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PRERVALENCE OF URINARY TRACT INFECTION IN CHILDREN

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ABSTRACT

Fifty eight episodes of urinary tract infection were demonstrated from 205 children attending to Kanti Children's Hospital, Maharajgunj, Kathmandu. The study was conducted during March 1996 to December 1996 with the aim to isolate the organism causing UTI in children and to correlate bacteriuria, pyuria and clinical feature during the infection. The project also aimed to determine antibiotics sensitivity test profiles of these organism.

Two hundred five urine samples were cultured with the standard bacteriological techniques. Of the 205 urine specimens 28% showed significant bacterial growth. Among the total isolate, *E. coli* was the predominant isolate (57%) followed by *Klebsiella pneumonia* (24%) and *Proteus sps* (10%).

Greater prevalence of bacteriuria was at age of 0-1 years in male, whereas 5-10 years in female children. Growth positivity with regards to genderwise distribution of the urine samples female children showed higher rate of growth positivity than male children. Invitro susceptibility test of these pathogens showed that almost all isolates were sensitive to Nitrofurantoin (88%) followed by Ciprofloxacin (81%),Nalidixic acid (69%)and Chloramphenical (60%). Cotrimoxazole and Amoxycillin were least effective antibiotics against these bacterial isolates.

Abbreviations

Gm	: Gram
-ve	: Negative
+ve	: Positive
LF	: Lactose Fermenting
NLF	: Non Lactose Fermenting
BA	: Blood Agar
NA	: Nutrient Agar
MA	: Mac Conkey Agar
MSU	: Mid Stream Urine
MR	: Methyl Red
VP	: Voges- Proskauer
TSI	: Triple Sugar Iron Agar
O/F	: Oxidative/Fermentative
SIM	: Sulphide Indole and Motility
R.B.C.	: Red Blood Cell
A/A	: Acid / Acid
Alk/A	: Alkali / Acid
Alk/A, H_2S	: Alkali/Acid, Hydrogen Sulphide
UTI	: Urinary Tract Infection
KCH	: Kanti Children's Hospital
Hrs.	: Hours
mm	: millimeter
ml	: millilitre
rpm	: revolution per minute

Abbreviations

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CHAPTER ONE

INTRODUCTION

Urinary tract infection (UTI) simply means the presence of bacteria undergoing multiplication in urine within the urinary drainage system. UTI encompasses a wide variety of clinical entities whose common denometer is microbial invasion of any tissues of the tract extending from renal cortex to the uretheral meateus. Infection of adjacent structure such as prostate and epididymidis are also included in the definition.

Infection may be expressed predominantly at a single site of the kidney (Pyelonephritis) bladder (Cystitis),prostate(Prostatitis),urethra(Urethritis)etc.

Urine is sterile ultra filtrate of blood. In absence of UTI, it is free from micro-organisms. During passage through the distal urethra, the small number of bacteria may enter the urinary stream as contaminant. In 1956, Kass gave a definition of true or significant bacteriuria by which means that a 1000,00 or more single species bacteria per/ml counts in a carefully collected samples of clean voided or mid stream urine made possible a clear distinction between infection and contamination. This much number indicates a multiplication of the organism in urinary tract. But in certain circumstances the properly collected sample shows even less than 10^5 CFU/ml is consider as low count significant bacteriuria such as if the sample was collected before the organism reached to the log phase or patient is under treatment or renal calculi or diabetic patients. Any bacterial growth from a specimen obtained by ureteric catherization, renal biopsy, suprapubic aspiration and post prostatic massage should be consider as significant. (Manzon et al, 1958. Meares and Stamy 1968).Usually there is single bacterial infection in urinary tract. The presence of more than one species usually indicates urethral or perineal contamination. Multiple strain however have been found to cause UTI in patients with in dwelling uretheral catheters.

However bacteria are most commonly responsible, although fungi & viruses may also occasionally produce UTI. Coliform group of bacteria specially *E.coli* has been found responsible for the majority of urinary tract infection. Which accounts for approximately 90% of the initial infection. *Klebsiella, Proteus, Pseudomonas, Staphylococcus, Enterococci* are largely responsible for most of the remaining 10 % infection in children.

Urine culture is generally accepted as an essential components of the diagnosis of UTI in paediatric patient. Urine for this purpose must be free from urtheral or genital tract contamination. Generally four different method of collection of urine samples are there. These include clean catch mid stream urine, suprapubic aspiration of urine, sterile urinary bag attached on the genital organ and catheterization sample.

In many parts of our country the facilities for culture & sensitivity is lacking which causes further diagnostic difficulty. The growing kidney might be scared and child may die from its complications. Virtullay we do not know common organisms causing UTI among children and antibiotics sensitivity test profile of these organisms. To my knowledge no systemic study so far has been done in this field in Nepal. Therefore, this study was done with the following objectives.

1. To observe the pattern of UTI in children attending in Kanti Children's Hospital.
2. To determine prevalence of organisms causing UTI among the children.
3. To determine antibiotics/sensitivity test profiles of the organisms causing UTI among children.

CHAPTER TWO

OBJECTIVES OF THE STUDY :

A. To observe the pattern of UTI in children attending in Kanti Children's Hospital.

B. To determine prevalence of organisms causing UTI among the children.

C. To determine antibiotic sensitivity test profile of these organisms causing UTI among children.

CHAPTER THREE

REVIEW OF LITERATURE

3.1 DEFINITION OF URINARY TRACT INFECTION

The urinary tract consists of the kidneys, ureters, the bladder & the urethra (Bailey & Scott, 1990). Urinary tract infection is one of the most common bacterial infections in infancy and childhood (Ering & G. Zobel et al, 1988). 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 urethral meatus (Sarman Singh et al, 1992). Infection of the adjacent structures such as prostate and epididymidis are also included in the definition (Bailey & Scott, 1990). The severity of 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 involve tissues also the out side of the urinary tract due to which there may be Gram -ve sepsis and death (Catherina Svanbergi et al.).

Bacterial UTI in neonates & children can be life threatening and can cause severe renal disease of adulthood. UTI in children may present with a typical symptom or may be symptomatic specially in recurrent attack, so early detection and treatment is extremely important (Gh Hashemi et al, 1985).

The occurrence of bacteria i.e. the growth of single species of bacteria within the urinary tract is the common denominator of these disorder (Catherina Svanbergi et al.). Conformation of UTI is based on finding appreciable numbers of pathogenic bacteria in the bladder urine (RHR White et al, 1977).

Kass provided systemic 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, can be regarded as unimportant if there is a mixed growth and may require repetition if associated with a single pathogen.

3.2 LAB DIAGNOSIS OF UTI IN CHILDREN

For diagnosis of UTI, urine specimen have been obtained by different way like midstream clean catch urine, catheterization, suprapubic aspiration, dipslide method etc.

One of the most useful tests for presumptive diagnosis of UTI is the microscopic examination of specimen for bacteria. Presence of at least one bacteria per oil immersion field in a mid stream clean catch urine, Gram stained of uncentrifused urine correlates with 10^5 bacteria or more in per millilitre of urine. This titre is regarded to represent significant bacteriuria. The absence of bacteria in several fields in a stained sedimented specimen indicates the probability of less than 10^4 bact/ml.

Microscopic examination of urine sediments, under per high power field, when shows 5-10 leukocytes indicated the significant bacteriuria.

3.3 LOW COUNT SIGNIFICANT BACTERIURIA :

Sometimes when a child is under treatment i.e. presence of antimicrobial agents in urine or if the sample was as collected before the organism reach to log phase, sample showed 10^4 CFU/ml of urine and it is low count significant bacteriuria. High urea concentration and high osmolarity of urine also shows lower bacterial

densities (Gupta Deepak et al, 1993). According to study done by Tonston Sandberg et al. possible causes of low count significant bacteriuria are outlined in Table 1.

Table 1: Some Causes Of Low Count Bacteriuria In Voided Urine Specimens.

Urine collected before the organisms reached to log phase.
High diuresis and frequent voiding
Urine with a low pH
Occurrence of fastididious organisms
Obstruction of urine flow in pyelonephritis

3.4 PREDISPOSING FACTOR FOR URINARY TRACT INFECTION:

Residual urine due to incomplete bladder emptying serve 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 indwelling 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 approximate for multiplication of pathogen (Winberg 1959 & Kontz 1961). The sufficient amount of amino acids in normal urine help in bacterial growth (Rozer Gabrial et al, 1988)

3.5 CLINICAL MANIFESTATION IN CHILDREN:

UTI in children tends to manifest with different symptoms. Unexplained fever and failure to thrive are common presenting sign in infants, besides nausea, vomiting and diarrhoea. In slightly older children in addition to the above increase frequency of micturition and nocturnal enuresis can be the other complaints. But sometimes the symptoms may remain asymptomatic (CRB Anapurmath and S. Jayman et al, 1994). Again in neonates the most common symptom of UTI are weight loss and lethargy (Jack Selder et al.). When children are above 5 years develop infection they are more likely to display localised symptoms such as

frequency dysuria, abdominal and flank pain (Jack D. Sobel & Donald Kaye et al.).

3.6 SOURCES OF ORGANISMS CAUSING URINARY TRACT INFECTION:

It is now firmly established that most infections are caused by the organisms belonging to *Enterobacteriaceae* family derive from the patient's own bowel (Horkness JL, Anderson & FM Dalton, 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. In the neonate, however the urinary tract infection may becomes colonised hematogenously (Jack S. elder et al). Constipation 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). Urine may possibly reflux from the urethra into the bladder carrying with it enterobacteria that colonized & caused urinary tract infection.

Majority of recurrent UTI were due to the re-infection, not persistence of the pathogen within the urinary tract and suggested that the colonic flora was the reservoir for these re-infecting strains (Russo, Thomos & A. Ann Slepton et al.) again prolong use of antibiotics in children will alter the intestinal flora & the development of resistant strains in the bowel will lead to recurrent UTI (Horkness JL, Anderson & FM Dalton et al, 1975).

