They do have a number of drug interactions it is suggested that these be given orally two hours before antacids or heavy metals. They are not recommended in people under 18 years of age or in pregnancy or breast feeding because they have caused orthopathy in lab animals but there have also been reports of tendinitis and tendon rupture in patients who have been receiving these drugs. Major uses are principally for gram negative infections and chronic otitis media.

Ciprofloxacin is used parenterally or orally for things like osteomyelitis, pneumonias, skin infections, bacterial diarrhoea (Medical Library Records, 2000). Ciprofloxacin may be considered as first-line treatment for a number of gram negative pathogens are proven and strongly suspected, including complicated urinary tract infections (Louie Thomas *et al.*, 1994). Norfloxacin is a drug that basically has been used for urinary tract infections.

Nitrofurantoin Compound :

This group consists of nitro group in the furan ring structure common examples are nitrofurantoin and furazolidone. For many years nitrofurantoin has been successfully and safely used for prevention of recurrent UTI in infants and children. The agent is active against many of the organisms causing infection but is ineffective against *Pseudomonas species*, *Enterococci*, and some strains of *Klebsiella*, *Enterobacter* and *Proteus*. Maximum bactericidal activity of nitrofurantoin occurs in acidic urine. Large concentrations occur in urine after oral administration of 1 to 2 mg/kg doses. It has been suggested that use of this drug be limited because of the relative high frequency of adverse reactions especially pulmonary, that occur in adolescent and adult females. Holmberg L, Bowman G *et al.*, (1980); Corragio *et al.*, (1989) reviewed the serious adverse reaction reports submitted to the Food and Drug Administration since 1953. There were 26 cases of adverse cases of serious reactions to nitrofurantoin in United States children and adolescents who were less than 20 years of age. Neurologic and hepatic reactions occurred in 7 and 9 patients, respectively, which equated to 0.8 and 1.0 cases/million uses, respectively. The total rate of serious adverse reactions was 3.0/million users (Corragio MJ *et al.*, 1989). On the basis of these data and of clinical experience it appears that nitrofurantoin is a safe and effective antibiotics for prophylaxis in infants and children with recurrent UTI.

Sulphonamides :

These are sulpha drug containing sulphanamido (SO_2NH_2) group. Common examples are sulphamethoxazole and sulphadiazine.

Cotrimoxazole :

Combination of sulphamethoxazole and trimethoprim. This is bactrim or sepra. This is used for things like otitis media. The problem with these drugs are mainly related to sulfa although trimethoprim can cause some bone marrow suppression. Journal of medical library (2000) antibiotics. TMP/SMX has been used for many years for prophylaxis of recurrent UTI.

When doses of 1 to 2 mg of TMP -5 to 10 mg of SMX per kg daily in one dose the agent has been well tolerated, safe and effective.

Miscellaneous Groups :

This mixed group consists of Chloramphenicol, Fusidic, Lincomycin, Vancomycin, Novobiocin and Cycloserine.

CHAPTER - THREE

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

Urine specimen was collected from 346 children, (attending OPD and hospitalized patients) between 2 days to 14 years of age, with suspected urinary tract infection; bacterial examination and sensitivity test was done at microbiology lab of Kanti Children's Hospital, Maharajgunj.

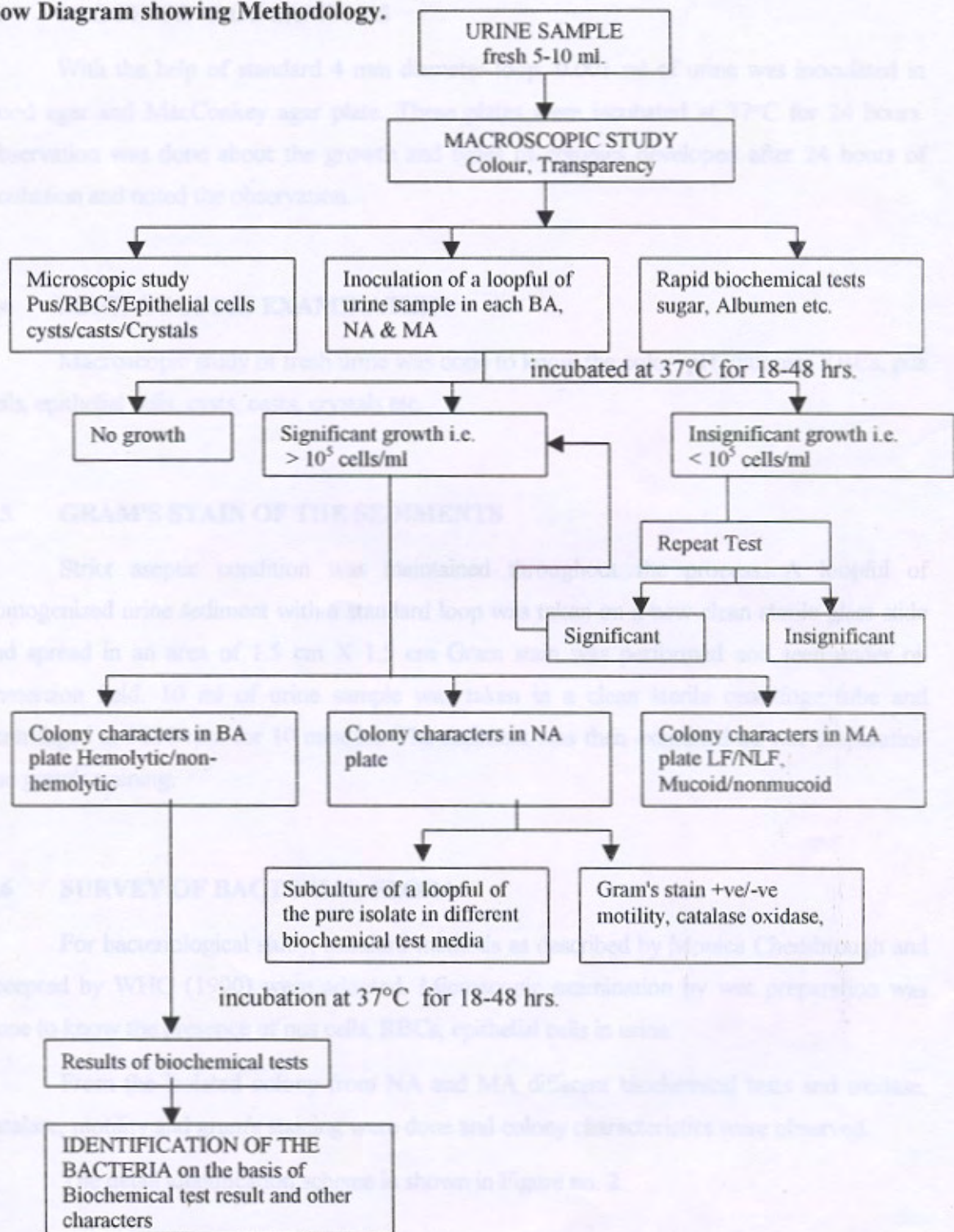
Urine secreted in the kidney is sterile unless the kidney is infected. Uncontaminated bladder urine is also normally sterile as stated by Jawetz 1998. The most important consideration for collection of a clinically relevant urine specimen is the prevention of contamination. Cheesbrough M. (1984), suggested to collect the clean catch mid stream urine specimen and process for study as soon as possible or store for overnight at 4°C.

Hendry (1991), important considerations are to be borne in mind relative to the collection of urine specimen for examination. As the result produced after microscopic examination, isolation and identification depends upon the method of sample collection. The patients were instructed carefully for collections of all urine specimens and the precaution that are to be taken during sample collection. Urine sample were collected in dean, dry and sterile wide mouthed container, cultured freshly, and in case of delayed analysis the specimens were refrigerated. In case of female the collection of sample was difficult than in the male due to the length of urethra. Therefore the patients was instructed that the normal urethral flora must be adequately flushed out by passing 5-10 ml of urine before collection. The 5-10 ml of mid-stream was collected in sterile clean wide mouthed container and tightened the closure.

3.2 SPECIMEN EVALUATION

Before proceeding with any testing, the urine specimens were evaluated in terms of their acceptability. Considerations included proper labeling visible sign of contamination and any transportation delays in getting the specimen to the laboratory and was performed culture of the urine sample.

Flow Diagram showing Methodology.



3.3 PROCESSING OF SAMPLES

With the help of standard 4 mm diameter loop, 0.001 ml of urine was inoculated in blood agar and MacConkey agar plate. These plates were incubated at 37°C for 24 hours. Observation was done about the growth and types of colonies developed after 24 hours of incubation and noted the observation.

3.4 MACROSCOPIC EXAMINATION

Macroscopic study of fresh urine was done to know the color, pH, glucose, RBCs, pus cells, epithelial cells, cysts, casts, crystals etc.

3.5 GRAM'S STAIN OF THE SEDIMENTS

Strict aseptic condition was maintained throughout the process. A loopful of homogenized urine sediment with a standard loop was taken on a new clean sterile glass slide and spread in an area of 1.5 cm X 1.5 cm Gram stain was performed and seen under oil immersion field. 10 ml of urine sample was taken in a clean sterile centrifuge tube and centrifuged at 3000 rpm for 10 minutes. The sediment was then examined by wet preparation and gram's staining.

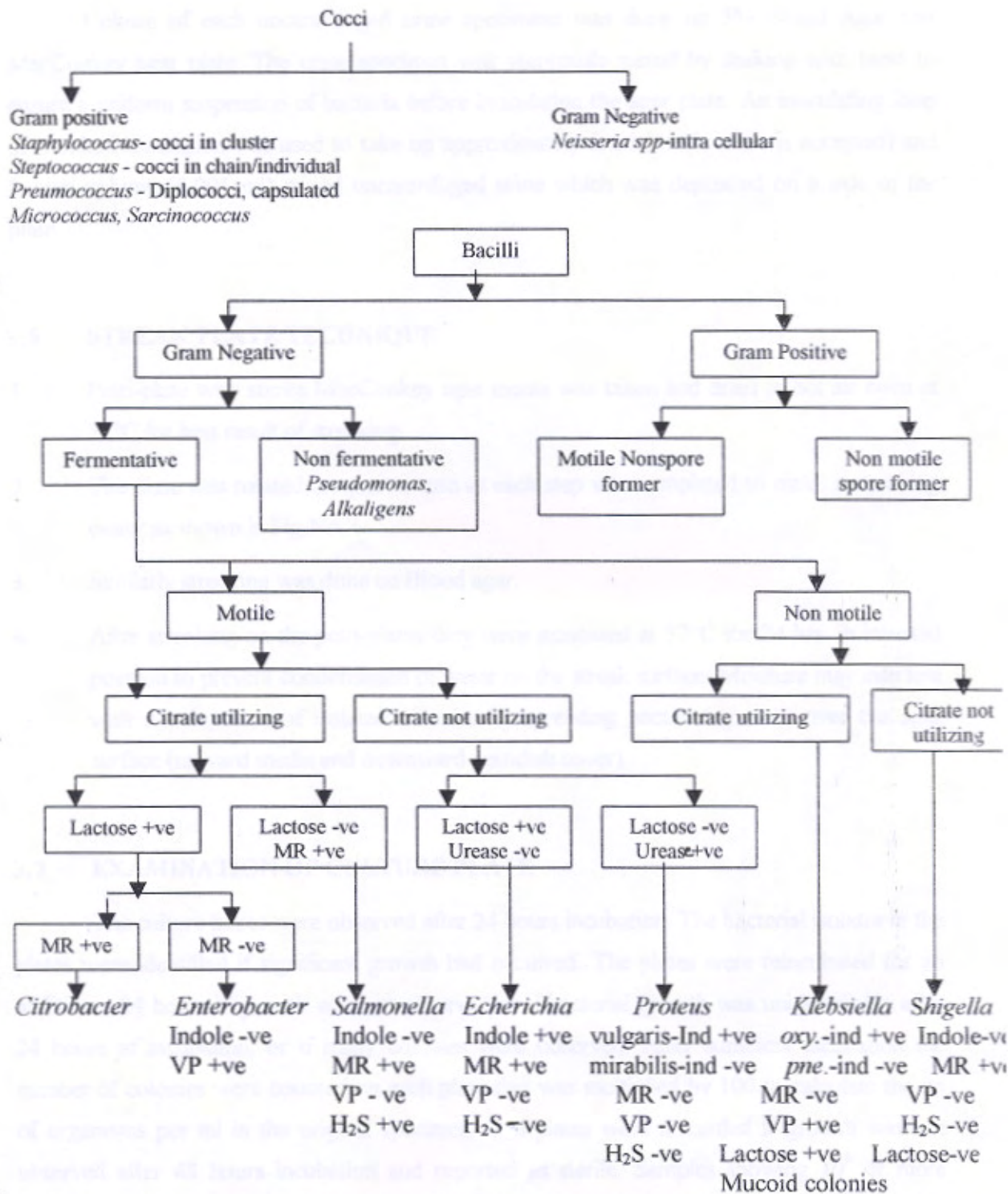
3.6 SURVEY OF BACTERIAL FLORA

For bacteriological study, standard methods as described by Monica Cheesbrough and accepted by WHO (1990) were adopted. Microscopic examination by wet preparation was done to know the presence of pus cells, RBCs, epithelial cells in urine.

From the isolated colony from NA and MA different biochemical tests and oxidase, catalase, motility and gram's staining were done and colony characteristics were observed.

The detail identification scheme is shown in Figure no. 2.

Flow Diagram Showing Identification Scheme of Bacteria Isolated from Urine.



Non motile spore former

Citrate not utilizing

Klebsiella
oxy.-ind +ve
pne.-ind -ve
 MR -ve
 VP +ve
 Lactose +ve
 Mucoid colonies

Citrate not utilizing

Shigella
 Indole -ve
 MR +ve
 VP -ve
 H₂S -ve
 Lactose -ve

NB : *Oxy.* = *Oxytoca*, *Pne.* = *Pneumoniae*, Source: Lab Manual in General Microbiology (Benson with Modification)

Figure No. 2

3.7 CULTURE OF URINARY SPECIMENS

Culture of each uncentrifuged urine specimens was done on 5% Blood Agar and MacConkey agar plate. The urine specimen was vigorously mixed by shaking with hand to ensure a uniform suspension of bacteria before inoculating the agar plate. An inoculating loop of standard dimension was used to take up approximately fixed ($\pm 10\%$ error is accepted) and known volume (0.001 ml) mixed uncentrifuged urine which was deposited on a side of the plate.