3.7 PATHOGENESIS OF URINARY TRACT INFECTION IN CHILDREN :

There are three possible routes by which bacteria can invade and spread within the urinary tract, these are the ascending, hematogenous and lymphatic pathway (Jack Sobel & Donald Kaye et al.). A long term follow up studies by various worker

confirm that bacteria may gain its way to urinary tract by the ascending route, it is the usual and believed one. UTI arises from ascending from the perineal area. 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. Adherence produced by pilli on the bacterial surface seen to interact with specific receptor on the urothelial surface.

The degree of pillion of the bacteria and the number of recepter sites on the urothelial cells, which is increased subject to reccurent UTI and renal parenchymal scorring. Adherence may also relate to the presence of certain glycolipids in human urothelial cells.

E.coli responsible to UTI are belong to a limited numbers of O:K:H sero types i.e. combination of lipopolysaccharide (K) and flagellar antigens (H). They carry fimbrial adhesion resistant to killing by serum and produce haemolysin.

Bacterial adhesion reflects the ability of an organism to bind to mucosal epithelial cells. In the urinary tract adherence may increase virulence by several mechanisms. A typical uropathogenic strain of *E.coli* posses P fimbrie. Which seen to be the major virulence factor possessed by *E.coli* (Gupta Deepak et al.).

In addition to this the surface agglutination of the *E.coli* pathogen appear to a key determinate 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 (R.R. Bailey et al.).

3.8 HOST DEFENSE MECHANISMS:

Defense mechanism are present throughout the urinary tract and contributes to resistance of the infection. In a book "A Practical Approach to Infectious Disease"

(1983). In chapter 12-Genitourinary Tract Infections, Thomas T. Ward & Stephen R. Jones et al. gave some examples such as :-

Complete Bladder Emptying :-

It is one of the most important defense mechanisms. When residual urine volumes are high, large number of bacteria may remain in the urine, to expel bacteria is complete when voiding is complete.

Vesicoureteral value :-

The vesicoureterial also provide a barrier to spread of infection by preventing reflux of bacteria once bladder bacteriuria is established.

The relatively long urethra :-

It is believed to protect against infection in a male child. The shorter urethra probably allow entry of bacteria from the bladder and this part may account for higher frequency of UTI in a female child.

Frequent voiding of urine also protect against infection because this may allow for wash out of bacteria. The actual defense mechanism of the mucosa is not well known but the mucosal cell secret a substance which kill the bacteria. Again inhibit the adhesion of bacteria on mucosal membrane (Vivaldi et al, 1965).

3.9 CLASSIFICATION OF UTI :

As a guide to the management of patients with UTI a characterisation of the type of infection will influence not only the choice of treatment, but also the degree of supervision need for urinary tract investigation.

Table 2 shows the classification of UTI (S. Ragnar Norrby)

Table no.2:- Classification of urinary tract infection (UTI).

Classification by	Groups	Definition
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 kidneys.
Complications	Uncomplicated	No identified anatomical defects, foreign bodies or tumours.
	Complicated	Identified anatomical defects, foreign bodies or tumours.
Recurrences	Sporadic	< 2 episodes of UTI in the preceding six months and < 3 episodes in the preceding year.
	Recurrent	>2 episodes of UTI in the preceding six months or > 3 episodes in the preceding year.

3.10 MICROBIOLOGY OF URINARY TRACT INFECTION :

Bacteria causing urinary tract infection :

1. *Escherichia coli (E.coli)*
2. *Proteus vulgaries*
3. *Proteus mirabilis*
4. *Pseudomonas aeroginosa*
5. *Klebsiella pneumoniae*
6. *Klebsiella aerogenes*
7. *Enterobacter sps*
8. *Serratia sps*
9. *Salmonella sps*
10. *Shigella sps*
11. *Micrococcus sps*
12. *Acinetobacter sps*
13. *Citrobacter freundi*
14. *Citrobacter diversus*

15. *Staphylococcus aureus*
16. *Staphylococcus haemolyticus*
17. *Staphylococcus epidermidis*
18. *Staphylococcus saprophyticus*
19. *Streptococcus faecalis*
20. *Klebsiella oxytoca*
21. *Streptococcus viridans*
22. *Streptococcus milleri*
23. *B-haemolytic streptococcus*

Fungi causing urinary tract infection :

1. *Candida albicans*
2. *Torulopsis glabarllata*
3. *Gardenella vaginalis*

Among the bacteria, the member of the family Enterobacteriaceae involved in causing urinary tract infection. In which *E.coli* accounts for up to 80% of all infections.

Fungi are isolated in patients receiving antibiotics and occasionally in patients who have had previous instrumentation's of the urinary tract.

There is indirect evidence to suggest that viruses may also be responsible for certain renal lesion, including glomerunephritis. The role of varicellor-zoster virus in haemorrhagic cystitis is well documented. Adeno virus type 8 has been associated with haemorrhagic cystitis in children.

CHAPTER FOUR

METHODS AND MATERIALS

For the purpose of this study, the urine samples were collected from 205 children patients suspected of UTI, who attended in Kanti Children's Hospital (KCH), Kathmandu were included. I surveyed children below 14 years of age. The study was conducted between March 1996 to December 1996. Details of relevant clinical information were taken on microbiological protocol (Annex) before investigation. The collected samples from KCH were brought to Tribhuvan University Teaching Hospital Microbiological Research Laboratory Kathmandu. Then those samples were subjected for routine investigation, culture and antibiotics sensitivity test.

4.1 COLLECTION OF SAMPLES :

Urine in a sterile plastic cup or bottle with tight lid was collected from a patient who had clinical feature of urinary tract infection at Kanti Children's Hospital and immediately brought to department of microbiology, Institute of Medicine which is located close to the hospital. Either midstream urine sample, catheterization specimen or urine collected into a sterile urinary bag attached on genital organ was used to culture and sensitivity test. All the specimens were labelled properly.

1. **Mid Stream Urine Specimen:** The genital was washed with mild soap and water. Patient was asked to pass few portion so that the normal urethral flora would be adequately flushed out. Then 5 to 10 ml of urine was collected in the clean sterile wide mouth container.
2. **Catheterization:** With the help of sterile catheter the urine sample was collected directly into the sterile urine container where rubber tube was passed in the bladder after cleaning external genitalia thoroughly.

3. **Sterile Urinary Bag Attached On The Genital Organ:** In infants the bag was applied after disinfection of genitalia. So that randomly voided urine would be collected in the urinary bag. The portion of this urine was collected for culture and sensitivity test. This method was seldom used due to incontinence and poor hygienic.

Since urine being excellent culture medium for bacterial growth, the urine sample was processed within half an hour of collection. Otherwise it was kept at 4^0 C until it was processed.

4.2 PHYSICAL EXAMINATION OF URINE :

First of all urine was examined macroscopically for it's colour and appearance.

4.3 CULTURING OF SPECIMEN :

During the course of processing of sample, first of all culturing of specimen was done to avoid the risk of contamination. For this the agar plates were dried in incubator at 45^0 C for best result of streaking. Then the uncentrifuged urine was thoroughly mixed before plating. After this, with the help of standard loop (4 mm diameter) which deliver a known volume of urine i.e. 0.001 ml of urine was inoculated in Mac Conkey agar plate and blood agar plate by standard quadrant streaking technique. After this inoculation, the plates were incubated on incubator, where temperature was 37^0 C for 24 hours. During incubation period the petri plates were placed in inverted position i.e. upward media and downward petri dish cover. This was done so that the condensation of water on the streaked surface prevented. If downward media and upward petri dish cover was placed, moisture may interfere the development of isolated colonies because if over moisture was there, the bacterial growth would spread all over the agar surface. After overnight incubation, colonies were counted on each plate. The number of Colonies Forming Unit (CFU) was multiplied by 1000 to determine the number of micro-organisms per

millilitre in the original specimens. Reincubation of plates were done while on growth or tiny colonies for an additional 24 hours because antimicrobial treatment or other factors may inhibit the initial growth, before discarding the plates. The result was interpreted as follows:

No. of colonies	Result
1. No growth	Negative
2. Less than 50 colonies	Not significant
3. Mixed growth	Not significant
4. 80-100 colonies	Significant bacteriuria

4.4 ROUTINE EXAMINATION OF URINE :

Microscopic Examination of Urine:

Five millilitre of urine sample was taken in a clean centrifuged tube, and centrifuged at 2500 revulation per minute (rpm) for 10 minutes. The supernatant was transferred into another clean test tube for sugar and albumin test. The deposit was then well mixed and a drop of sediment was placed on a clean slide and covered with cover slip. The deposit was then examined under the microscope using 10x and 40x power for examining presence of pus cells, RBC, crystals and other cellular materials if any.

4.5 CHEMICAL EXAMINATION OF URINE :

This tests was done to detect the presence of sugar and albumin in the urine.

(a) Test for presence of sugar in the urine :

Benedicts Method:

1. 5 ml of benedicts reagent was added then it was heated in burner.
2. About 8 drops of supernatant urine was added with 5 ml Benedicts reagent previously placed in a clean test tube and was mixed thoroughly and boiled

over Bunsen burner for 5 minutes. Positive and negative control was run parallely. The result was interpreted as follows:

RESULT:

Appearance of solution	Sugar concentration
Blue colour	Nil
Green, no precipitate	Trace
Green with precipitate	+
Yellow	++
Orange	+++
Brick red	++++

(b) Detection of Albumin in the urine:

Sulphosalicyclic acid Method :

Sulphosalicyclic acid solution (3%) precipitates protein in the urine specimens irrespective of the type i.e. albumin. It is an anion precipitant that works by the neutralisation of the protein cation.

1. Two millilitre of supernatant from centrifuged urine specimens was taken in a clean test tube.
2. Two drops of 3% sulphosalicylic acid was added.
3. The test tube was shook gently and the result was interpreted as follows :

RESULT :

No cloudiness	Negative
Slight cloudiness	+
Moderate cloudiness	++
Marked cloudiness	+++
Marked cloudiness with curdy precipitate	++++

4.6 IDENTIFICATION OF PATHOGENS CAUSING UTI :

With the use of standard microbiological technique, identification of bacterial pathogen was done, which included the colonial morphology, Grams reaction, catalase, oxidase test, biochemical properties and serology if necessary.

BIOCHEMICAL TEST :

A single isolated culture from Mac Conkey agar (MA) plate was inoculated into the peptone water and incubated at 37^0 C for 4 hours. From 4 hours culture, the organism was inoculated into different required types of biochemical medium for the different biochemical test with or without addition of reagent as required by the test. And result of biochemical test was noted then according to these biochemical test organism was identified. Details of biochemical media and its preparation is given in Annex.

4.8 ANTIBIOTIC SENSITIVITY TEST :

After identifying the bacteria sensitivity test was performed by disc diffusion method. Here Kirby bauer method was done. Only pure culture was used for sensitivity testing. During this study pure culture was taken from subculture i.e. 4 hours incubation culture. The single colony from nutrient agar plate was transferred to peptone water and incubated at 37^0 C for 4 hours, until the turbidity of bacterial growth was similar to that of Mc Farlane tube no. 0.05. Which give the idea about the organism approximately 10^5 CFU/ml. Then a sterile cotton swab was dipped into the tube containing culture and pressing the swab against inside wall of the tube to remove excess of inoculum. This inoculum containing cotton swab was streaked in dried Muller-Hinten agar (MHA) plates.After this about 8 to 9 routine different antibiotics were removed with the help of flamed forceps and carefully placed them 15 mm away from the edge at equal distance then finally press lightly with forceps for complete contact of medium. These plates were incubated for 24 hours. Following overnight incubation the culture was examined

incubated for 24 hours. Following overnight incubation the culture was examined for areas of no growth around the disc i.e. zone of inhibition. Bacterial strains sensitive to the antimicrobial were inhibited at a zone was measured and compared against a previously prepared scale. The scale correlates zone size with the minimum inhibition concentrate. Here is the study zone size was compared with the standard interpretation table developed by Kirby-Bauer.

CHAPTER FIVE

RESULTS :-

During the study period 205 urine samples from UTI suspected children patient were collected from KCH. Growth of more than one species i.e. mixed growth and not significant bacterial growth were considered as contaminants such cultures were excluded. Only single species of significant bacterial growth was included and further study was done for such urine samples.

5.1 PATTERNS OF CULTURE RESULTS :

The patterns of growth indicated by table no. 3 and fig: 1, where growth negative was 33%, significant bacteriuria was found in 28% of sample, not significant and mixed bacterial growth were present in 25% and 13% of samples.

Table 3: Patterns of cultural results

S.N.	Growth	Number	Percentage
1	Positive	58	28
2	Negative	68	33
3	Mixed	27	13
4	No significant	52	25

5.2 PATTERN OF THE DIFFERENT SPECIES OF THE PATHOGENS ISOLATED FROM INFECTED URINE.

Table no. 4, and fig 2, shows pattern of bacterial growth from urine sample. Among Gram negative bacterial isolates *E.coli* was the predominant one (57%), followed by *Klebsiella pneumoniae* (24%). In case of Gram positive bacterial isolates *Staph. aureus* and *Streptococcus feacalis* were the only isolates in the urine samples.

Fig. 1: PATTERNS OF CULTURE RESULTS

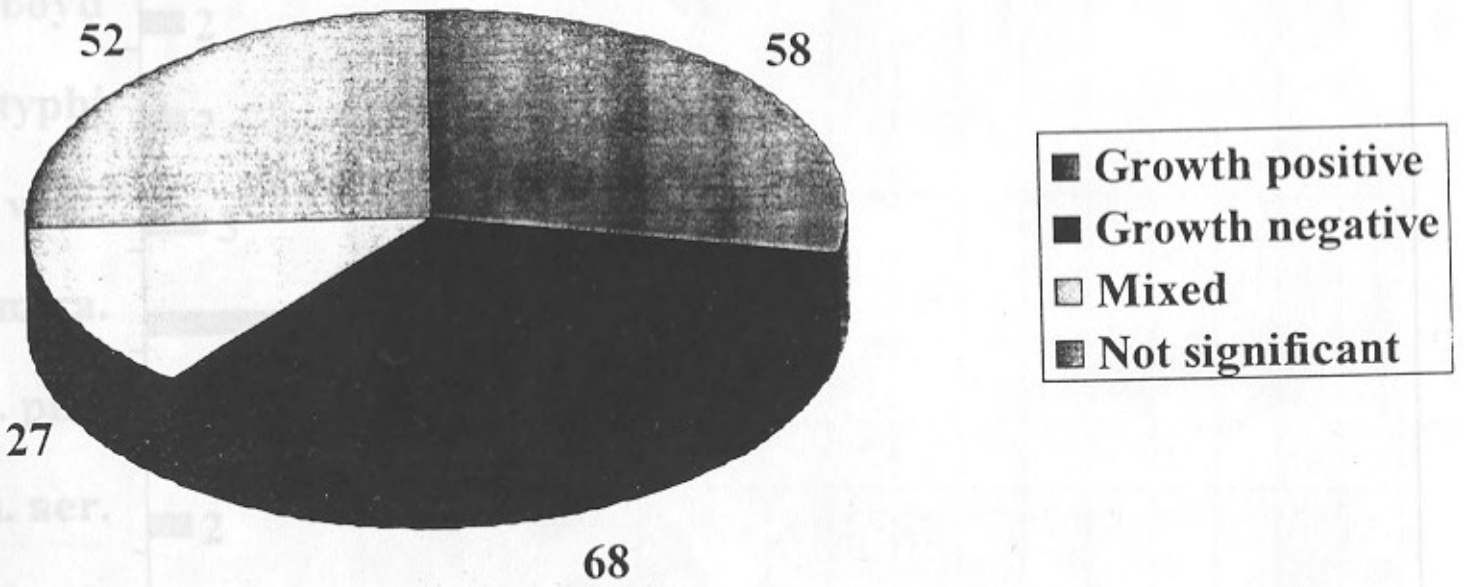


Fig. 2: PERCENTAGE OF DIFFERENT SPECIES OF THE PATHOGENS ISOLATED FROM INFECTED URINE

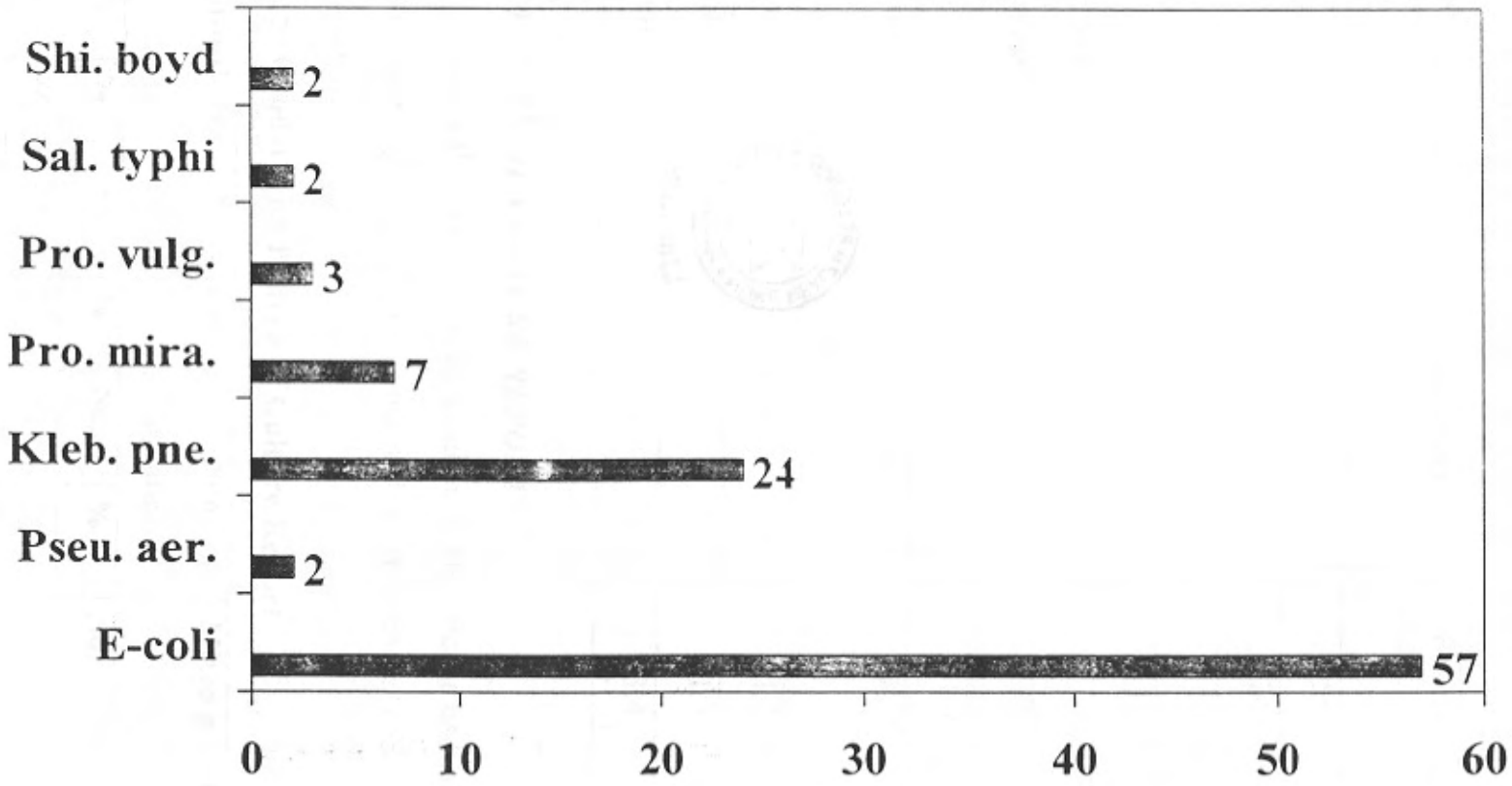


Table 4: **Pattern of Different Species of the Pathogenes Isolated from Infected Urine**

Type of organisms	Number	Percentage
Gm -ve rod		
E.coli	33	56.8
Klebsiella pneumoniae	14	24.1
Proteus mirabilis	4	6.8
Proteus vulgaris	2	3.4
Pseudomonas aeuroginosa	1	1.7
Salmonella typhimurium	1	1.7
Shigella boydii	1	1.7
Total	56	96.2
Gm +ve cocci		
Staphylococcus aureus	1	1.7
Streptococcus faecalis	1	1.7
Total	2	3.4

GENDER WISE PATTERN OF CULTURE REPORT

Table no. 5 and fig 4 shows the gender wise variation of significant bacterial growth. Higher percentage of growth was obtained from female children compared to male children.