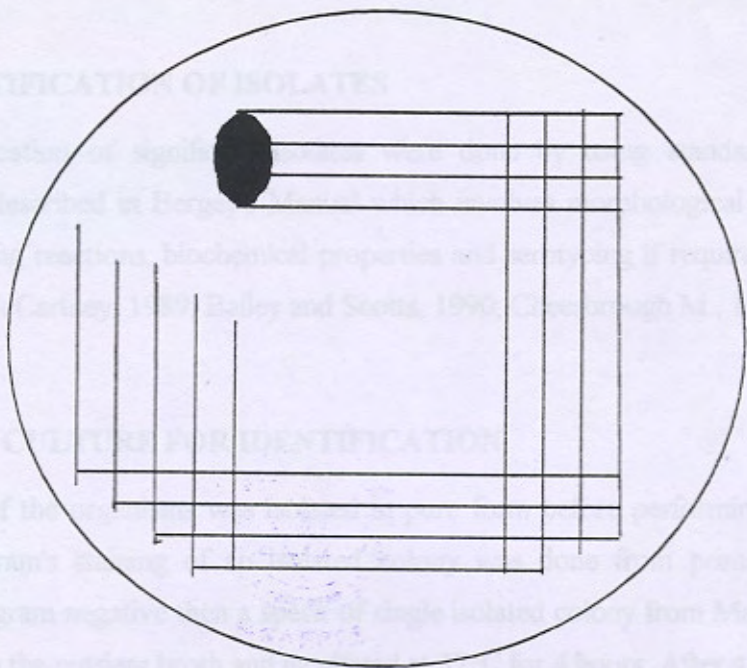
3.8 STREAK PLATE TECHNIQUE

1. Petri-plate with sterile MacConkey agar media was taken and dried in hot air oven at 37°C for best result of streaking.
2. The plate was rotated on quarter turn as each step was completed to make a streaking easier as shown in Fig.No.3.
3. Similarly streaking was done on Blood agar.
4. After streaking on the petri-plates they were incubated at 37°C for 24 hrs. in inverted position to prevent condensation of water on the streak surface. Moisture may interfere with development of isolated colonies by spreading bacterial growth over the agar surface (upward media and downward petridish cover).

3.9 EXAMINATION OF CULTURE PLATE

The culture plates were observed after 24 hours incubation. The bacterial isolates in the plates were identified if significant growth had occurred. The plates were reincubated for an additional 24 hours if growth was not observed or if bacterial growth was unidentifiable after 24 hours of incubation, or if many colonies were observed. After sufficient incubation the number of colonies were counted on each plate that was multiplied by 100 to calculate the no of organisms per ml in the original specimen. The plates were discarded if growth was not observed after 48 hours incubation and reported as sterile. Samples showing 10^5 or more organisms per ml of uncentrifuged urine were taken as significant. Colony counts less than this was considered non significant. The blood agar plate was observed for haemolysis where as MacConkey agar plate was observed for lactose fermentation.

STREAKING METHOD



Streak Plate Pattern

1. Primary Streaking
- 2 and 3. Secondary streaking
4. Final streaking

Figure No. 3

3.10 IDENTIFICATION OF ISOLATES

Identification of significant isolates were done by using standard microbiological techniques as described in Bergey's Manual which involves morphological appearance of the colonies, staining reactions, biochemical properties and serotyping if required in specific cases (Mackie and McCartney, 1989; Bailey and Scotts, 1990; Cheesbrough M., 1984).

3.11 PURE CULTURE FOR IDENTIFICATION

Each of the organisms was isolated in pure form before performing biochemical and other tests. Gram's staining of an isolated colony was done from primary culture. If the organism was gram negative then a speck of single isolated colony from MacConkey agar was transferred into the nutrient broth and incubated at 37°C for 4 hours. After 4 hours incubation it was sub cultured on dried nutrient agar plate, which was then incubated at 37°C for 24 hours. Thus obtained over night incubated culture of organism on nutrient agar was used to perform catalase, oxidase, biochemical and antibiotic sensitivity test.

3.12 MEDIA USED IN ISOLATION AND IDENTIFICATION OF BACTERIA

Table No. 8 :

S.No.	Classification of Media	Name of Media
1.	Basal Media	1. Nutrient agar 2. Nutrient broth 3. Peptone water
2.	Selective Media	1. MacConkey agar 2. Sal-Shigella agar 3. Deoxycholate citrate agar 4. Mannitol salt agar
3.	Enriched Media	1. Blood agar 2. Mueller-Hinton agar
4.	Differential Media	Eosin Methylene Blue agar
5.	Biochemical Media	1. Indole medium 2. MR-VP medium 3. Nitrate borth 4. Phenylalanine-Diaminase test-medium 5. Simmon citrate agar 6. O/F medium 7. Triple sugar iron agar 8. Urease broth

Different test were done for identification and isolation of bacteria which cause urinary tract infection. Some of the tests are as follows:

3.13 REAGENTS AND CHEMICALS USED FOR IDENTIFICATION OF ORGANISMS

1. Hydrogen Peroxide
2. Acetone
3. Safranin
4. Gram's iodine
5. Kovac's reagents
7. Methyl red indicator
8. Barritt's reagents
9. Nitrate reduction test reagent solution A and B
10. 1% tetra methyl P- phenylene-deamine dihydrochloride
11. Crystal violet
12. 10% Ferric chloride solution

3.13.1 Gram's Staining

The procedure of Gram's staining is based on the ability of microorganisms to retain the dye crystal violet during decolorization with alcohol. Gram-negative bacteria are decolourized by the alcohol losing the purple colour of crystal violet. Gram positive are not decolourized and remain purple after decolorization. Safranin-red counter stain is used to impart pink colour to the decolorized gram-negative organisms.

The CVI complex formed inside cell wall in bacteria is prevented from leaching in gram-positive bacteria and hence becomes purple while it is not prevented in gram-negative bacteria because of thin cell wall.

Bacterial smear + Covering with crystal violet

20 secs —→ washing with 95% ethanol

for 20 secs —→ cover with Safranin for 20 secs.

Gram positive bacteria —→ Purple e.g. *streptococcus spp.*

Staph. spp

Gram negative bacteria —→ Pink - *E.coli, Bacillus spp, Pseudomonas spp.*

3.13. II Motility Test

Motility test is very useful in the identification of bacteria. Bacteria are motile by means of flagella. Flagella occur primarily among the bacilli, however; a few coccal forms are motile. Motile bacteria may contain a single or many flagella; in addition, their location varies with bacterial species and cultural condition non-motile organisms lack flagella.

Procedure

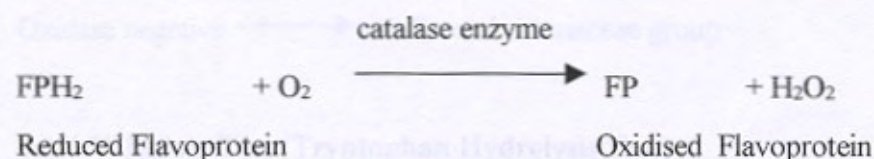
Motility of organism was determined by hanging drop method and cultural characteristics. In cultural method the test organism was stabbed in the SIM medium and incubated at 37°C for 48 hours. Motile organisms migrate from the stab line and diffuse into the medium, causing turbidity. Non-motile bacterial growth was accentuated along the stab line.

Motile bacteria - *Citrobacter spp*, *Enterobacter spp*, *Salmonella spp.*, *Escherichia coli*, *Proteus spp*.

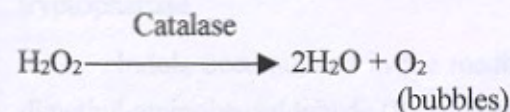
Non-motile bacteria - *Klebsiella spp*, *Shigella spp*, *Bacillus spp*.

3.13. III Catalase Test

This test is done to demonstrate the presence of catalase, which converts hydrogen peroxide to water and oxygen. Catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria. These bacteria utilize oxygen and produce hydrogen peroxide, which is toxic to their own metabolism. Reduced Flavoprotein reacts directly with gaseous oxygen to form hydrogen peroxide.



Catalase enzyme in bacteria decomposes hydrogen peroxide into water and oxygen.



Procedure:

A speck of the culture to be tested was picked from nutrient agar (pure culture) plate with a clean glass rod and inserted into hydrogen peroxide solution in a small clean tube. Catalase positive case (observed for 10 secs) was indicated by the production of gas bubbles.

Positive: *Staphylococcus spp.*

Negative: *Streptococcus spp, Clostridium*

3.13. IV Oxidase Test

This test was done to determine bacterial production of oxidase enzyme. This oxidase reaction is due to the presence of cytochrome oxidase system, which activates the oxidation of reduced cytochrome by molecular oxygen, which in turn acts as an electron acceptor in the terminal stage of the electron transfer system.

Organisms capable of growing in the presence of oxygen i.e. aerobic or facultative anaerobic are mostly oxidase positive and at the same time they produce the enzyme catalase. Obligate anaerobic organisms lack oxidase activity since they are unable to live in the presence of atmospheric oxygen and do not possess a cytochrome oxidase system.

All *Pseudomonas* and *Neisseria spp.* produce an oxidase enzyme which when in the presence of atmospheric oxygen, cytochrome C and an oxidase reagent, oxidise the reagent to form a coloured compound, idophenol. A nature of cytochrome a, cytochrome a₃, is termed cytochrome oxidase.

Procedure:

Filter paper coated with kovac's reagent when streaked with the organism gives purple colour in case of oxidase positive organisms.

Oxidase positive → *Pseudomonas spp, Neisseria spp.*

Oxidase negative → All Enterobacteriaceae group

3.13. V Indole Test (Tryptophan Hydrolysis Test)

This test demonstrates the ability of certain bacteria e.g. *E.coli* decompose the amino acid tryptophan into indole and pyruvic acid, the enzyme causing such hydrolysis is tryptophanase.

Indole accumulated in the medium is then tested for by colorimetric reaction with p-dimethyl-aminobenzaldehyde (Kovac's reagent).

Positive organism - *E.coli, Proteus vulgaris, K. oxytoca*

Negative organism - *Kleb. pneumoniae, Enterobacter, Salmonella spp.*

3.13. VI Methyl red Test (Mixed Acid Fermentation)

A considerable number of gram-negative intestinal bacteria can be differentiated on the basis of the end products produced when they ferment glucose in MR-VP medium. Generally bacteria such as *Escherichia coli*, *Salmonella spp*, *Proteus spp* and *Aeromonas* ferment glucose to produce large amount of lactic, acetic, succinic and formic acids, plus carbondioxide, hydrogen and ethanol. The accumulation of these acids lowers the pH of the medium to 5.0 and less. If methyl red added to such a culture, the indicator turns red; an indication that the organism is a mixed acid fermenter. These organisms are generally great gas producers too, because they produce the enzyme formic hydrogenylase, which splits the formic acid into equal parts of CO₂ and H₂.



MR Positive \longrightarrow Red -- *E.coli, Salmonella spp.*
(colour of media)

MR Negative \longrightarrow Yellowish --- *Klebsiella spp. Entero. aerogens.*
(colour of media)

3.13. VII Voges-proskauer Test (Butanediol Fermentation)

Many bacteria ferment carbohydrate with the production of acetyl methyl carbinol (acetoin) or its reduction product 2,3 butylene glycol (2,3 butanediol). The substance can be tested for by a colorimetric reaction between diacetyl (formed during the test by oxidation of acetone or 2,3 butanediol) and a guanidine group under alkaline conditions. This test is usually done in conjunction with the methyl red test since the production of acetone or 2,3 butanediol usually results in insufficient acid accumulating during fermentation to give a methyl red positive reaction. An organism of the enterobacterial group is usually either methyl red positive or Voges Proskauer - positive.

Procedure:

The VP test medium (glucose phosphate peptone water) was inoculated with test organism and incubated at 37°C for 48 hours Barritt's reagent (1 ml of 40% potassium hydroxide and 3 ml of a 5% solution of α -naphthol in absolute ethanol) was added to the incubated suspension. A positive reaction was indicated by the development of a pink colour in 2-5 min, becoming crimson in 30 min.

VP. Positive <i>enterocolitica</i>	→	Red (Pink) - (colour of media)	<i>Entero. aerogen, Kleb.pneumoniae, Yers.</i>
VP Negative	→	Yellowish - (colour of media)	<i>E.coli, Enterob. cloacae</i>

3.13. VIII Citrate Utilization Test

The ability of some organism to utilize citrate as a sole source of carbon energy for growth and an ammonium salt as a sole source of nitrogen is determined by citrate utilization test. Ammonium salts are broken down to ammonia with resulting alkalinity, which causes the change in colour of indicator (bromothymol blue) used in the medium.

Procedure:

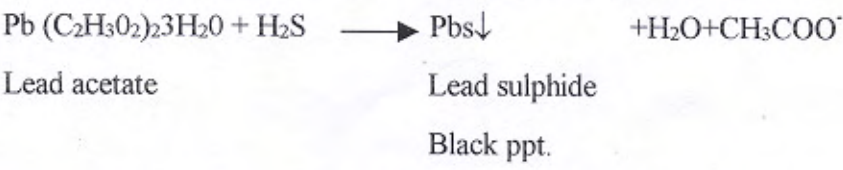
The test organism was streaked in the slant of Simmon's citrate medium and incubated at 37°C for 24 to 48 hours. Positive test was indicated by growth with a Prussian blue colour on the slant.

Citrate positive	→	<i>Sal. typhimurium, Klebsiella spp., E. aerogens</i>
Negative	→	<i>E.coli, Proteus spp.</i>

3.13. IX H₂S-production (TSI Agar Test)

This test was done to determine the ability of an organism to attack a specific carbohydrate incorporated in a basal growth medium, with or without the production of gas along with the determination of possible hydrogen sulphide (H₂S) production.

H₂S gas is produced either through the reduction of an inorganic sulphur source such as thiosulphate (-S₂O₃) or the reduction of organic sulphur supplied by the R-SH functional group of the amino acid cysteine, which is present in peptone. Lead acetate, the salt of the metal, is an indicator of H₂S production. H₂S is a colourless gas which, upon contact with lead acetate, produces the sulphide of lead, a black precipitate, indicated by a visible black colour reaction.



TSI medium contains three carbohydrates. Lactose and sucrose in 1% concentration and glucose in a 0.1% concentration. Some organisms have the ability to ferment all the

carbohydrates where as others ferment only glucose, still others are capable of fermenting neither of the sugars present in TSI agar. Carbohydrate fermentation may occur either with or without gas production ($\text{CO}_2 + \text{H}_2$).

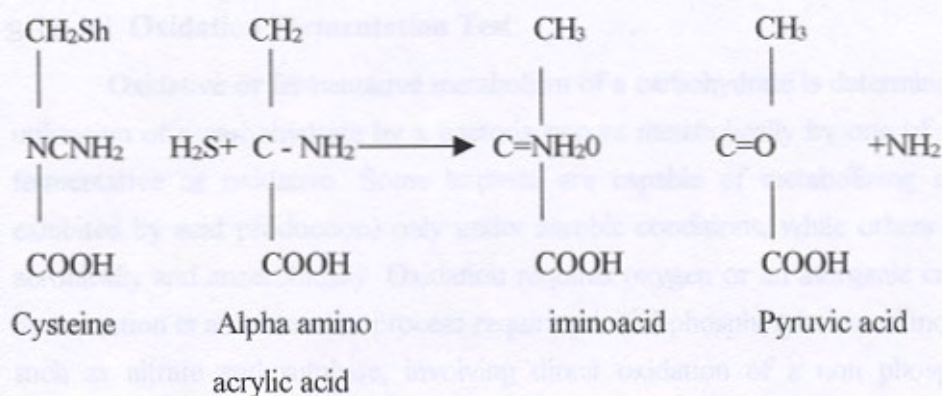
TSI reaction can be interpreted in following ways depending on the bacteria being tested.

Table No. 9 : Interpretation of TSI Test.

S. No.	Reaction Slant/Butt	Sugars attacked
1.	Alkaline/Acid (Red/Yellow)	Only glucose attacked.
2.	Acid/Acid (Yellow/Yellow)	Glucose and lactose attacked; glucose and sucrose attacked; glucose, lactose and sucrose attacked
3.	Alkaline/Alkaline (Red/Red)	Neither glucose nor lactose attacked; peptone utilized
4.	Alkaline/No change (No change in colour in slant and butt).	Neither glucose nor lactose attacked, peptones utilized.

Procedure:

With the help of straight wire TSI agar butt and slant was inoculated with the organism. These tubes were examined for colour change, gas production and the production of H_2S after 18 hours of incubation at 37°C . The result after 18 hours was interpreted as tabulated above.

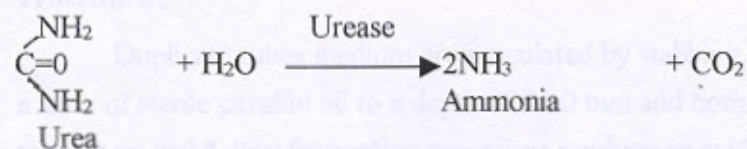


H₂S positive \longrightarrow Black ppt. - *P. vulgaris* and minerals

H₂S negative \longrightarrow *E.coli*, *Klebseilla spp.*

3.13. X Urea Hydrolysis Test

The purpose of urease test is to determine the ability of the organism to split urea forming ammonia (alkali) by the action of the enzyme urease.



The occurrence of urease can be tested for by growing the organism in the presence of urea and testing for alkali (NH₃) production by means of a suitable pH indicator.

Procedure:

The organism to be tested was inoculated heavily in urea broth and incubated at 37°C. The tubes were examined after four hours and then further incubated for four days. urease positive cultures changed the colour of the indicator to pink.

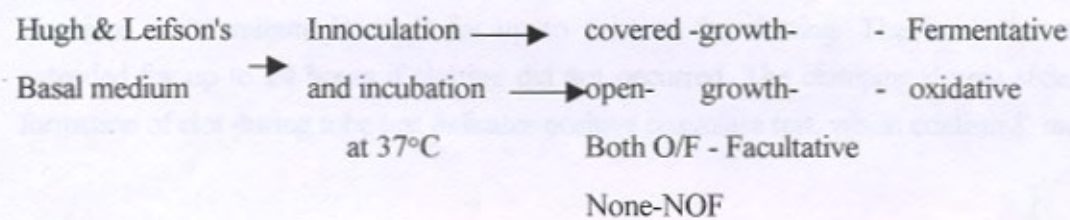
Urea hydrolysis Positive \longrightarrow *P. vulgaris*

Urea hydrolysis Negative \longrightarrow (Yellow) - *E.coli*

3.13. XI Oxidation Fermentation Test

Oxidative or fermentative metabolism of a carbohydrate is determined by this test. The utilization of a carbohydrate by a bacteria occurs metabolically by one of the two processes, fermentative or oxidative. Some bacteria are capable of metabolizing a carbohydrate (as exhibited by acid production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Oxidation requires oxygen or an inorganic compound where as fermentation is an anaerobic process requiring initial phosphorylation of inorganic compounds such as nitrate and sulphate, involving direct oxidation of a non phosphorylated glucose molecule. Fermentation produces a higher acidity than the oxidative metabolic process.

The main difference between fermentative metabolism and oxidative metabolism of a carbohydrate depends upon the requirement for atmospheric oxygen and initial phosphorylation.



Procedure :

Duplicate tubes medium are inoculated by stabbing; one tube is promptly covered with a layer of sterile paraffin oil to a depth of 5-10 mm and both are incubated at 37°C for 48 hours to even up to 14 days fermenting organisms produce an acid reaction throughout the medium in the covered (anaerobic) as well as the open (aerobic) tube. Oxidizing organisms produced an acid reaction only in open tube; this begins at the surface and gradually extends downwards.

3.13. XII Coagulase Test

This test is performed to test the ability of an organism to clot plasma by the action of the enzyme coagulase. This test is used to differentiate species within the genus *Staphylococcus*; *Staph. aureus* (usually +ve) from *S. epidermidis* and *S. saprophyticus* (-ve). Two types of coagulase are produced by most strains of *S. aureus*.

- Free coagulase is detected by the appearance of a fibrin in the tube test. Free coagulase converts fibrinogen to fibrin by activating a coagulase reacting factor present in plasma.
- Bound coagulase is detected by the clumping of bacterial cells in the rapid slide test. Bound coagulase converts fibrinogen directly to fibrin without requiring a coagulase reacting factor.

Procedure

Slide test : A drop of physiological saline (0.85% NaCl) was placed on each end of a slide. A speck of colony of the test organism was emulsified in each of the drops to make two thick suspensions. A drop of human plasma was added to one of the suspensions and mixed gently by the loop. The slide was observed for the appearance of coarse clumping visible to the naked eye within 10 seconds. The suspension to which plasma was not added acts as negative control. In the same way, the positive control was carried out using *S. aureus* (Standard organism).

Tube test : Human plasma was diluted i.e. 0.2 ml of plasma was mixed with 1.8 ml of saline 0.5 ml of the diluted plasma was taken in a tube to which 0.1 ml of 24 hour broth culture of test organism was added. After mixing gently the tube was incubated at 37°C. The tube was examined at 30 minute intervals for up to 6 hours for clotting. The incubation time was extended for up to 24 hours if clotting did not occurred. The clumping during slide test and formation of clot during tube test indicates positive coagulase test, which confirm *S. aureus*.

3.13. XIII Litmus Milk Reduction Test

This test was done for the identification of *Enterococci*. It is based on the ability of most strains of *Enterococci* to reduce litmus milk by enzyme action shown by a decolourization of the litmus.

Procedure

Into 0.5 ml of sterile litmus milk medium a heavy inoculum of the test organism was inoculated and incubated at 37°C for 4 hours, examining at half hour intervals for a reduction reaction as shown by a color change from mauve to white or pale yellow. The reduction of litmus (i.e. white or pale yellow colour) suggest *Enterococcus*.

3.13. XIV Growth in 6.5% NaCl for Enterococci

This test is done for the identification of *Enterococcus*. Two or three colonies were inoculated into a tube of nutrient broth with 6.5 NaCl and incubated at 37°C for 3 days. The growth was judged by the turbidity seen after dispersing any sediment.

3.14 PURITY PLATE CULTURE

To observe whether the tests were proceeded in an aseptic condition or not, purity plate culture of each biochemical test was performed. The 4 hours incubated broth culture prepared for biochemical test was inoculated in half of the nutrient agar plate just before proceeding the biochemical test. The other half of the same nutrient agar plate was inoculated immediately after completing the biochemical test. This plate incubated at 37°C for overnight the pure growth of same organism in both the pre and post-inoculated portion of the plate is the indication of maintenance of aseptic condition through out the experiment.

3.15 ANTIBIOTIC SENSITIVITY TEST

Antibiotic sensitivity testing is an *in vitro* method for estimating the activity of drugs against an infecting microorganism *in vivo*. Sensitivity testing is done to select effective antimicrobial drugs (Cheesbrough, 1984).

All isolated organisms were subjected to *in vitro* antimicrobial susceptibility test by disc diffusion method as described by NCCLS (National Committee for Clinical Laboratory Standard) modified Kirby-Bauer method. Kirby-Bauer method is based on the observation that the degree of inhibition of bacterial growth on agar medium surrounding and antimicrobial containing disc correlates with susceptibility to the agent. Paper disc impregnated with standardized amounts of a antimicrobial agents and specifically certified for sensitivity testing were used. The antibiotic discs used in this study were gentamycin (30 mcg), tetracycline (30mcg), ciprofloxacin (5 mcg), cotrimoxazole (25mcg), nalidixic acid (30 mcg), nitrofurantoin (300 mcg), norfloxacin (10 mcg), cephalexin (30 mcg), ampicillin (10 mcg), amikacin (30 mcg), chloramphenicol (10 mcg).

Procedure

Pure isolated colonies of identified organism were transferred into 5 ml nutrient broth and incubated at 37°C for 4 hours. The prepared inoculum was compared with Mc Failand tube No.1. With the help of a sterile swab the inoculum was inoculated over the entire surface of dried Mueller-Hinton agar plate. The selected antibiotic paper discs were gently pressed into the organism carpeted on MHA plate. After 30 minutes the plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition was measured and compared with standardized zone size interpretative chart provided by the respected company.

CHAPTER - FOUR

RESULTS

In order to determine antibiotic resistant gram negative urinary pathogens in pediatric patients, 346 urine samples were investigated from UTI suspected pediatric patients from Kanti Children's Hospital. Mixed growth and not significant bacterial cultures were excluded. Significant bacterial growth of single species was included and further study was undertaken.

4.1 MICROBIOLOGICAL RESULTS

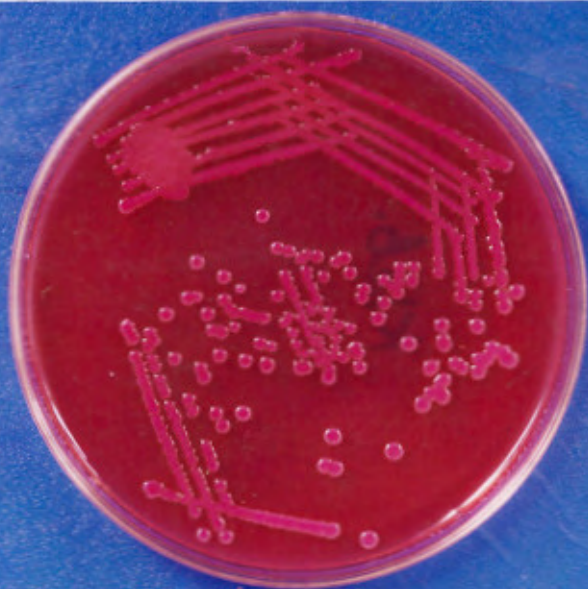
This study was done in 346 pediatric patients, suspected with UTI, and subjected to bacteriological investigation of age two days to 14 years. 149 were female patients and 197 were male children. Table no 10 shows 90 bacteria were isolated from 49 boys and 41 girls infected with UTI. The major isolate 98% percentage was gram negative bacteria where as only 2% was gram positive. Among gram negative bacteria 94% bacteria were the members of family Enterobacteriaceae. *E.coli* (78%) was the primary isolate from both male and female patients. followed by *Klebsiella pneumoniae* (9%), *Pseudomonas aeruginosa* (3%), *Proteus mirabilis* (2%), *Proteus vulgaris* (2%), *Citrobacter spp.* (2%), *Streptococcus faecalis* (2%) and *Enterobacter spp* (1%). Among the isolates *E. coli* was found more prominent in boys 53% than in girls 47%.

Table No. 10 :

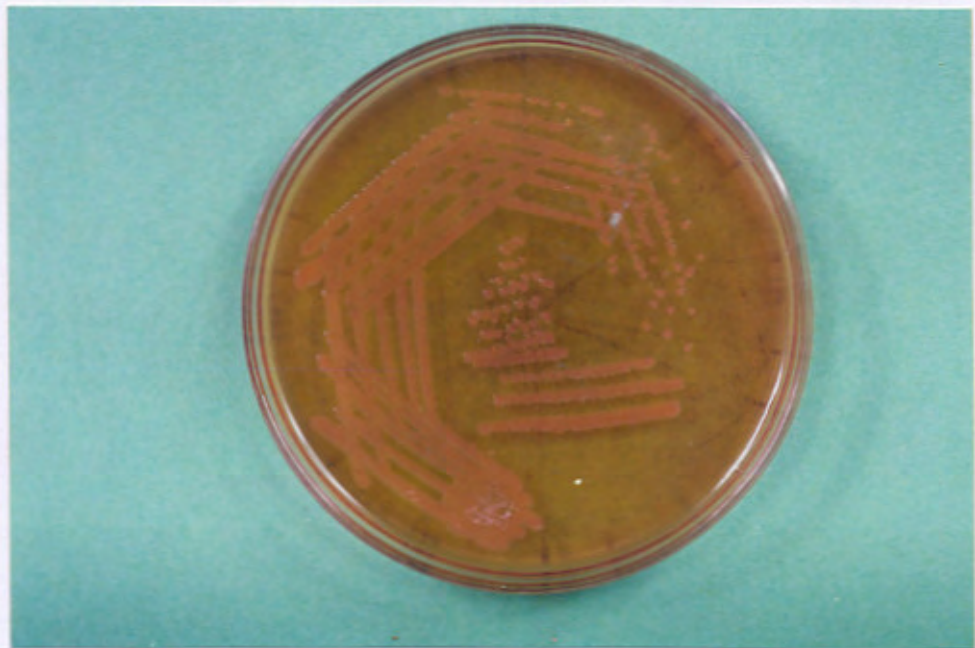
Bacteria	Male	%	Female	%	Total	%
<i>E. coli</i>	36	51	34	47	70	78
<i>Klebsiella</i>	5	62	3	38	8	9
<i>Proteus mirabilis</i>	2	100	0	0	2	2
<i>Proteus vulgaris</i>	1	50	1	50	2	2
<i>Pseudomonas</i>	2	67	1	33	3	3
<i>Citrobacter</i>	1	50	1	50	2	2
<i>Streptococcus faecalis</i> (<i>Enterococci</i>)	1	50	1	50	2	2
<i>Enterobacter</i>	1	100	0	0	1	1
Total	49	25%	41	28%	90	100



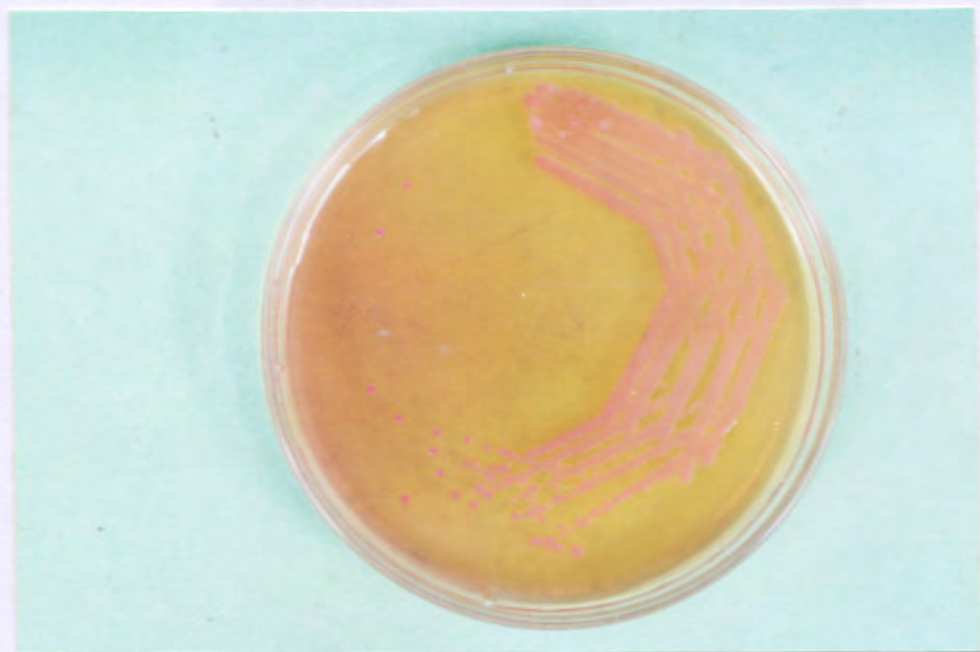
Lactose fermenting colonies of *E.coli* on MacConkey Agar.