Table 5: Gender wise Pattern of Culture Report

S.N.	Sex	Growth +ve		Growth -ve		Non significant		Mixed growth	
		No.	%	No.	%	No.	%	No.	%
1	Female	30	52	39	57	30	58	17	63
2	Male	28	48	29	43	22	42	10	37
	TOTAL	58	100	68	100	52	100	27	100

Fig. 3: GENDERWISE PATTERN OF CULTURE REPORT

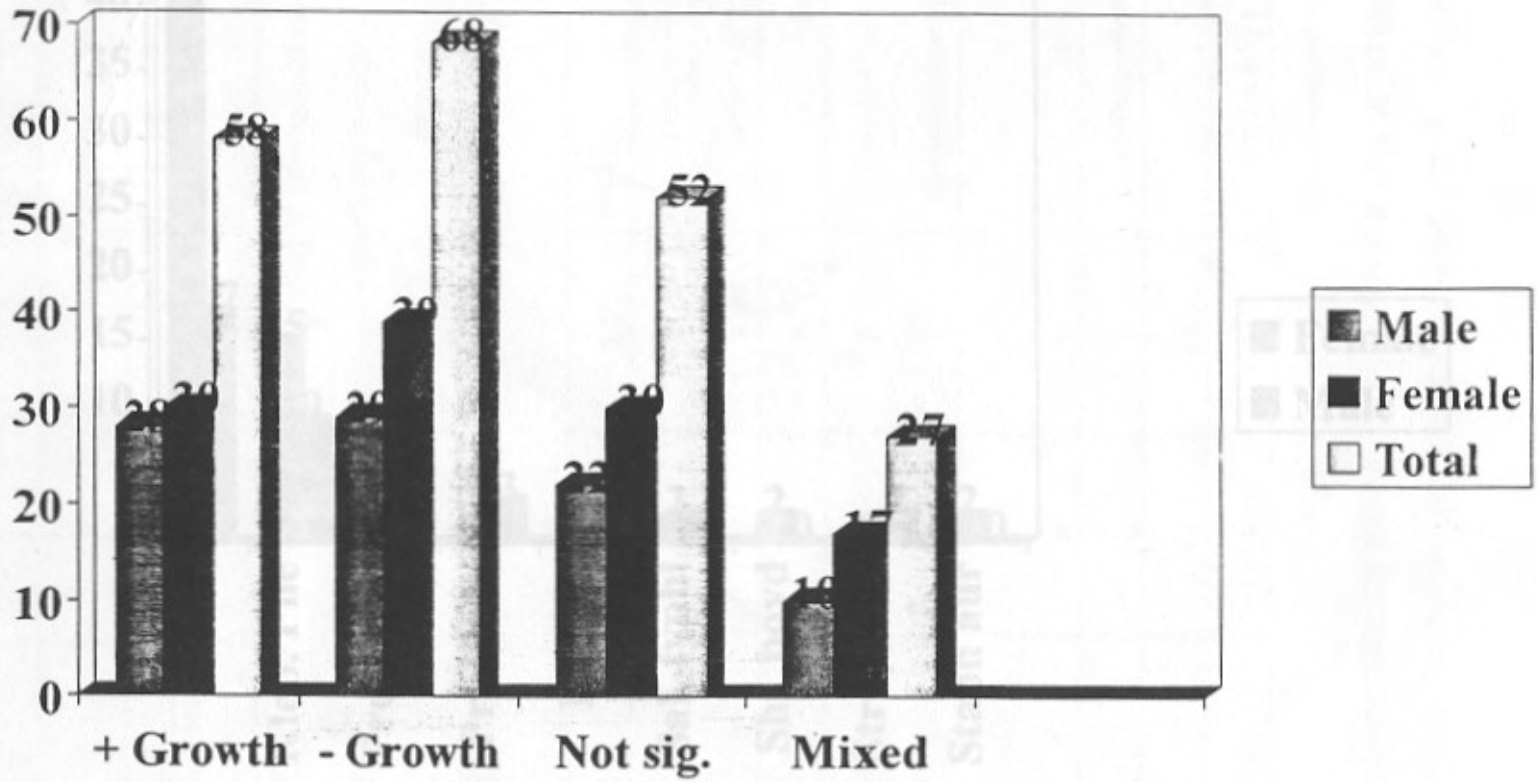
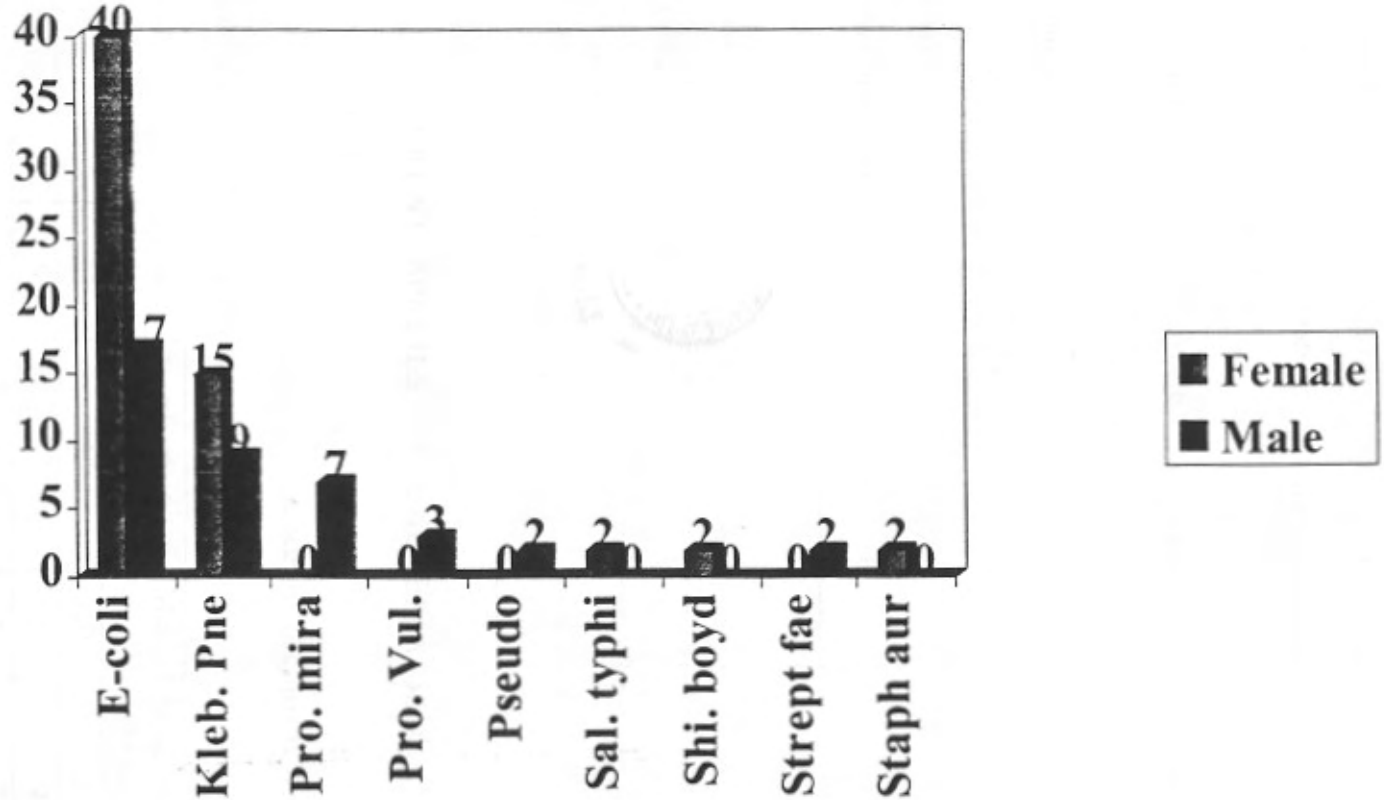


Fig. 4: PERCENTAGE OF DIFFERENT SPECIES OF BACTERIA ISOLATED FROM THE URINE SAMPLE



GENDER WISE PATTERN OF BACTERIA ISOLATED FROM THE URINE SAMPLE.

From the above table no. 6, the bacterial aetiological agents isolated was highest in female children. In both the sexes, *E.coli* showed the higest number, followed by *Klebsiella pneumoniae*.