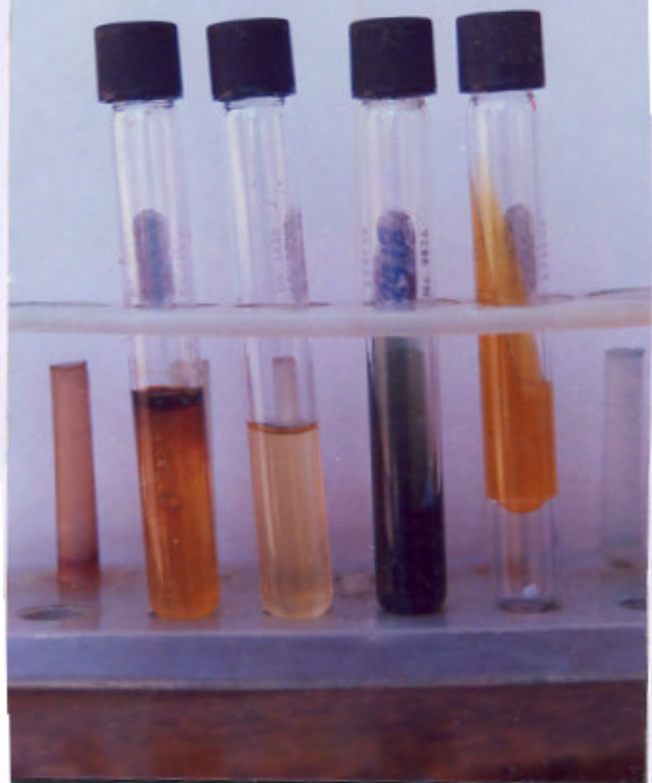
Lactose fermenting, viscous colonies of *Klebsiella* on MacConkey agar.



Non Lactose fermenting colonies of *Proteus* on MacConkey agar.



on Lactose fermenting colonies of *Pseudomonas aeruginosa* on MacConkey agar.



Biochemical tests of *E. coli*

SIM - Sulphur -ve, Indole negative, Motile

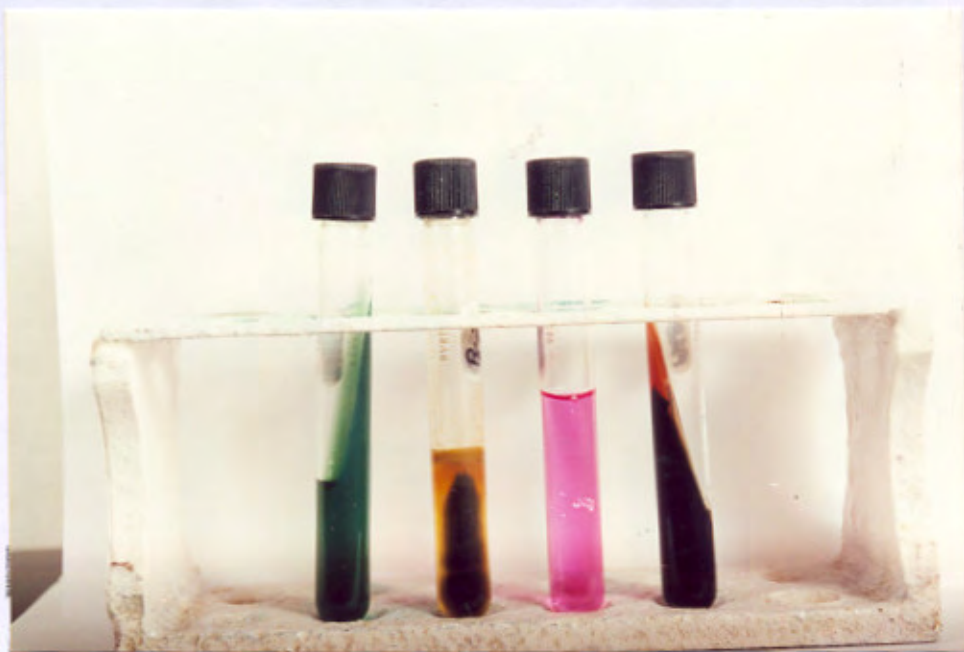
Urease - Negative

Citrate - Not utilizing

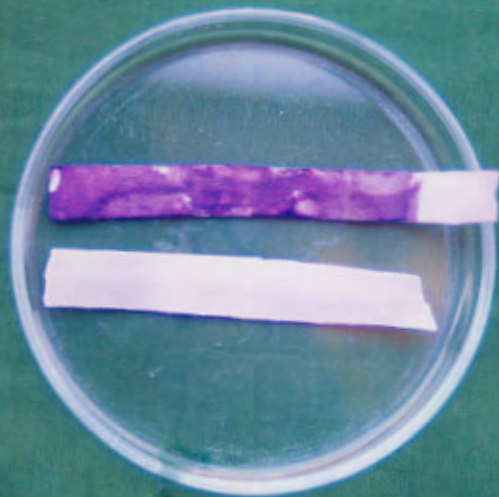
TSI - Acid/Acid, H₂S-ve, Gas +ve



Biochemical Test of *Klebsiella*. Urease positive; TSI: A/A H₂S-ve Gas +ve; Citrate Utilization -ve
 SIM - Sulphur negative, Indole -ve, non - motile



Biochemical tests of *Proteus*. Citrate not utilizing, SIM: Sulphur Positive, motile, indole -ve, urease. Positive



Oxidase positive test of *Pseudomonas aeruginosa*



Pseudomonas showing oxidative in O/F media.



O/F Media showing facultative result.

4.2 PATTERN OF CULTURE RESULTS

Table No. 11 :

S.No	Growth	Number	(%) percentage
1	Positive	90	26
2	Negative	152	44
3	Mixed	38	11
4	Not significant	66	19

The Pattern of growth indicated by table no. 11 and Fig. no. 4 where growth negative was 44% of samples, not significant was 19%, mixed growth was 11% and 26% was growth positive.

Table No. 12

S.No	Isolated Bacteria	Number	(%) percentage
1	Gram - Negative	88	98
2	Gram - Positive	2	2
3	Family of enterobacteriaceae	85	94

Table no. 12 and Fig. no. 5 shows that the majority of the isolated urinary pathogens were gram - negative rods 98% and only 2% were gram - positive. 94% of the isolates are the member of enterobacteriaceae family.

4.3 AGEWISE AND GENDERWISE DISTRIBUTION

4.3.1 Agewise Distribution of UTI

The clinical and laboratory features of urinary tract infections in 346 children aged two days to 14 years were presented. 36% of the patients were below three years of age and positive growth was 33% (male and female). Out of patients between 3 to 6 years of age, sample was 20% and 14% was positive growth. 21% of the patients were between 6 to 9 years age group, 11% was positive growth. In infancy and childhood, male has higher prevalence of UTI 63% than female 37%. Prevalence of UTI was highest 75% in older

Figure No. 4: Pattern of Culture Results.

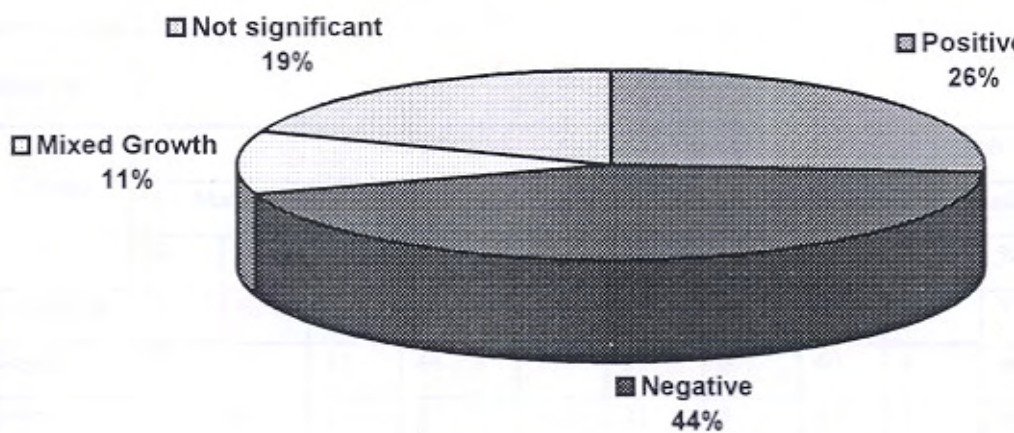
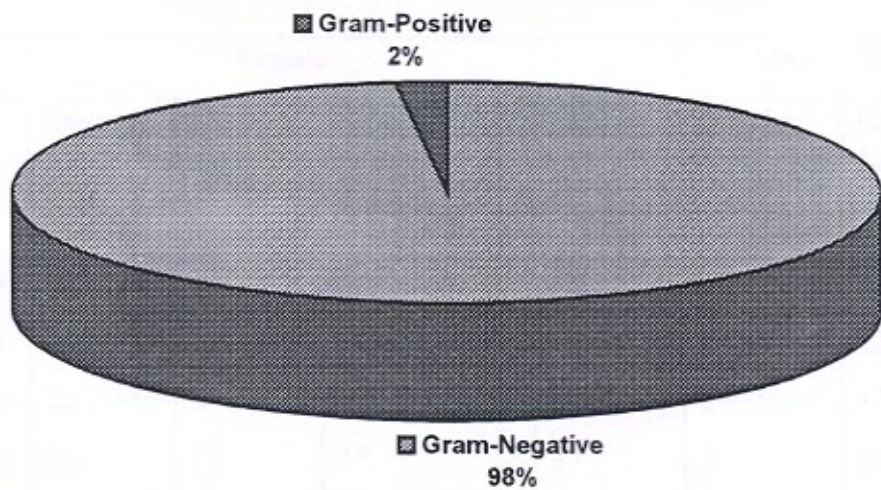


Figure No. 5 : Pattern of Isolated Organisms.



girls of age group 12-14 years, while in boys of same age group the percentage of positive growth was 25%.

Table No. 13 :

Age Groups	Sample						Positive					
	Male		Female		Total		Male		Female		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
2 days-3yrs	78	62	47	38	125	36	26	63	15	37	41	33
3-6yrs	39	56	31	44	70	20	6	60	4	40	10	14
6-9yrs	45	63	26	37	71	21	8	67	4	33	12	17
9-12yrs	27	46	32	54	59	17	8	35	15	65	23	39
12-14yrs	8	38	13	62	21	6	1	25	3	75	4	19
Total	197	57	149	43	346		49	54	41	46	90	26

Table no. 13 shows the age wise variation of significant urinary tract infection. In an average highest percentage of growth positive (39%) cases were obtained from the children of age group 9-12 years.

4.3.2 Genderwise Distribution of UTI in Pediatric Patient

Table No. 14:

Age group	Female			Male		
	Sample	Positive	Rate of Infection per 100 cases	Sample	Positive	Rate of infection per 100 cases
2days-3 yrs	47	15	32	78	26	33.3
3 yrs - 6yrs	31	4	13	39	6	15.3
6 yrs - 9 yrs	26	4	15	45	8	17.7
9 yrs-12 yrs	32	15	47	27	8	29.6
12yrs-14yrs	13	3	23	8	1	12.5
Total	149	41	28	197	49	25

Figure No. 6 : Age-wise Distribution of UTI

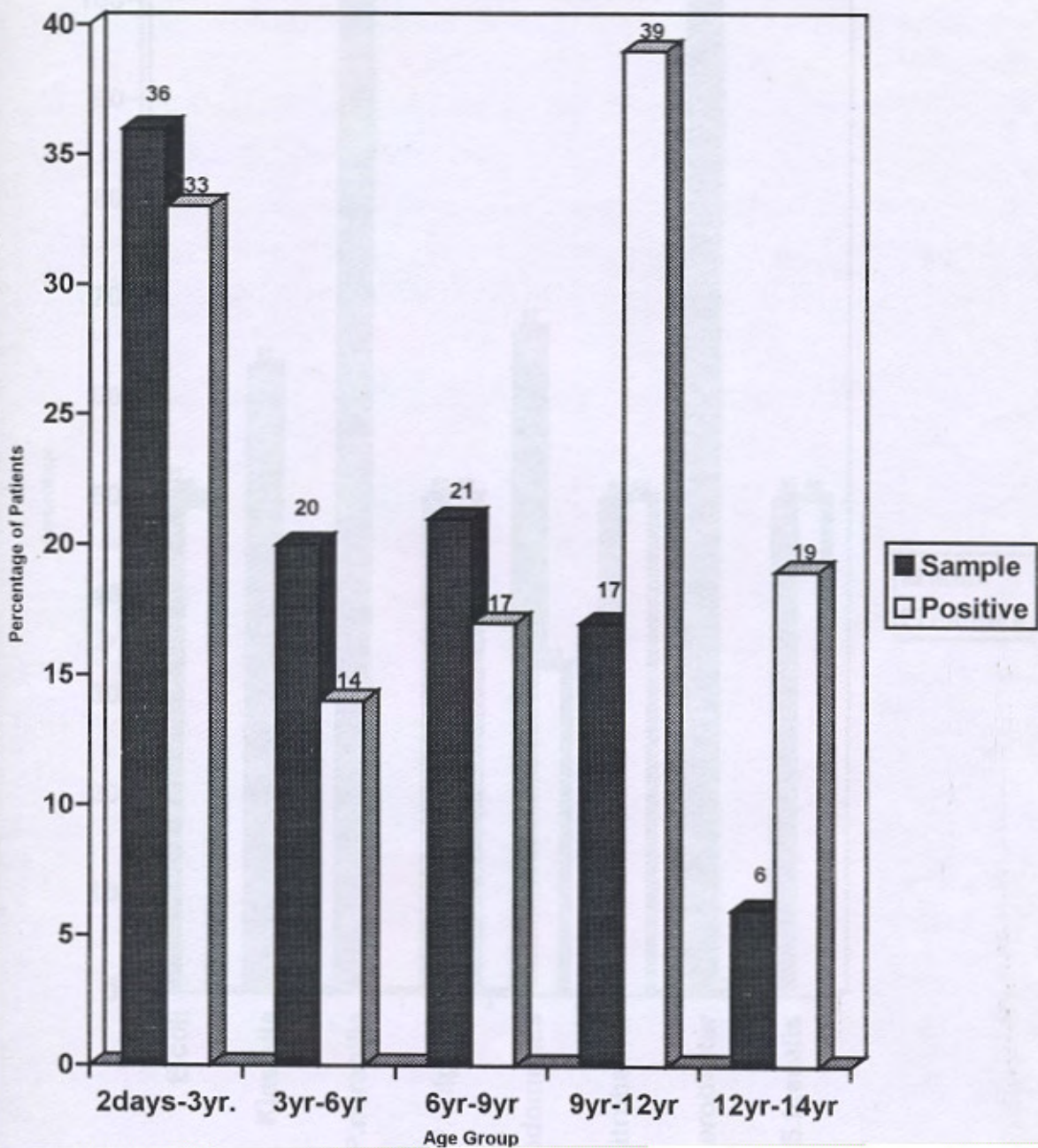


Figure No. 7 : Genderwise Distribution of UTI in Pediatric Patients.

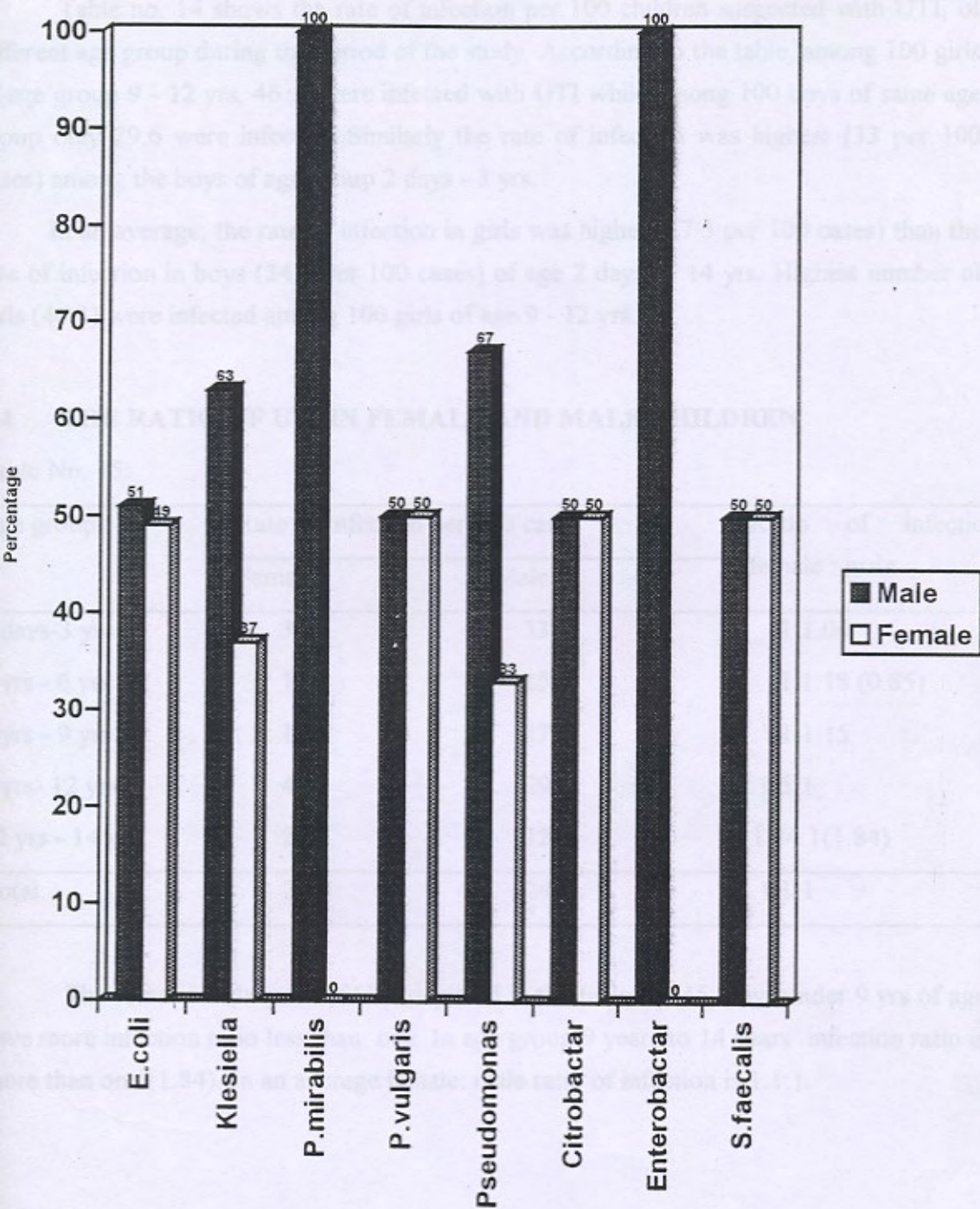


Table no. 14 shows the rate of infection per 100 children suspected with UTI, of different age group during the period of the study. According to the table, among 100 girls of age group 9 - 12 yrs, 46.8 were infected with UTI while among 100 boys of same age group only 29.6 were infected. Similarly the rate of infection was highest (33 per 100 cases) among the boys of age group 2 days - 3 yrs.

In an average, the rate of infection in girls was higher (27.5 per 100 cases) than the rate of infection in boys (24.8 per 100 cases) of age 2 days to 14 yrs. Highest number of girls (46.8) were infected among 100 girls of age 9 - 12 yrs.

4.4 THE RATIO OF UTI IN FEMALE AND MALE CHILDREN

Table No. 15:

Age group	Rate of Infection per 100 cases		Ratio of infection female : male
	Female	Male	
2 days-3 yrs	31.9	33.3	1:1.04
3 yrs - 6 yrs	12.9	15.3	1:1.18 (0.85)
6 yrs - 9 yrs	15.3	17.7	1:1.15
9 yrs- 12 yrs	46.8	29.6	1.5:1
12 yrs - 14 yrs	23	12.5	1.84:1(1.84)
Total	27.5	24.8	1.1:1

The female: male ratio of UTI depicted in the table no. 15, boys under 9 yrs of age have more infection ratio less than one. In age group 9 years to 14 years infection ratio is more than one (1.84). In an average female: male ratio of infection is 1.1:1.

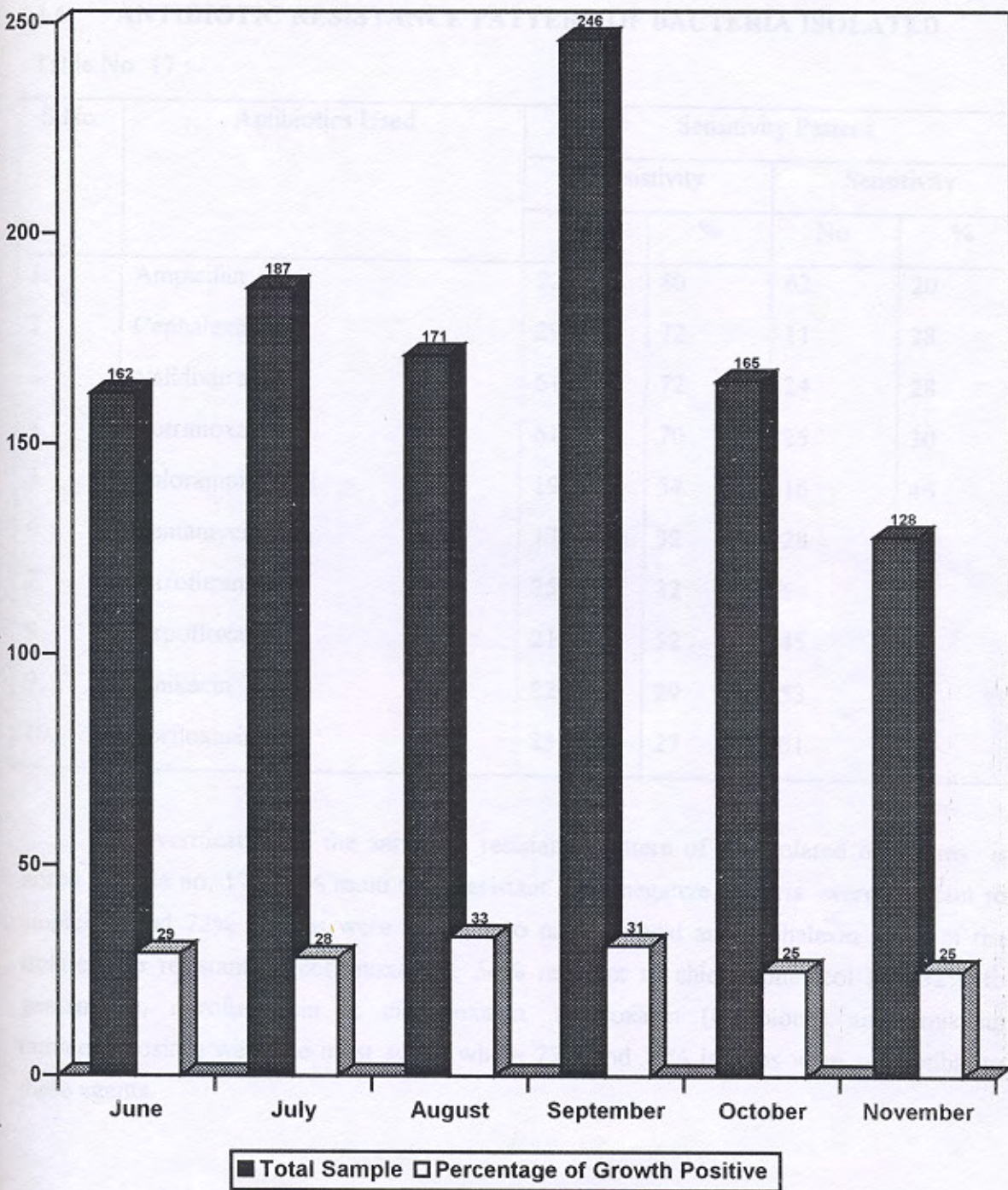
4.5 FREQUENCY OF THE ORGANISMS ISOLATED ACCORDING TO SEASON AND ITS PERCENTAGE

Table No. 16 :

Month	June		July		August		September		October		November	
Total sample	162		187		171		246		165		128	
	No	%	No	%	No	%	No	%	No	%	No	%
<i>E. coli</i>	34	72	40	77	38	67	55	71	34	81	27	84
<i>Proteus</i>	3	6	-	-	3	5	4	5	3	7	1	3
<i>Pseudomonas</i>	1	2	7	13	4	7	10	13	4	10	3	9
<i>Klebsiella</i>	7	15	5	10	8	14	4	5	1	2	1	3
<i>Citrobacter</i>	2	4	-	-	-	-	1	1	-	-	-	-
<i>Enterobacter</i>	-	-	-	-	2	4	3	4	-	-	-	-
<i>Strep. faecalis.</i>	-	-	-	-	1	2	-	-	-	-	-	-
Growth Positive	47	29	52	28	56	33	77	31	42	25	32	25

Table no. 16 and Fig. no. 8 shows the variation of frequency of UTI in six months (from June to November). The percentage of bacteria isolated is highest in the month of August (33%) and the lowest percentage (25%) is in the month of October and November.

Figure No. 8 : Frequency of Organism Isolated.



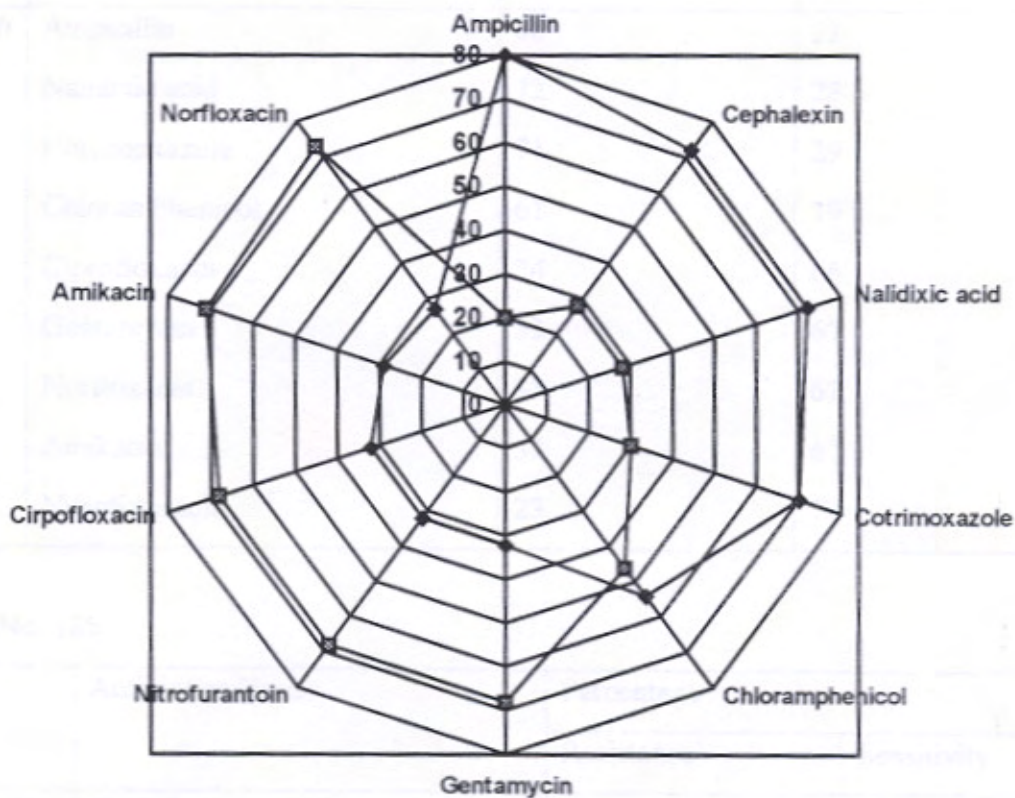
4.6 ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED

Table No. 17 :

S.No.	Antibiotics Used	Sensitivity Pattern			
		Resistivity		Sensitivity	
		No.	%	No.	%
1.	Ampicillin	22	80	62	20
2.	Cephalexin	29	72	11	28
3.	Nalidixic acid	61	72	24	28
4.	Cotrimoxazole	61	70	25	30
5.	Chloramphenicol	19	54	16	46
6.	Gentamycin	13	32	28	68
7.	Nitrofurantoin	25	32	54	68
8.	Ciprofloxacin	21	32	45	68
9.	Amikacin	22	29	53	71
10.	Norfloxacin	23	27	61	73

The verification of the antibiotic resistance pattern of the isolated organisms is noted in table no. 17. 80% multi drug resistant gram negative bacteria were resistant to ampicillin and 72% isolates were resistant to nalidixic acid and cephalexin. 70% of the isolates are resistant to cotrimoxazole, 54% resistant to chloramphenicol and 32% to gentamycin, nitrofurantoin & ciprofloxacin. Norfloxacin (quinolone) and amikacin (aminoglycoside) were the most active where 73% and 71% isolates were susceptible to these agents.

Figure No. 9 : Antibiotic Resistance Pattern of Gram Negative UTI Pathogens.



◆ Resistance ■ Sensitivity

4.7 VERIFICATION OF ANTIBIOTIC SENSITIVITY PATTERN OF DIFFERENT GRAM NEGATIVE UTI PATHOGENS

Table No. 18a

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>E. coli</i>	Ampicillin	88	22
	Nalidixic acid	72	28
	Cotrimoxazole	71	29
	Chloramphenicol	61	39
	Ciprofloxacin	34	66
	Gentamycin	33	67
	Norfloxacin	33	67
	Amikacin	33	67
	Nitrofurantoin	23	77

Table No. 18b

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>Klebsiella spp</i>	Cephalexin	83	17
	Nalidixic acid	62	38
	Gentamycin	40	60
	Nitrofurantoin	33	67
	Chloramphenicol	33	67
	Ciprofloxacin	0	100
	Norfloxacin	0	100
	Amikacin	0	100

Table No. 18c

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>Proteus spp.</i>	Cotrimoxazole	50	50
	Cephalexin	50	50
	Chloramphenicol	50	50
	Ciprofloxacin	50	50
	Gentamycin	0	100
	Amikacin	0	100
	Norfloxacin	0	100

Table No. 18d

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>Pseudomonas spp.</i>	Ciprofloxacin	50	50
	Norfloxacin	25	75
	Tetracyclin	0	100
	Gentamycin	0	100
	Chloramphenicol	0	100
	Amikacin	0	100

Table No. 18e

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>Citrobacter spp.</i>	Chloramphenicol	50	50
	Nalidixic acid	50	50
	Cephalexin	0	100
	Gentamycin	0	100
	Cotrimoxazole	0	100
	Ciprofloxacin	0	100
	Norfloxacin	0	100



Antibiotic sensitivity test of *Proteus vulgaris* on MHA.



ing resistance to Nitrofurantoin, Amikacin, Norfloxacin, Gentamycin, Ciprofloxacin,
Chloramphenicol, Cotrimoxazole, Nalidixic acid.