S.No.	Isolated bacteria	Male	Female	Total
1	*E.coli*	10	23	33
2	*Klebsiella pneumoniae*	5	9	14
3	*Proteus mirabilis*	4	0	4
4	*Proteus vulgaris*	2	0	2
5	*Pseudomonas aeuroginosa*	1	0	1
6	*Salmonella typhimurium*	0	1	1
7	*Shigella boydii*	0	1	1
8	*Streptococcus faecalis*	1	0	1
9	*Staphylococcus aureus*	0	1	1
	Total	23	35	58

PERCENTAGE OF DIFFERENT SPECIES OF BACTERIA ISOLATED FROM THE URINE SAMPLE.

Fig no.5 and table no. 7 shows the gender wise distribution of organism where *E.coli* shows the highest percentage in both the sexes.

S.No.	Organism Isolated	Female	% of organism in Female	Male	% of organism in Male
1	*E.coli*	23	40	10	17
2	*Klebsiella pneumoniae*	9	15	5	9
3	*Proteus mirabilis*	0	0	4	7
4	*Proteus vulgaris*	0	0	2	3
5	*Pseudomonas aeuroginosa*	0	0	1	2
6	*Salmonella typhimurium*	1	2	0	0
7	*Shigella boydii*	1	2	0	0
8	*Streptococcus faecalis*	0		1	2
9	*Staphylococcus aureus*	1	2	0	0

RESULT OF ANTIBIOTICS SUSCEPTIBILITY PATTERN AGAINST BACTERIAL ISOLATED FROM URINE SAMPLE.

For the bacterial isolates the most frequently effective antibiotic was Nitrofurantoin (87.9%), followed by Ciprofloxacin (81%) and Chloramphenical (60.3%) etc.

Antibiotics used	Sensitivity		Intermediate		Resistant		TOTAL
	Number	%	Number	%	Number	%	
Norfloxacin	27	46	2	3	27	46	56
Ampicillin	0	0	-	-	52	90	52
Ciprofloxacin	47	81	3	5	7	12	57
Nalidixic acid	40	69	-	-	18	31	58
Nitrofurantoin	51	88	1	2	8	14	60
Cotrimoxazole	16	27	-	-	39	67	55
Amoxycillin	8	14	1	2	31	88	60
Cephalaxin	19	33	4	7	37	64	60
Chloramphenical	35	60	-	-	23	40	58

ANTIBIOTICS SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM URINE SAMPLE.

Among 33 strain of *E.coli* isolates Nitrofurantoin (100%) & Ciprofloxacin (78%) were most effective drug followed by Nalidixic acid (70%) & Chloramphenical (64%). Ampicillin was found to be the least effective against UTI causing *E.coli.* For *Klebsiella pneumonia*, the most effective antibiotic was Ciprofloxacin (86%) & Nalidixic acid (71%). Nitrofurantoin & Norfloxacin were equally efficient against the Gm -ve rod, efficiency was 64%. Cortimoxazole was found to be less effective drug 21%.

Table 9: Antibiotics Susceptibility Pattern of Bacteria Isolated from Urine Sample.

Isolated organism	Antibiotics used	Sensitivity patterns					
		Sensitivity		Intermediate		Resistant	
		No.	%	No.	%	No.	%
E.coli							
	Ciprofloxacin	26	78	1	3	6	18
	Nalidixic acid	23	70	-	-	10	30
	Cephalaxin	9	27	2	6	22	67
	Ampicillin	4	12	-	-	29	88
	Nitrofurantoin	33	100	-	-	0	-
	Cotrimoxazole	11	33	-	-	22	67
	Norfloxacin	10	30	1	3	22	67
	Amoxycillin	0	0	-	-	33	100
	Chloramphenical	21	64	-	-	12	36
Klebsiella pneumoniae							
	Ciprofloxacin	12	86	1	7	1	7
	Nalidixic acid	10	71	2	14	2	14
	Cephalaxin	5	36	1	7	8	57
	Ampicillin	0	-	-	-	14	100
	Nitrofurantoin	9	64	-	-	5	35
	Cotrimoxazole	3	21	1	7	10	71
	Norfloxacin	9	64	-	-	5	35
	Amoxycillin	34	57	-	-	10	71
	Chloramphenical	8	57	-	-	6	43
Proteus mirabilis							
	Ciprofloxacin	4	100	-	-	0	-
	Cephalaxin	1	25	-	-	0	-
	Ampicillin	0	-	-	-	4	100
	Nitrofurantoin	3	75	-	-	1	25
	Cotrimoxazole	1	25	-	-	3	75
	Norfloxacin	4	28	-	-	0	-
	Amoxycillin	0	-	-	-	4	100
	Chloramphenical	2	50	-	-	2	50
Proteus vulgaris							
	Ciprofloxacin	2	100	-	-	0	-
	Nalidixic acid	2	100	-	-	0	-
	Cephalaxin	0	-	-	-	2	100
	Ampicillin	0	-	-	-	2	100
	Nitrofurantoin	2	100	-	-	0	-
	Cotrimoxazole	2	100	-	-	0	-
	Norfloxacin	2	100	-	-	0	-
	Amoxycillin	0	-	-	-	0	100
	Chloramphenical	2	100	-	-	2	100

Pseudomonas aeuroginosa							
	Ciprofloxacin	1	100	-	-	0	-
	Nalidixic acid	0	-	-	-	1	100
	Cephalaxin	0	-	-	-	1	100
	Ampicillin	0	-	-	-	1	1000
Pseudomonas aeuroginosa	Nitrofurantoin	0	-	-	-	1	100
	Cotrimoxazole	0	-	-	-	1	100
	Norfloxacin	0	-	-	-	1	100
	Amoxycillin	0	-	-	-	1	100
	Chloramphenical	0	-	-	-	1	100
Staphylococcus aureus							
	Ciprofloxacin	1	100	-	-	0	-
	Nalidixic acid	0	-	-	-	1	100
	Cephalaxin	1	100	-	-	0	-
	Ampicillin	1	100	-	-	0	-
	Nitrofurantoin	1	100	1	100	0	-
	Cotrimoxazole	0	-	-	-	1	100
	Norfloxacin	-	-	1	100	-	-
	Amoxycillin	-	-	1	100	-	-
	Chloramphenical	1	100	-	-	0	-
Streptococcus faecalis							
	Ciprofloxacin	0	-	-	-	1	100
	Nalidixic acid	0	-	-	-	1	100
	Cephalaxin	1	100	-	-	-	-
	Ampicillin	1	100	-	-	-	-
	Nitrofurantoin	-	-	1	100	-	-
	Cotrimoxazole	0	-	-	-	1	100
	Norfloxacin	0	-	1	100	-	-
	Amoxycillin	1	100	-	-	-	-
	Chloramphenical	1	100	-	-	-	-
Shigella boydii polyvalent							
	Ciprofloxacin	1	100	-	-	0	-
	Nalidixic acid	1	100	-	-	0	-
	Cephalaxin	1	100	-	-	0	-
	Ampicillin	0	-	-	-	1	100
	Nitrofurantoin	1	100	-	-	0	-
	Cotrimoxazole	0	-	-	-	1	100
	Norfloxacin	1	100	-	-	-	-
	Amoxycillin	0	-	-	-	1	100
	Chloramphenical	0	-	-	-	1	100
Salmonella typhimurium							
	Ciprofloxacin	1	100	1	100	0	-
	Nalidixic acid	0	-	-	-	1	100

	Cephalaxin	0	-	1	100	1	100
	Ampicillin	0	-	-	-	1	100
	Nitrofurantoin	1	100	-	-	0	-
	Cotrimoxazole	0	-	-	-	1	100
	Norfloxacin	1	100	-	-	0	-
	Amoxycillin	0	-	-	-	1	100
	Chloramphenical	0	-	-	-	1	100