Table No. 18f

S.No.	Antibiotics Used	Percentage	
		Resistance	Sensitivity
<i>Enterobacter spp.</i>	Cotrimoxazole	100	0
	Chloramphenicol	100	0
	Nalidixic acid	100	0
	Nitrofurantoin	50	50
	Ciprofloxacin	0	100
	Norfloxacin	0	100

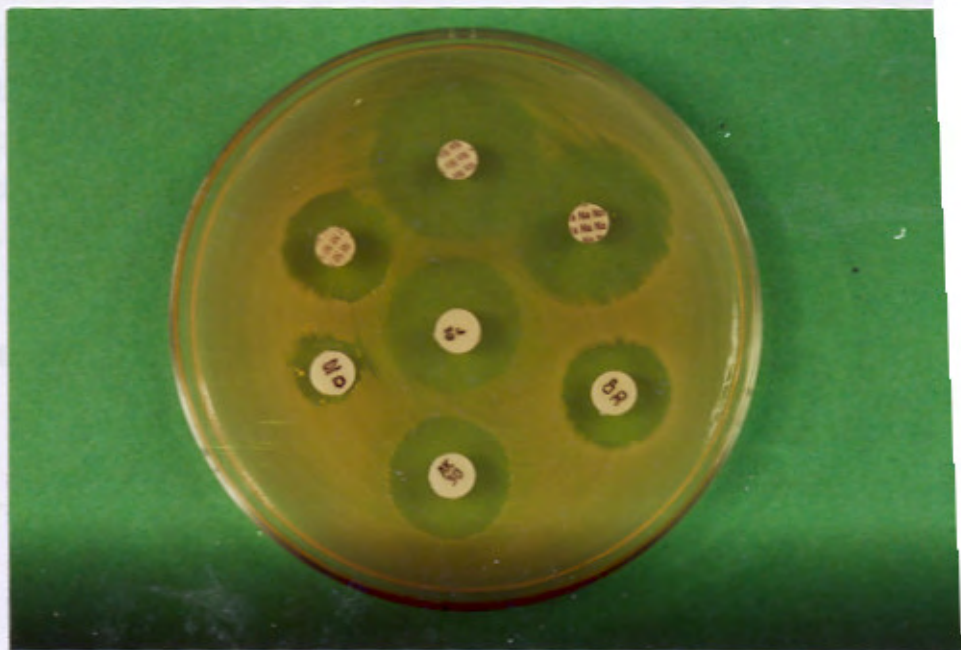
Table no. 18 shows the pattern of antibiotic resistance of different gram negative urinary pathogens. 88% *E. coli* was resistant to ampicillin and 72% to nalidixic acid. Similarly 71% multi drug resistant *E. coli* were resistant to cotrimoxazole and 61% to chloramphenicol. Nitrofurantoin is the most effective nitrofurans; 77% of *E. coli* were sensitive to this agent. Amikacin/gentamycin (aminoglycosides) and norfloxacin were the second most effective, where 67% *E. coli* were susceptible to these agents. Resistance to ciprofloxacin was 34%.

Resistivity of *Klebsiella spp* is documented in table no. 18b, 83% was resistant to cephalexin followed by 62% resistant to nalidixic acid and 40% to gentamycin, 33% to nitrofurantoin & chloramphenicol. All the isolated *Klebsiella spp* was sensitive to ciprofloxacin, norfloxacin and amikacin hence these are most effective antibiotics.

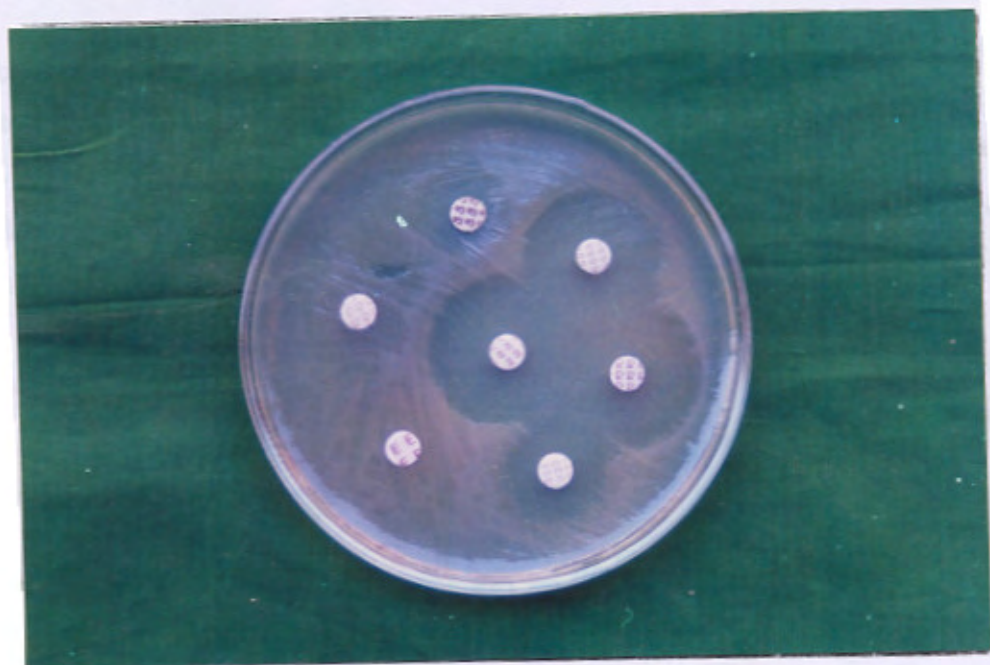
Table No. 18c shows 50% *Proteus spp* were resistant to cotrimoxazole, cephalexin, chloramphenicol and ciprofloxacin. Most effective antibiotics for *Proteus* infection were gentamycin, amikacin and norfloxacin; 100% isolates were susceptible to these agents.

Table No. 18d. shows 50% and 25% *Pseudomonas spp.* was resistant to ciprofloxacin and norfloxacin respectively. All isolated *Pseudomonas spp* was sensitive to tetracyclin, gentamycin, chloramphenicol and amikacin.

Documentation in table no. 18e shows 50% multi drug resistant *Citrobacter spp* was resistant to chloramphenicol and nalidixic acid. All the isolated *Citrobacter spp* was sensitive to cephalexin, gentamycin, cotrimoxazole, ciprofloxacin and norfloxacin.



Antibiotic susceptibility test of *Citrobacter* on MHA.



Antibiotic sensitivity test of *Enterobacter* on MHA.

In table no. 18f all the isolated *Enterobacter spp* was resistant to cotrimoxazole, chloramphenicol & nalidixic acid and 50% was resistant to nitrofurantoin. Ciprofloxacin and norfloxacin (quinolones) are the drug of choice for *Enterobacter* infection, Since 100% isolates were sensitive to these agents.

4.8 CENT PERCENT RESISTANT GRAM NEGATIVE BACTERIA CAUSING UTI

Table No. 19 : Cent percent Antibiotic Resistant Gram Negative Bacteria.

S.No	Bacteria	Total no.	Cent percent resistant number	Percentage
1	<i>E. coli</i>	70	4	6%
2	<i>Pseudomonas aeruginosa</i>	3	1	33%
	Total Isolates	88	5	6%

Among 70 isolated *E. coli*, 6% isolates were cent percent resistance to antibiotics used at the lab. The isolates were resistant to nitrofurantoin, amikacin, norfloxacin, gentamycin, ciprofloxacin, chloromphenicol, cotrimoxazole, nalidixic acid and ampicillin.

Table no. 19 shows, 1% isolate of *Pseudomonas aeruginosa* was found cent percent resistant to antibiotics used at the lab, which were amikacin, chloramphenicol, gentamycin, tetracycline, norfloxacin, ciprofloxacin, nalidixic acid and nitrofurantoin.

41% of the isolates were multi drug resistant (resistant to more than four antibiotics). In an average 6% of gram negative bacteria were cent percent resistant against the antibiotics used at the lab. The verification of resistance pattern of these bacteria are tabulated in table no. 20 and 21.

4.9 ANTIBIOTIC RESISTANCE PATTERN OF CENT PERCENT RESISTANT ISOLATES

Table No. 20 :

S.No	Code No.	Bacteria	Antibiotics								
			Nf	Ak	Nx	G	Cf	C	Co	Na	Am
1	E ₃₅₂	<i>E. coli</i>	R	R	R	R	R	R	R	R	R
2	E ₂₀₃₆	<i>E. coli</i>	R	R	R	R	R	R	R	R	R
3	E ₂₂₆₈	<i>E. coli</i>	R	R	R	R	R	R	R	R	R
4	E ₂₃₃₅	<i>E. coli</i>	R	R	R	R	R	R	R	R	R

Table No. 21:

S.No.	Code No.	Bacteria	Antibiotics							
			Ak	C	G	T	Nx	Cf	Na	Nf
1	P ₂₀₄₂	<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	R	R	R

Here,

Nf = Nitrofurantoin

Ak = Amikacin

Nx = Norfloxacin

G = Gentamycin

Cf = Ciprofloxacin

C = Chloramphenicol

Co = Cotrimoxazole

T = Tetracycline

Na = Nalidixic acid

Am = Ampicillin

CHAPTER - FIVE

DISCUSSION

According to the annual report of "Department of Health Services, Kathmandu" (1996/1997), in Nepal the morbidity rate by urinary tract infection is 0.42% of total population and urine sample appears as the second most common sample (18%) to be submitted to the laboratory after blood (48%) for examination.

The diagnosis and management of urinary tract infection in infants and children are usually routine and outcome is generally good. Although the bladder and upper urinary tract are usually sterile, urine obtained per urethra can easily be contaminated especially in infants and young children by bacteria that colonize the urethral meatus, periurethral sites (eg. prepuce of uncircumcised males) and perineum. Collection of sterile urine from young patient poses a potential problem if a plastic receptacle is used. Percutaneous suprapubic bladder aspiration or bladder catheterization is usually performed only in very young infants who require immediate antimicrobial therapy or in those in whom sterile collection of urine by bag technique is thought to be unlikely.

Management of pediatric patients with UTI is confounded by inadequate follow up to establish the structural integrity of the urinary system by radiographic techniques, to document recurrence of infection and to prevent renal damage. The ultimate goal of managing children with UTI occurs most commonly in adolescence or young adult life. The pediatricians mistakenly believe that the long-term therapy is uniformly excellent and that rigorous attention to detail of diagnosis and follow up is unnecessary.

Bacteria in infancy and early childhood, with or without symptoms, may lead to the development of renal scarring and vesicoureteral reflux (Hodson and Kincaid Smith, 1979). Failure to recognize unexplained episodes of fever and delay in effective treatment are major factors leading to renal damage. Most renal scars are developed by late childhood (Winberg *et al.*, 1974; Smellic and Orskoy *et al.*, 1976). Pyelonephritis of infective etiology is still one of the commonest cause of end stage renal failure requiring dialysis or transplantation (Leigh, 1990).

This study was carried out in six months period on 346 pediatric patients at Kanti Children's Hospital, Kathmandu. Their age ranged from two days to 14 years with suspected UTI.

In the earlier chapter (Chapter - V) the different data of various microbiological and antibiotic sensitivity analysis are tabulated.

In this study among the causative bacteria *E. coli* was the commonest which is 78%. Table no 10 shows, after *E. coli*, *Klebsiella* was second most important causative organism which was 9% followed by *Proteus* (4%), *Pseudomans* (3%), *Citrobacter* (2%), *Streptococcus faecalis* (2%), *Enterobacter* (1%).

Such a higher percentage of *E. coli* was found on previous study done by Gautam *et al.* (1998); Achari 1989(India); Manandhar *et al.*, 1996; Ghimire *et al.*, 1995; Tuladhar *et al.*, 1989; Jha and Yadav 1992; Dolan *et al.*, 1989 (USA); Ayhan 1988 (Turkey); Alos and Chacon 1988 (Spain); Kuchen and Stalder, 1980 (Switzerland); Ceddia *et al.*, 1979 (Italy). *Escherihia coli* is the most frequent etiological agent of acute uncomplicated UTI in infants and children, accounting for 85 to 90% of all pathogens recovered from urine cultures (McCracken *et al.*, 1989).

E. coli binds to glycolipid and glycoprotein receptors on the surface of uroepithelial cells via various adhesins especially the genetically related p and F adhesins (Antelo *et al.*, 2000). *E. coli* can bind to the glyconjugate receptor (Gal $\alpha_1 \rightarrow 4$ Gal) of the epithelial cells of human urinary tract (Block *et al.*, 1985), so it can initiate infection itself to colonize the urethral meatus (Schaeffer and Chmiel, 1983).

Thus ability of *E. coli* encourage being the predominant organism in UTI in both sexes. The second commonest organism *Klebsiella spp* was more in boys (63%) compared to girls (38%). Our study resemble to the study done by Dhakal *et al.*, 1999 ; Manandhar *et al.*, 1996; Gautam *et al.*, 1998; Sharma 1982. In our study, *Kleb. pneumoniae* was isolated although *Kleb. oxytoka* may cause bacteriuria in children. During the study 2% infection was encountered by *Proteus mirabilis* and all the isolation was from boys. Another 2% of isolate were *Proteus vulgaris*. Gautam *et al.* (1998) isolated 10% *Proteus sps* from 205 UTI suspected children The production of urease form *Proteus spp* help in invading the renal pelvis and its ability to split urea release alkali which damage the urinary epithelium can ease renal abnormalities particularly calculi and in hospital patients it may cause urinary tract infection in association with obstruction or use of instrument (Leigh, 1990).

Pseudomonas aeruginosa play important role as the primary pathogen in compromised hosts (Dolan *et al.*, 1989; Fowler *et al.*, 1984) and hospitalized patients (Lohr *et al.*, 1989; Coronado *et al.*, 1995); in complicated urinary tract infection (Kosakai *et al.*, 1990; Ogata *et al.*, 1989) and in the bladder carcinogenesis (Kaji *et al.*, 1994). In our study, *Pseudomonas aeruginosa* and *Citrobacter spp* accounted for 3% and 2% respectively. Species of *Citrobacter* have been isolated from human urine and a variety of serious infections, especially those of infants and immunocompromised patients. (Crichton P B *et al.*, 1996)

During study period 2% *Streptococcus faecalis* was isolated. *Streptococcus faecalis* is more frequent in complicated urinary tract infections (Leigh, 1990)

In this study only 1% of *Enterobacter spp.* was isolated. McCracken *et al.*, (1989) states, after *E. coli* other etiological agents include *Streptococcus*, *Klebsiella*, *Enterobacter species* and occasionally *Enterococci*. *Staphylococcus saprophyticus* can cause UTI in adolescent girls. Chronic infections of UTI that occur in those who are receiving antimicrobial prophylaxis are frequently caused by *Enterococci*, *Proteus species*, *Pseudomonas aeruginosa* or *Candida species* (McCracken G *et al.*, 1989).