Fig 5: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM URINE SAMPLE

Isolated organism - Klebsiella Pneumoniae

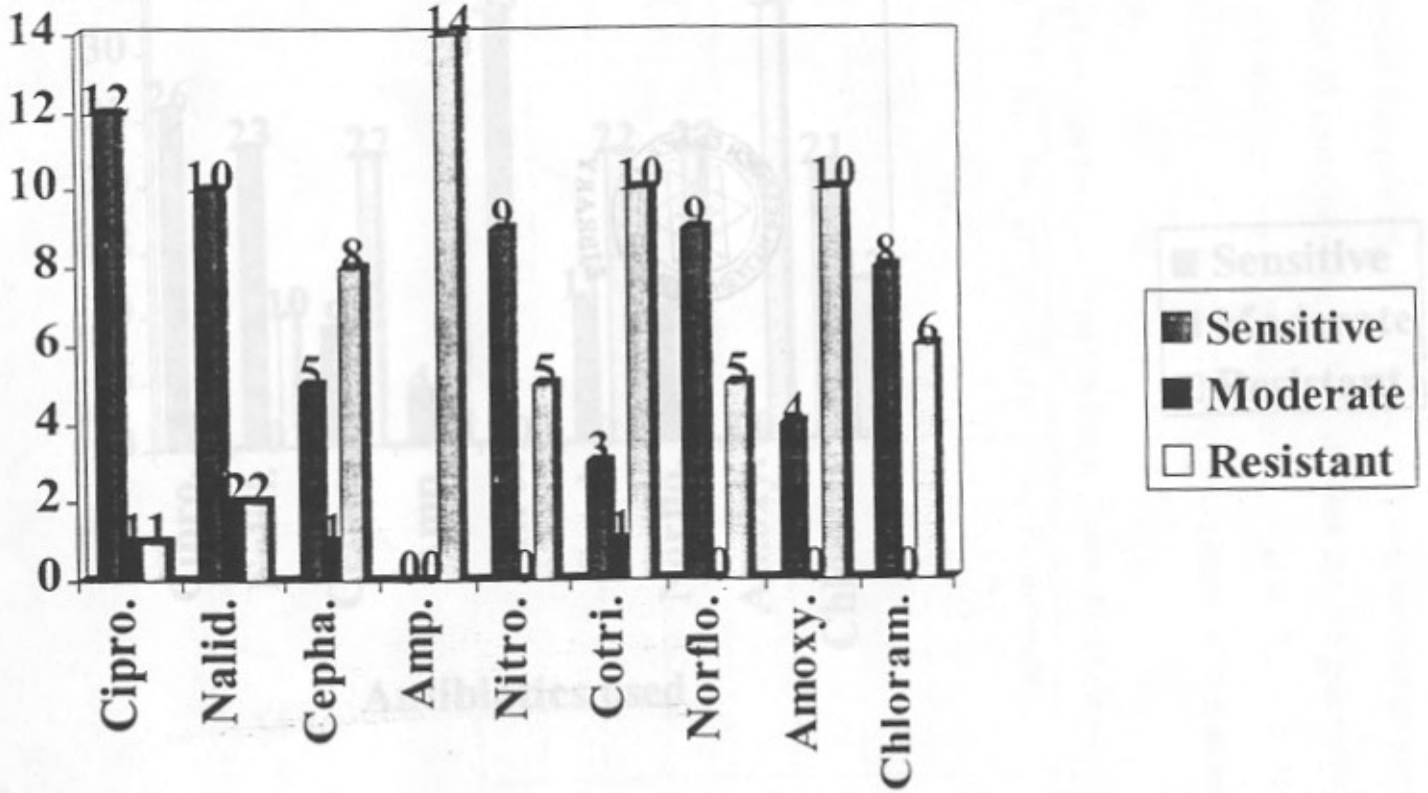


Fig: 6: ANTIBIOTICS SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM URINE SAMPLE

Isolated organism - E. Coli.

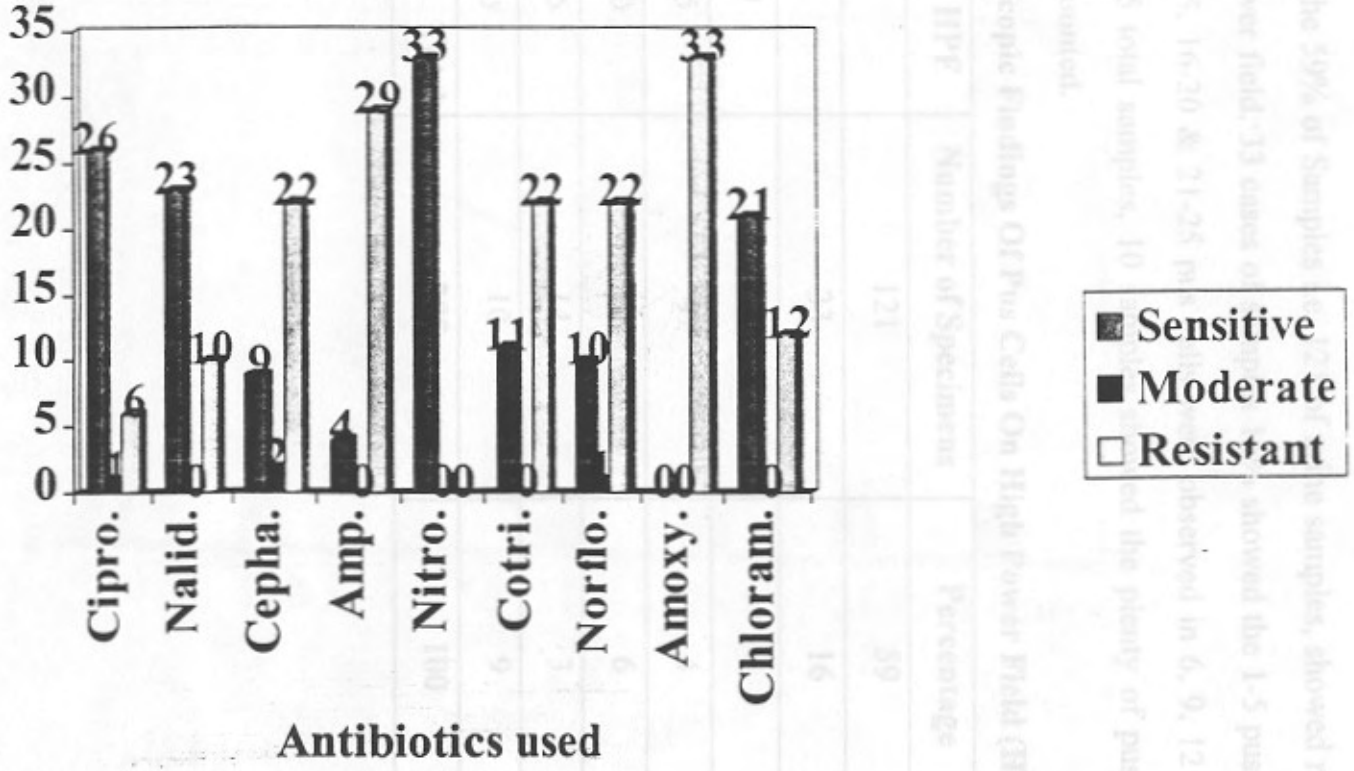


Fig. 7: MICROSCOPIC FINDING OF PUS CELLS ON HIGH POWER FIELD (HPF)

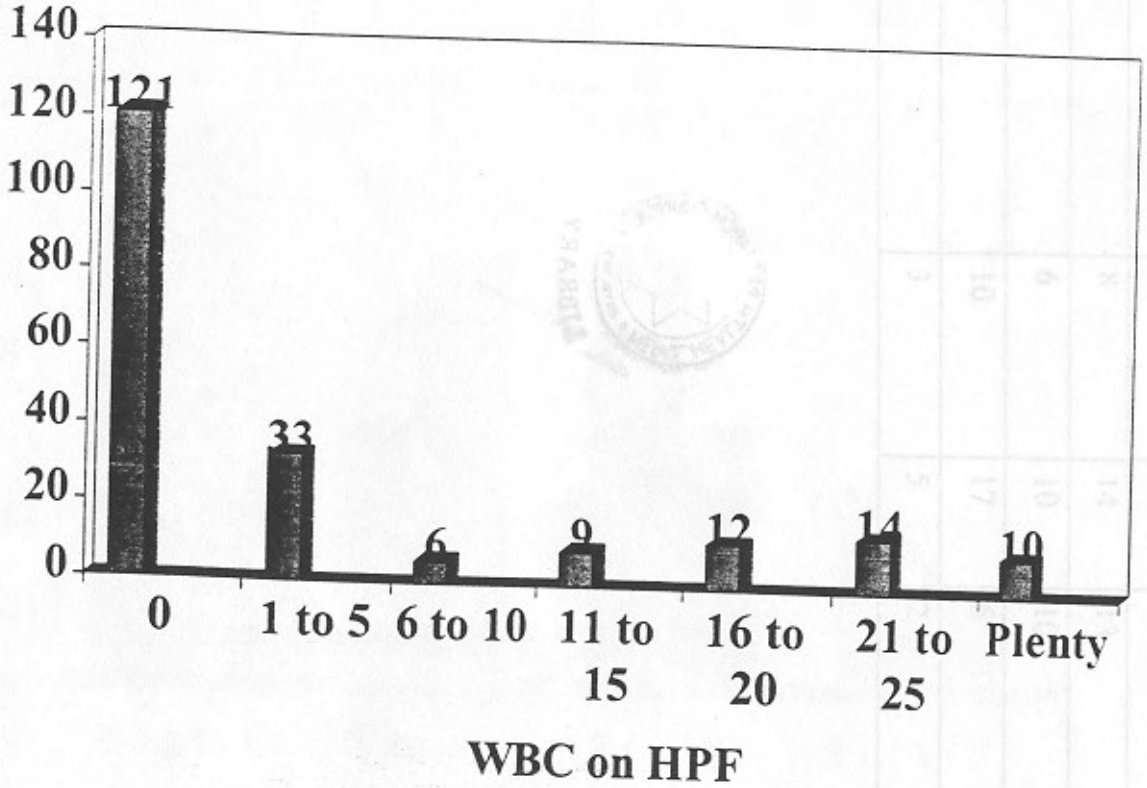


Fig. 8: URINE CULTURE POSITIVE CASES ACCORDING TO AGE

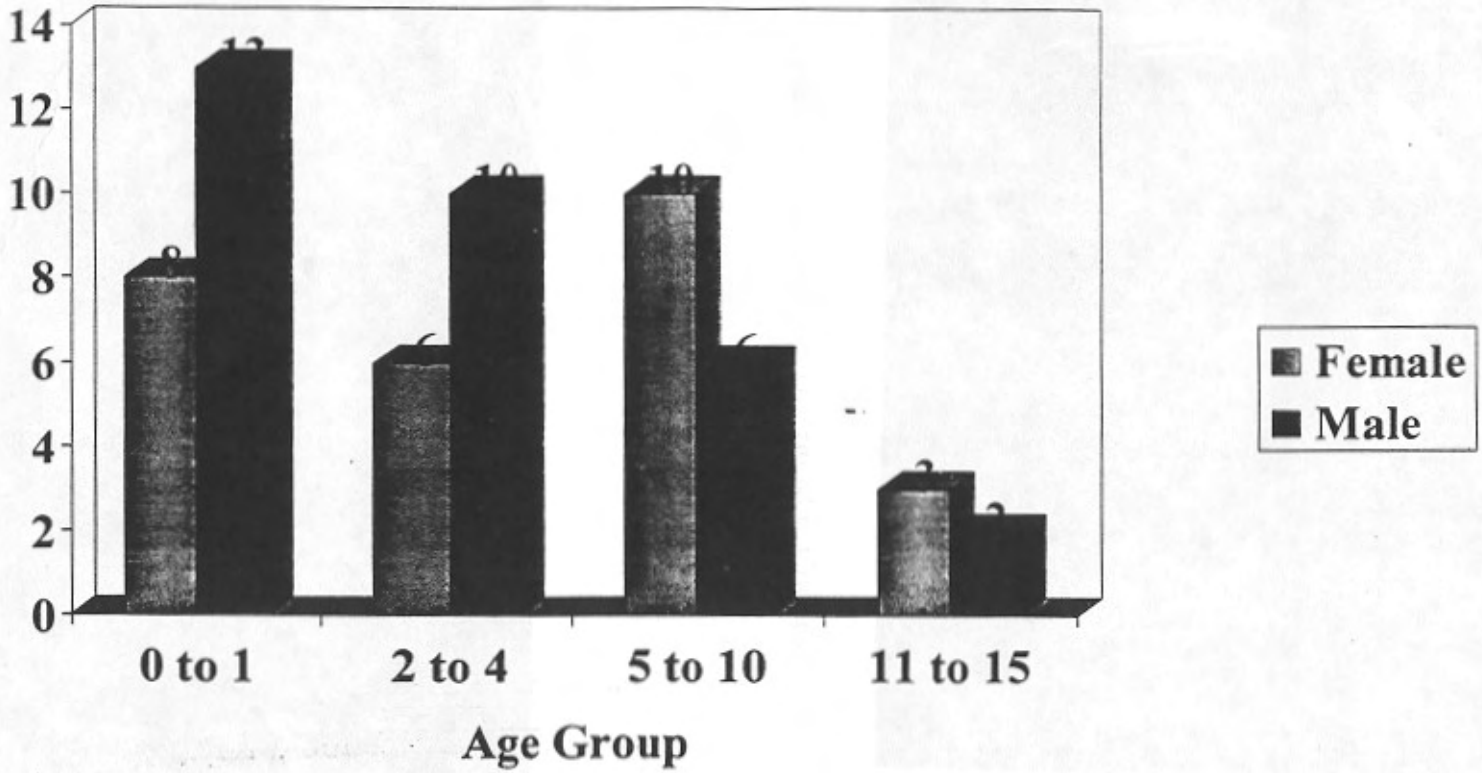
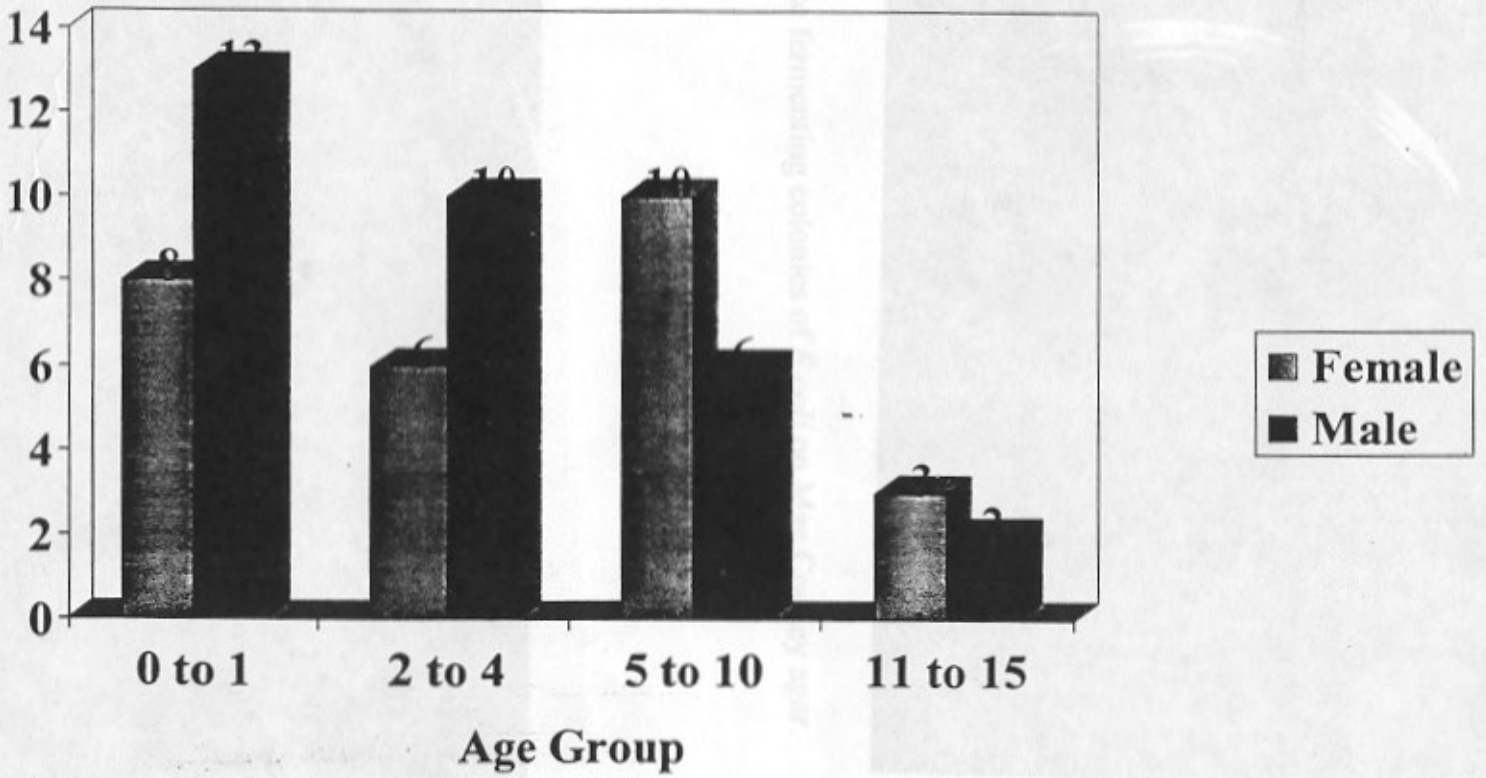
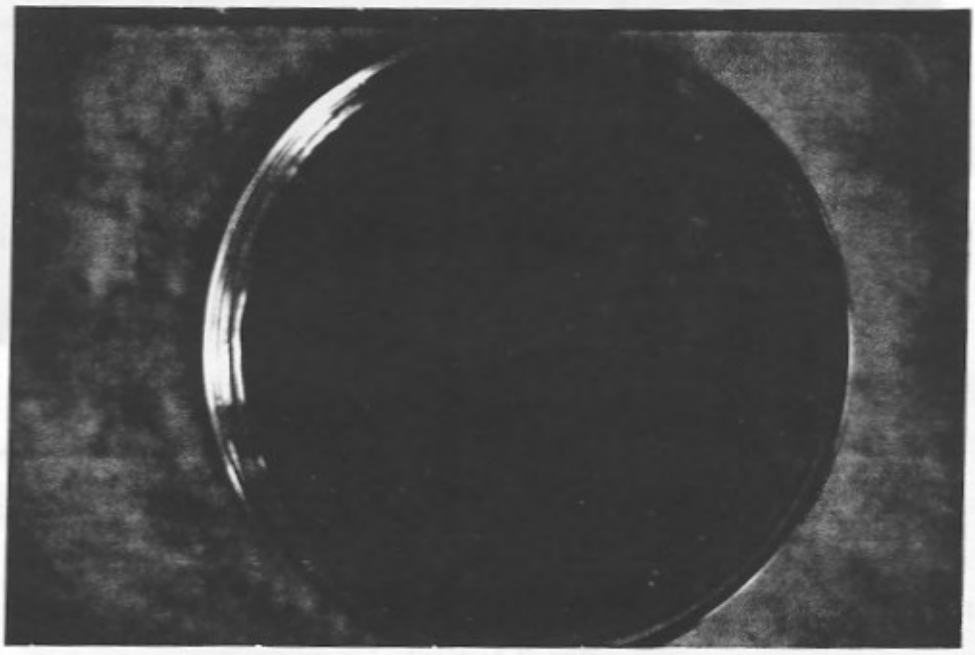