However in our study no *Staphylococci sps* was isolated, Gautam *et al.*, 1988 isolated about 2% *Staphylococcus aureus* from 205 patients attending at Kanti Children's Hospital. Our isolation correlate with the study done by Sharma *et al.*, (1983) on "Urinary infection" in 100 children patients of age group of four days to 14 years at Kanti Children's Hospital. *E. coli* was predominant (98%) followed by *Klebsiella* (19%), *Proteus* (16%) *Streptococcus faecalis* (13%), *Citrobacter* (4%). Davis Dele, Jones Ford *et al.* (1992) isolated *E. coli* (26%) as the most common etiological agent followed by *Enterococcus species* (10%) and coagulase negative staphylococci (9%). The study was performed in Canada about the "Nosocomial urinary tract infection" at a pediatric hospital. Adeyemo, Onyemenem and Ekwoezer *et al.*, 1994 isolated *Klebsiella sps* (52.8%) as the commonest etiological agent from 65 Nigerian children.

Table no 11 shows the pattern of culture results where 26 percentage of samples showed the positive growth and 49 percentage was negative growth. Such low rate of growth positive was observed by Gautam *et al.*, 1998; Manandhar *et al.*, 1995; Levett (1993); Dhakal *et al.*, 1999; Obi *et al.*, 1996. The strong reason of low growth rate could be the prior use of antibiotics.

According to a study done by Kaji, 1994 in Japan suggest that urinary tract infection caused by bacteria possessing nitrate reducing ability could be a causative factor in the development of bladder cancer in terms of the formation of presumably novel direct acting mutagen or carcinogen.

Table no. 12 shows that gram negative bacteria cause 98% of UTI in pediatric patients while gram positive bacteria cause only 2% of UTI. 94% of the isolated gram negative bacteria was the member of family Enterobacteriaceae only 3% was other gram negative bacteria. In case of adult use of spermicide could also be contributory factor for enhancement and encourage the growth of Enterobacterial organism. Use of diaphragms for contraception of the vagina often invaded by Enterobacterial organism rather than the normal flora.

UTI is more frequent in girls than in boys, however in infancy and childhood it is more prone to boys. In table no 13, boys below 9 years of age have faced more incidence of UTI (63 to 67 per 100 cases) than girls. In older girls above 9 Years of age UTI was more frequent (65 to 75 per 100 cases). The higher prevalence of UTI in girls suggests that shorter urethra of girls facilitates the ascendant via of infection. Such a high rates of infection in girls was documented by Gautam *et al.*, 1998; Nelson J, McCracken G *et al.*, 1984; Antelo *et al.*, 2000; Kosakai *et al.*, 1990. Fowler *et al.*, (1984) states that the prevalence of infection in females correlates directly with age. While toilet trained older girls may be more likely to get a UTI if they wipe themselves from back to front because this can expose the vaginal and meatal area to bacteria which colonize the rectum most commonly *Enterobacteriaceae*, *Pseudomonas* and rarely *Staph. aureus*. The acid vaginal pH is an important fact to the lack of colonization in female (Antelo *et al.*, 2000).

Table no. 13 shows, out of total samples 149 samples were from female and 197 were from males. UTI is decidedly more common in girls than in boys because of the favorable anatomical factors that render female urinary tract (is) more susceptible to ascending infection. Presence of congested anomalies takes bladder neck obstruction, neurogenic bladder or urethral valves predisposes to recurrent UTI. Infection or skin may also act as a focus for hematogenous spread of bacteria to the urinary tract.

Table no. 14 shows that the rate of UTI was higher in girls (27.5 per 100 cases) than boys (24.8 per 100 cases) which reflects that infection was more common in female than male. In childhood, UTI is more frequent in girls (from 4 girls: 1 boy to 20 girls: 1 boy) but in the first year of life it is more frequent in male (Antelo *et al.*, 2000).

We documented that female: male ratio was 1.1:1 in children (table no. 15). Other investigators observed such high ratio of UTI in girls. McCracken *et al* (1989) estimated that approximately 3% of Swedish girls and 1% of Swedish boys have symptomatic UTI before the age of 11 years.

In our study in first few years of life, the incidence of UTI was more in boys than in girls (table no. 14). Similar observation was documented at Parkland memorial Hospital in Dallas, that the incidence rate of UTI in infants 6 months of age and younger was 1.65 cases/1000 live births in 1972 to 1975 and 2.04 cases/1000 live births in 1977 to 1980. (Nelson J *et al.*, 1984). The male: female ratio were 2.7:1 and 5:1, in the two time periods (Nelson J *et al.*, 1984; Ginsburg CM *et al.*, 1982)

Ginsburg *et al.*, 1982 discussed about the possible factors related to the predominance of male infants with urinary tract infection. Uncircumcised boys are more likely to get an infection than circumcised boys because bacteria survive in warm, moist areas like the area under the foreskin. While in colonization of glans and foreskin *E.coli* the receptor density is increased (Antelo *et al.*, 2000).

In developing country like Nepal the trend of antibiotic resistance is increasing especially by the overuse and misuse of antibiotics. Thus in vitro sensitivity tests are done to determine the degree of resistivity or sensitivity of the pathogens to antibiotics. However it should be always considered that the response to therapy in vivo might not always reflect the result of testing the sensitivity of the pathogen in vitro.

Bacterial resistance to antimicrobial agents is a major health problem in many tropical countries (Farrar *et al.*, 1985; Amyes *et al.*, 1992). There are several reasons for the development of resistance of bacteria to antimicrobial agents. A number of reports are there that the resistant bacteria produce antibiotic destroying enzymes. Hamilton Miller and Smith (1979) had extensively studied and reviewed the types of β -lactamases produced by bacteria that destroy the β -lactam antibiotics.

The exchange of genetic material of drug resistance by natural transformation system has a limited hold mostly from gram negative bacteria (Hugo & Russel, 1995). The selection of most appropriate antibiotic is dependent on the susceptibility of the pathogen.

In our study (table no. 17), norfloaxain was the most effective antibiotic against isolated gram negative bacteria. Extensive studies with norfloxacin have demonstrated that its broad spectrum, high urine concentration and oral administration make it a drug with a

promising future in the treatment of urinary tract infections (Hugo & Russel, 1995). Nalidixic acid has been used for several years as a clinically important drug in the treatment of urinary tract infections (Hugo & Russel, 1995). Ciprofloxacin may be considered as first line treatment for a number of infections in which gram negative pathogens are proven or strongly suspected including complicated urinary infections, bacterial prostatitis, cystic fibrosis (Thomas J. Louie, 1994). Most of the isolates of *E coli*, *Klebsiella* and *Enterobacter spp* (Table no. 18a, 18b and 18f) are sensitive to nitrofurantoin, ciprofloxacin and norfloxacin.

Norfloxacin was the most sensitive drug against UTI gram negative pathogen followed by amikacin. Nitrofurantoin, ciprofloxacin and gentamycin were 68% sensitive antibiotics against the isolates (table no 17). In our study nitrofurantoin has good coverage against *E coli*, *Kleb. spp* and *Enterobacter spp*. Similar observation was documented by Gautam *et al.*, 1998; Dhakal *et al.*, 1999; Manandhar *et al.*, 1996; Jha and Yadav *et al.*, 1992; Levett *et al.*, 1993; Antelo *et al.*, 2000, and report of pediatric database (1994). Nitrofurantoin should be considered as drug of choice for acute uncomplicated UTI particularly in view that it continues to show such low in vitro resistance (Obi *et al.*, 1996; Spencer *et al.*, 1994). Maximum bactericidal activity of nitrofurantoin occurs in acidic urine. Large concentrations occur in urine after oral administration. On the basis of clinical experience it appears that nitrofurantoin is a safe and effective antibiotic for prophylaxis in infants and children with recurrent UTI (McCracken G *et al.*, 1989). Nitrofurantoin and nalidixic acid are tested only against isolates from the urinary tract (Collee *et al.*, 1989; Block *et al.*, 1986). Nitrofurantoin is as effective as cotrimoxazole (Johnson, 1990).

Ampicillin, cotrimoxazole, norfloxacin, and nalidixic acid have been the most extensively evaluated drugs with the majority of studies and Schultz *et al.*, 1984 suggests that cotrimoxazole is preferable. We documented the high resistivity of cotrimoxazole against *E coli* (71%), *Proteus spp* (50%) and *Enterobacter spp* (100%).

Bemer Melchier *et al.* (1995) observed high level of penicillinase producer *E coli* (92.1%) from clinical samples. We observed high resistance of *E coli* to nalidixic acid (72%) which correlate with the result reported by Dhakal *et al.*, 1999, Amatya *et al.*, 1998.

Highest percentage of *E coli* (88%) was resistant of ampicillin followed by nalidixic acid (72%), cotrimoxazole (71%) and chloramphenicol (61%) Gautam *et al.* (1998) observed 88% and 67% of *E coli* was resistant to ampicillin and cotrimoxazole respectively followed by to nalidixic acid (30%) and chloramphenicol (36%). Dhakal *et al.*, 1999 observed 88.8%,

66.6% and 55.5% *E coli* were resistant to ampicillin, cotrimoxazole and nalidixic acid respectively. Such higher percentage of resistance to ampicillin/amoxicillin, and cotrimoxazole was observed by Amatya *et al.* (1998) and Ghimire *et al.* (1995). Ciprofloxacin showed high sensitivity (34%) to *E coli*.

Table no 18b shows highest (83%) percentage of *Klebsiella spp* was resistant to cephalixin, to nalidixic acid (62%) and to gentamycin (40%) Gautam *et al.*, (1998) observed 57% . *spp* was resistant to cephalixin. Dhakal *et al.* (1998) observed 66.6% *Kleb. pneumoniae* and 100% *Kleb. oxytoca* was resistant to nalidixic acid. Norfloxacin, ciprofloxacin and amikacin were most sensitive antibiotics to *Klebsiella spp*.

Klebsiella sps isolated (in table 18b) from children were highly resistant to cephalixin (83%) and nalidixic acid (62%). This result is similar to Gautam *et al.*, (1998) and Dhakal *et al.*, (1999) isolated higher resistance to nalidixic acid. Amikacin, norfloxacin and ciprofloxacin were the most sensitive antibiotics for *Klebsiella spp*. Such high sensitivity of *Klebsiella spp* to flouroquinolones were observed by Gautam *et al.*, (1998) and Dhakal *et al.*, (1999). Gentamycin, nitrofurantoin and chloramphenicol are moderately sensitive to *Klebsiella* infection.

Norfloxacin appears to be effective drug with excellent safety profile for the treatment of complicated, recurrent (Schaeffer 1993) and lower uncomplicated UTIs; Miano *et al.*, (1990). 61.4% of *Peud. aeruginosa* isolated by Mroz *et al.*, 1993 in Poland were susceptible to norfloxacin. In this study, norfloxacin has been recommended as highly effective antimicrobial agent for UTI. Chattopadhyay Smandal 1993, Esko and Renkonen 1985, Kaomoka *et al.*, 1986. Dhakal *et al.* (1999) documented the high resistivity of norfloxacin (57.89%) against UTI isolates.

In our study 100% *Proteus spp* was sensitive to norfloxacin amikacin and gentamycin. Poudel *et al.*, 1999 isolated 100% *Proteus sps* sensitive to norfloxacin. In contrast our study, Poudel documented 100% *Proteus spp* were sensitive to chloramphenicol from poultry culture.

50% *Citrobacter spp* were resistant to chloramphenicol and nalidixic acid. All the isolated *Citrobacter sps.* were sensitive to norfloxacin, ciprofloxacin, cotrimoxazole, gentamycin and cephalixin. In contrast to our result Poudel *et al.*, 1999 documented 100% sensitive *Citrobacter sps* were to chloramphenicol and nalidixic acid. Ciprofloxacin and norfloxacin are the most effective antibiotics against *Enterobacter spp*.

6.1 SUMMARY

The followings were the findings of this study:

1. Ninety positive cases obtained from total 346 urine samples of children; there were 98% (88 out of 346) gram negative bacteria and 2% (2 out of 346) gram positive bacteria. Altogether there were 8 different types of bacteria isolated.
2. Among gram negative bacteria *E.coli* was most predominant isolate (78%) followed by *Klebsiella pneumoniae* 9%, *Pseudomonas aeruginosa* (3%), *Proteus mirabilis* (2%), *Proteus vulgaris* (2%), *Citrobacter spp* (2%) and *Enterobacter spp* (1%).
3. In childhood UTI was more frequent in girls (1.1 girl : 1 boy) than boys, but in infancy and childhood (2 days - 3 years age group) it was more frequent in boys (63%) than girls (37%).
4. The antibiotic sensitivity profile of gram negative bacteria showed that 41 % of the isolates were multi drug resistant and 6% were cent percent resistant to the antibiotics used in the laboratory.
5. Eighty percentage of the organisms were resistant to ampicillin and seventy two percentage resistant to cephalixin and nalidixic acid. Seventy percentage of the isolates were resistant to cotrimoxazole and fifty four percentage resistant to chloramphenicol. Ciprofloxacin, gentamycin and nitrofurantoin were 32% resistant against gram negative isolates. Norfloxacin offered highest sensitivity (73%) against UTI pathogens followed by amikacin 71%.
6. Nitrofurantoin was the drug of choice for *E.coli* infection since it was most effective (77% sensitive) against *E coli*. Second most effective antibiotics were amikacin, gentamycin and norfloxacin which were 67% sensitive against *E. coli*. 88% *E coli* were resistant to ampicillin, 72% resistant to nalidixic acid, 71% resistant to cotrimoxazole, 61% to chloramphenicol and 34% to ciprofloxacin.
7. Most resistant antibiotic against *Klebsiella spp* was cephalixin 83% followed by nalidixic acid 62%, gentamycin 40% and nitrofurantoin & chloramphenicol 33%. The drug of choice for *Klebsiella* infection were amikacin, ciprofloxacin and norfloxacin.
8. 50% *Proteus sps* were resistant to cotrimoxazole, cephalixin, chloramphenicol & ciprofloxacin.

9. 50% *Pseudomonas spp* was resistant to ciprofloxacin, followed by norfloxacin 25%. Tetracyclin, gentamycin, chloramphenicol and amikacin were most effective antibiotics against *Pseudomonas* infection.
10. 50% *Citrobacter spp* was resistant to chloramphenicol and nalidixic acid.
11. All the isolated *Enterobacter spp* were resistant to cotrimoxazole, chloramphenicol & nalidixic acid. 50% of it was resistant to nitrofurantoin.

6.2 RECOMMENDATIONS

1. *E.coli* was the most commonly encountered organism in our study. Thus this pathogen should be borne in mind when antibiotic treatment is considered necessary for UTI in children.
2. This type of study should be conducted in other part of country such as Terai, Mountain and Himalaya region of Nepal to get information about geographical variation of organisms causing UTI.
3. It would be much better if extensive research could be carried out on mechanism of antibiotic resistance in gram negative pathogens and antimicrobial management of UTI in children.
4. The main reason for the low standard of health of people is lack of public awareness on health matters. So health education programmer should be set up to upgrade the health standards of the majority of rural population. Basic primary health services should be extended in rural areas to provide opportunity to the rural people to enable them to obtain benefits to modern medical facilities.
5. Awareness should be generated among health workers and public that the misuse and overuse of antibiotics increases resistance problem.
5. Before starting the antibiotic therapy in pediatric patients, the toxicity of the particular antibiotic should be in mind with the degree of necessity of that antibiotic.

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Appendix - I
Questionnaire Model of the Patient

1. Clinical Profile :

Sample No. : Lab No. :
 Patients Name : Age: Sex:
 I.P. No. : OPD No. :
 Ward : Bed No. : Cabin No.:
 Address:

Brief Clinical History :

Any underlying condition

Provisional Diagnosis

Investigation requested
 Type of specimen
 Method of collection

Date of collection :
 Time of collection :

1. Microbiological Profile

Colour:

Appearance : i) Clear ii) Cloudy

Microscopic Examination

Microscopic Examination of specimen from centrifuged urine

- i. White cells (Pus cells)
- ii. Red cells (RBC)
- iii. Yeast cells
- iv. Casts
- v. Crystals
- vi. Epithelial cells
- vii. Other if any

2. Culture of Specimen

Day 1

Culture media	No. of colonies	Nature of colony
Blood agar (BA)		
MacConkey agar (MA)		

Day 2

Biochemical Tests

S.No.	Test employed	Result
a	Catalase	
b	Oxidase	
c	O/F	
d	TSI	
e	SIM	
f	Citrate utilization	
g	MR/VP	
h	Urease	
i	Decarboxylase test	
j	Coagulase test	
k	Litmus milk reduction test	
l	Others	

3. Serotyping if any:

4. Organism isolated :

5. Antibiotic Sensitivity profile

Antibiotic	Zone of inhibition (ZOI)	Remarks

Appendix - II
List of the equipment used during the study.

- | | | |
|-----|--------------------|--------------------|
| 1. | Autoclave | Japan |
| 2. | Centrifuge | R4C Bombay - India |
| 3. | Distillation plant | Kayagaki - Japan |
| 4. | Freeze | Sanyo - Japan |
| 5. | Incubator | Sanyo - Japan |
| 6. | Laminar flow | Japan |
| 7. | Microscope | Olympus - Japan |
| 8. | Oven | FC-410 - Japan |
| 9. | Shaken heater | Thermonus - India |
| 10. | Water bath' | Chyomp 300 - Japan |
| 11. | Ehghing balance | Horida - Japan |
| 12. | pH meter | Japan |

Appendix - III

A. COMPOSITION AND PREPARATION OF DIFFERENT TYPES OF CULTURE MEDIA ARE GIVEN BELOW:

1. Nutrient agar (Hi-media)

Ingredients	gm/litre
Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	15
Final pH (at 25°C)	7.4±0.2

14 grams powder was suspended in 500 ml distilled water and then boiled to dissolve completely then the medium was sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

2. Nutrient Broth (Hi-media)

Ingredients	gm/litre
Peptone	5.0
Sodium chloride	5.0
Yeast extract	15
Beef extract	1.5
Final pH (at 25°C)	7.4±0.2

13 grams nutrient broth powder was dissolved in 1000 ml distilled water and autoclaved at 121°C (15 lbs pressure) for 15 minutes.

3. MacConkey agar (Hi-media)

Ingredients	gm/litre
Peptone	20.0
Lactose	10.0
Bile salt	5.0
Sodium taurocholate	5.0
Neutral red	0.04
Agar	20
Final pH (at 25°C)	7.4±0.2

26 grams MacConkey agar powder was suspended in 500 ml distilled water then boiled to dissolve completely and the medium was sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

4. Blood Agar (Hi-media)

Blood agar base + 5% blood

Ingredients	gm/litre
Peptone	15.0
Liver digest	2.5
Yeast extract	5.0
Sodium chloride	5.0
Agar	5.0
Final pH (at 25°C)	7.4±0.2

40.5 grams powder was suspended in 1000 litre distilled water and boiled to dissolved the medium then it was sterilized by autoclaving at 121°C for 15 minutes. After cooling to 40°C- 50°C, 5-10% blood was added aseptically and mixed well before pouring.

B. ANTIBIOTIC SENSITIVITY TESTING MEDIUM

5. Mueller Hinton Agar (Hi-Media)

Ingredients	gm/litre
Beef, infusion form	300.0
Casein Acid hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25°C)	7.4±0.2

19 grams powder was suspended in 500 ml distilled water and the medium was boiled to dissolve completely then autoclaved at 121°C (15 lbs pressure) for 15 minutes.

6. Sulfide Indole Motility (SIM) (Oxoid)

Ingredients	gm/litre
Beef extract	3.0
Peptic digest of animal tissue	30.0
Peptonized iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0
Final pH (at 25°C)	7.3±0.2

36 grams was suspended in 1000 ml distilled water and dissolved completely. Then 5 ml of the medium was distributed in each tubes and sterilized at 121°C (5 lbs pressure) for 15 minutes.

7. Simmon Citrate Agar (Hi-Media)

Ingredients	gm/litre
Magnesium sulphate	0.2
Ammonium dehydrogen phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Agar	15.0
Bromothymol blue	0.08
Final pH (at 25°C)	6.8±0.2

24.2 gram was dissolved in 1000 ml distilled water and boiled to dissolve the medium completely and it was distributed about 3 ml in each test tubes and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

8. Triple Sugar Iron Agar (TSI) (Hi-Media)

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0

Saccharos	10.0
Dextrose	1.0
Ferrous sulphate	0.2
Sodium Chloride (NaCl)	5.0
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
pH (at 25°C)	7.4±0.2

65 grams of powder was dissolved in 1000 ml distilled water and boiled to dissolve the medium completely then sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of length.

9. Urea Broth (Hi-Media)

0.9 gm powder was suspended to 95 ml distilled water and sterilized by autoclaving for 20 minutes at 10 lbs per square inch pressure (115°C). Cooled at 55°C and 5 ml of sterile 40% Urea Solution (SR20) was aseptically introduced and mixed well. About 10 ml of the media was distributed into sterile containers.

10. Litmus Milk Medium

Ingredient

Skimmed milk powder	2 gms
Distilled water	20 ml
Litmus (indicator)	small amount

Sufficient to give a blue-mauve colour to the milk. Skimmed milk powder was dissolved in water, and litmus was added to it. The medium was dispensed in small test tubes in 0.5 ml amount and autoclaved at (15 lbs pressure) 121°C for 15 minutes.

C. STAINING REAGENTS

1. For Gram's Stain

(i) Crystal Violet Stain

Solution A

2.0 gm of crystal violet was dissolved in 20 ml of 95% ethyl alcohol.

Solution B

0.8 gm of ammonium oxalate was dissolved in 80.0 ml of distilled water.

Then the solutions A and B were mixed.

(ii) Gram's Iodine

20 gms of potassium iodide was dissolved in 300 ml of distilled water and then 10 gm of iodine crystals was added to it. The volume was adjusted up to 1000 ml by adding distilled water.

(iii) Decoloriser

70% acetone

(iv) Safranin (Counter stain)

2.5 grams safranin was dissolved in 1000 ml of 95% ethanol and the solution was diluted 1:10 with distilled water.

2. Test Reagents

(i) Barritt's Reagent

Solution A

□ - naphthol 5 gms.

Ethyl alcohol (absolute) 100 ml.

Solution B

Potassium hydroxide 40 gms.

Distilled water 100 ml.
1 ml of solution B and 3 ml of solution A was added to the test suspension.

- (ii) **Catalase Reagent (3% H₂O₂)**
Conc. Hydrogen peroxide 3 ml
Distilled water 97 ml.
- (iii) **Kovac's Reagent (Indol test)**
Pure amyl or isoamyl alcohol 150 ml
Conc. Hydrochloric acid 50 ml
Para dimethyl aminobenzaldehyde 10 g.
- (iv) **Methyl Red Indicator Solution**
Methyl red 0.1 gm
Ethyl alcohol (95%) 300 ml.
Distilled water 200 ml.
- (v) **Oxidase Reagent**
Tetraethyl para phenylene
diamine dihydrochloride 1 gm
Distilled water 100 ml.

Small filter paper strips were soaked, dried and then kept aseptically.

D. MICROBIAL SENSITIVITY DISCS

Antibiotic discs used were from different companies such as

- Oxoid Limited
Basingstoke, Hampshire, England
- Span Diagnostics Ltd.
GIDC, Sachin-394 230 (Surat) India.
- Hi Media Laboratories Pvt. Ltd., Bombay, India.
L.B.S. Marg, Bombay-400086, India.

Zone Size Interpretative Chart.

Antimicrobial Agents	Diameter of Zone of Inhibition			
	Sensitive mm or more	<i>E.coli</i> ATCC 25922	<i>Pseud. aeru.</i> ATCC 27853	<i>S. aureus</i> ATCC 25923
Amikacin (Ak 30 mcg)	17	19.26	18-26	20-26
Ciprofloxacin (Cf 5 mcg)	21	30-40	25-33	22-30
Nitrofurantoin (Nf 300 mcg)	17	20-25	-	18-22
Gentamycin (G 10 mcg)	15	19-26	16-21	19-27
Chloramphenicol (C 10 mcg)	18	21-27	-	19-26
Cotrimoxazole (Co 25 mcg)	16	-	-	-
Nalidixic acid (Na 30 mcg)	19	22-28	-	-
Cephalexin (Cp 30 mcg)	18	-	-	-
Tetracycline (T 30 mcg)	19	18-25	-	24-30
Ampicillin (A 10 mcg)	17	16-22	-	27-35
Norfloxacin (Nx 10 mcg)	17	39	33	28

Appendix - IV

BIOCHEMICAL FEATURES OF SOME GRAM NEGATIVE BACTERIA ISOLATED FROM URINE SAMPLE

Bacteria	O	Ca	I	MR	VP	Ci	H ₂ S	Motility	O/F	U	TSI	LD	LF
<i>E.coli</i>	-	+	+	+	-	-	-	M	F	-	A/A Gas	+	+
<i>Klebsiella pneumoniae</i>	-	+	-	-	+	+	-	NM	F	+	A/A Gas	+	+
<i>K. oxytoca</i>	-	+	+	V	V	+	-	NM	F	+	A/A Gas	+	+
<i>Proteus Vulgaris</i>	-	+	+	+	-	V	+	M	F	+	A/A Gas H ₂ S	-	-
<i>P. mirabilis</i>	-	+	-	+	V	-	+	M	F	-	Alk/A Gas H ₂ S		-
<i>Pseudomonas aeruginosa</i>	+	+	-			+	-	M	O	V	Alk/NC	-	-
<i>Alkalescens Dispar group</i>	-	+	+	+	-	-	-	NM	F	-	Alk/A	+	
<i>Citrobacter</i>	-	+	+	+	-		+	M	F	V	Alk/A H ₂ S	-	V
<i>Enterobacter</i>			-	-	+	+	-	M	F	V	A/A Gas	+	+

Note:

O	Oxidase test
Ca	Catalase test
I	Indole Production test
MR	Methyl Red Test
VP	Voges Proskauer test
Ci	Citrate utilization test
H ₂ S	H ₂ S production test
M	Motile
NM	Non-motile
O/F	Oxidation-fermentation test
U	Urease production test
LD	Lysine Decarboxylase test
V	Variable
TSI	Triple Sugar Iron test
A	Acid
Alk	Alkaline
NC	No change
LF	Lactose Fermentation

Source: ?

Appendix - V
CHART SHOWING MECHANISM OF ACTIONS OF SOME ANTIBIOTICS

Antibiotics	Rout of administrati on	Antibact erial activity	Mechanism of Action	Particular Indication
β- Lactam	-	-	Cell Wall (Peptidoglycan Synthesis)	
i. Ampicillin	Oral & Parenteral	Broad	Peptidoglycan; an essential component of bacterial wall is absent from mammalian cells. D-alanyl - D- alanine is unique to peptidoglycan.	UTI, RTI. Gall Bladder and bowel infections
ii. Cephalexin	Oral	Broad not <i>Pseud. aeruginosa</i>		
Aninoglycoside			Ribosome function (protein synthesis)	
i. Amikacin	Parenteral	Broad gm -vc	Selective for bacterial 30s subunit, active uptake	UTI
ii. Gentamycin			..	<i>Pseudomonas</i> infection
Tetracycline	Oral	Broad	Active uptake by bacteria, exclusion from mammalian cells	RTI, <i>Mycoplasma Cloxiell</i> & rickettsial Infections
Chloramphenicol	Oral & Parenteral	Broad	Selective for bacterial 50s subunit	Bacterial, <i>Salmonella</i> , rickettsial & <i>haemophiles</i> infections
Quinolones			Chromosome function (replication and transcription of DNA)	
i. Norfloxacin	Oral & Parenteral	Broad	DNA gyrase unique to bacteria	UTI
ii. Ciprofloxacin iii. Nalidixic acid	Oral	Broad Gram -ve	..	RTI, UTI UTI
Nitrofurantoin	Oral	Narrow Gram -ve	Converted to active form in bacteria	UTI
Cotrimoxazole:			Folate metabolism	
Sulphonamide	Oral	Gram -ve not <i>Proteus</i>	Dihydropteroic acid synthetase unique to bacteria	UTI & RTI
Trimethoprim	Oral & Parenteral	Broad	Selective inhibition of bacterial DHER	

DHFR : Dihydrofolate reductase

RTI : Respiratory tract infection

UTI : Urinary Tract Infection

Source: Hugo and Russel, 1981 (Modified)

Appendix - VI

MORPHOLOGY AND CULTURE CHARACTERISTICS OF BACTERIA ISOLATED FROM URINE SAMPLE.

Bacteria	Morphological Characteristics	Cultural Characteristics
1. <i>E.coli</i>	Gram-negative, rod of 1-3 μ m x 0.4-0.7 micron size, aerobic and facultative anaerobic, motile, spore forming, non encapsulated.	On Blood agar : Large 1-4 mm in diameter, greyish white, moist, smooth, convex, opaque. The colonies may appear mucoid some strains show haemolysis. On MA : Bright pink colonies due to lactose fermentation, smooth, glossy and translucent.
2. <i>Klebsiella spp.</i>	Gram-negative short rods of 1-2 x 0.8 μ m in size, capsulated, non sporing, non motile.	Large raised dome shaped, moist and usually mucoid colonies when cultured on Blood agar. On EMB agar circular smooth convex colonies having a striking blue purple or black metallic sheen. MacConkey agar- Most <i>Klebsiella</i> stains are pink lactose fermenting mucoid shining.
3. <i>Pseudomonas aeruginosa</i>	Gram-negative, rod 1.5-3 x 0.6 μ m non sporing, non capsulated, motile with a single polar flagellum, strict aerobe.	On MA : Pale or colourless translucent non lactose fermenting colony. On NA : Irregular to large spreading thin green or blue green fluorescent colonies with distinctive aromatic (misty) grape like odour.
4. <i>Proteus sps</i>	Gram-negative rod varying in length from short coccobacilli to long filaments 0.5-1x1-3 μ m in size actively motile, non sporing, no capsulated.	On NA : Fishy smell and swarming appearance which is thin, colourless and transparent film. On MA: Pale or colourless translucent non lactose fermenting. Colonies Swarming is inhibited.
5. <i>Streptococcus faecalis</i>	Gram-positive, spherical or ovoid, 0.5-1 μ m in diameter, facultative anaerobic, nonsporing, non motile and are arranged in pairs or short chains.	On BA : Small (0.5-1 mm), round, low convex, translucent and non pigmented. Colonies alpha or beta or non-haemolytic beta-haemolytic or alpha-haemolytic On MA : Small (0.5-1 mm) red pigmented colonies.

Source : ?