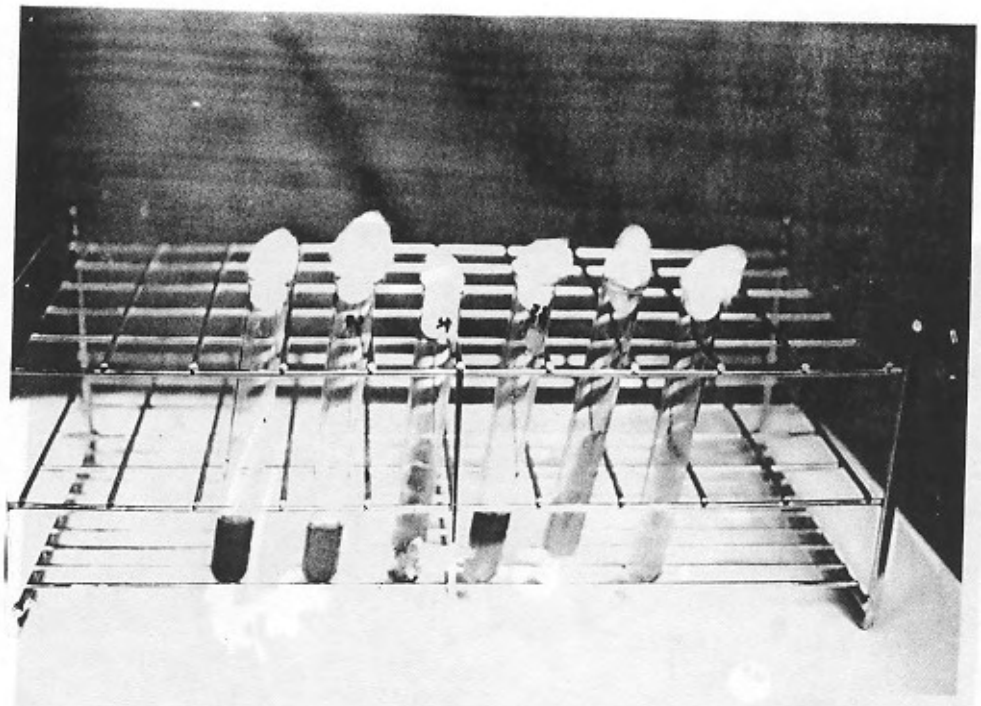
Fig. 8: URINE CULTURE POSITIVE CASES ACCORDING TO AGE



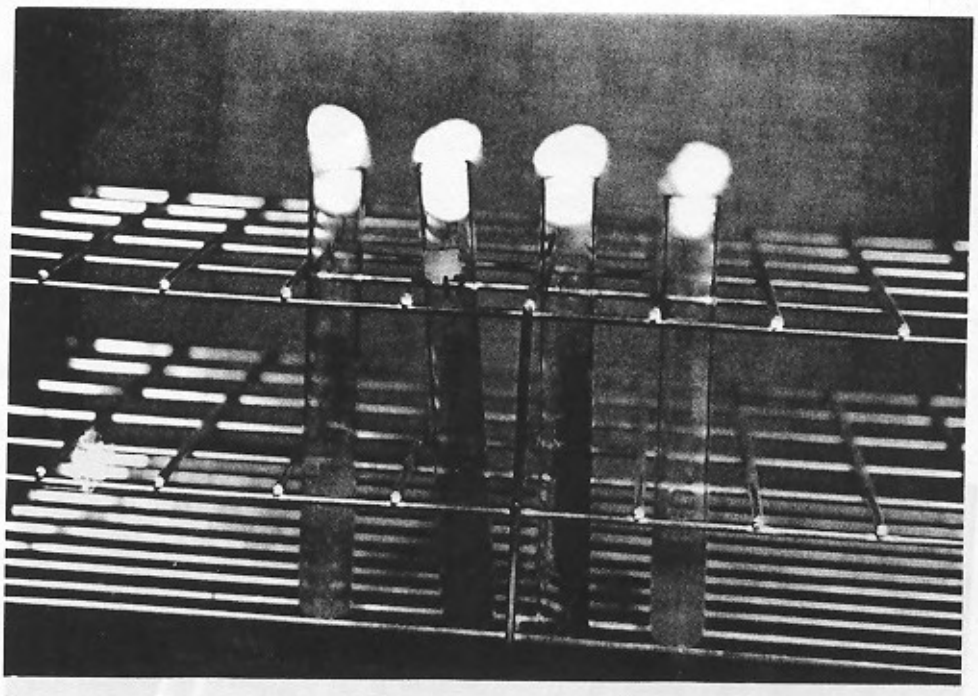


1. Lactose fermenting colonies of *E.coli* on Mac Conkey agar.

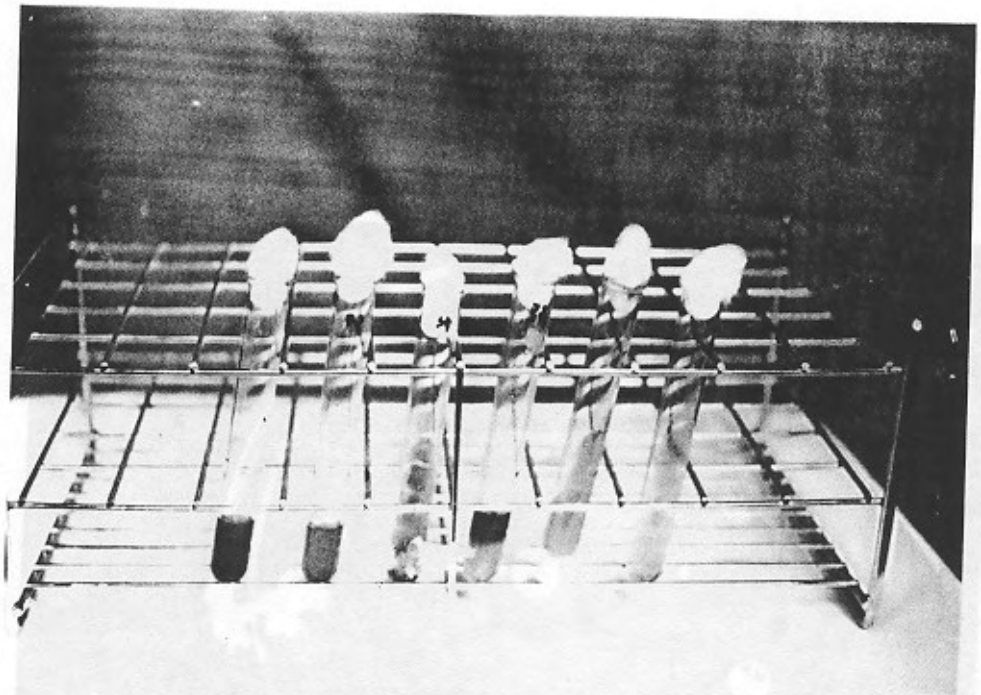
2. Mucoid colonies of *Klebsiella pneumoniae* on Mac Conkey agar.



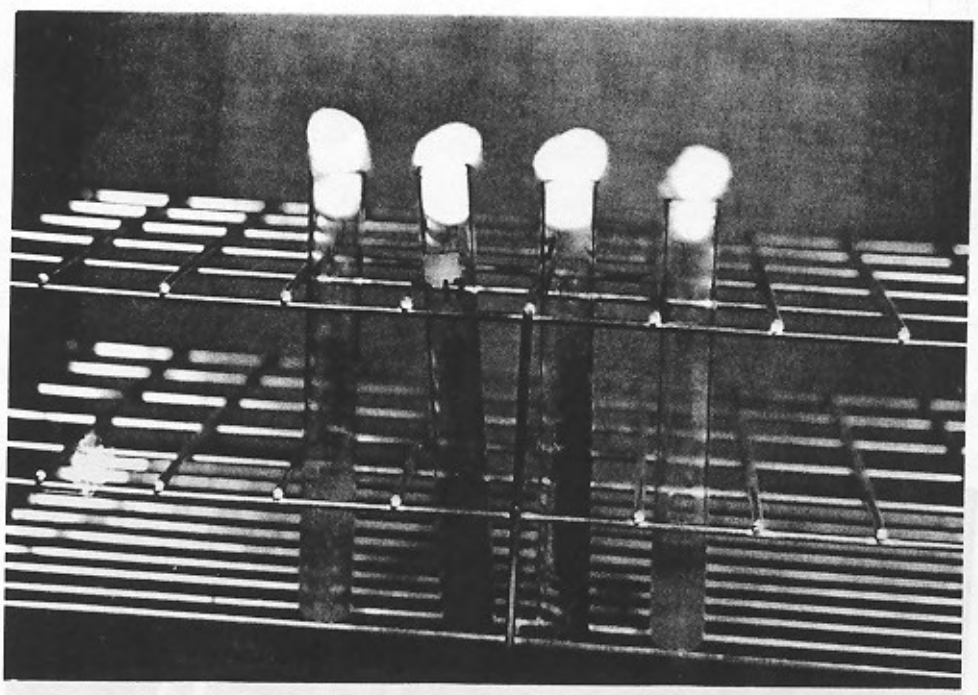
3. Biochemical test of *E. coli*.
(MR: +ve, VP: -ve, Citrate: not utilized, Indole: +ve, TSI. A/A, gas, Urease: -ve)



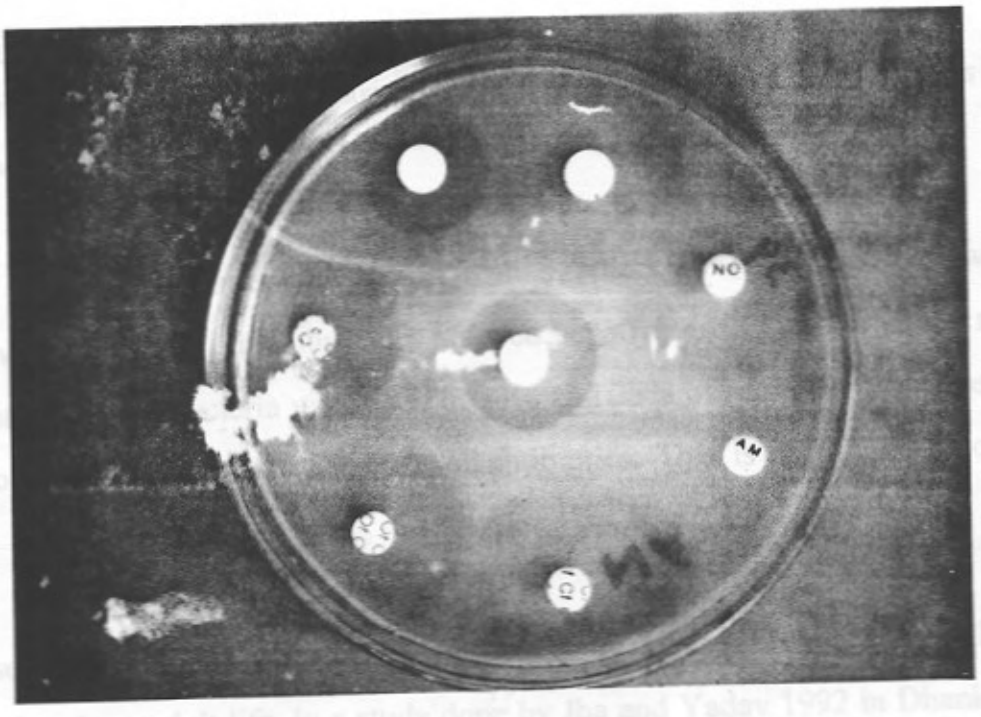
4. Biochemical test of *Klebsiella pneumoniae*
(TSI: A/A, Urease: +ve, Citrate: utilized, Indole: -ve)



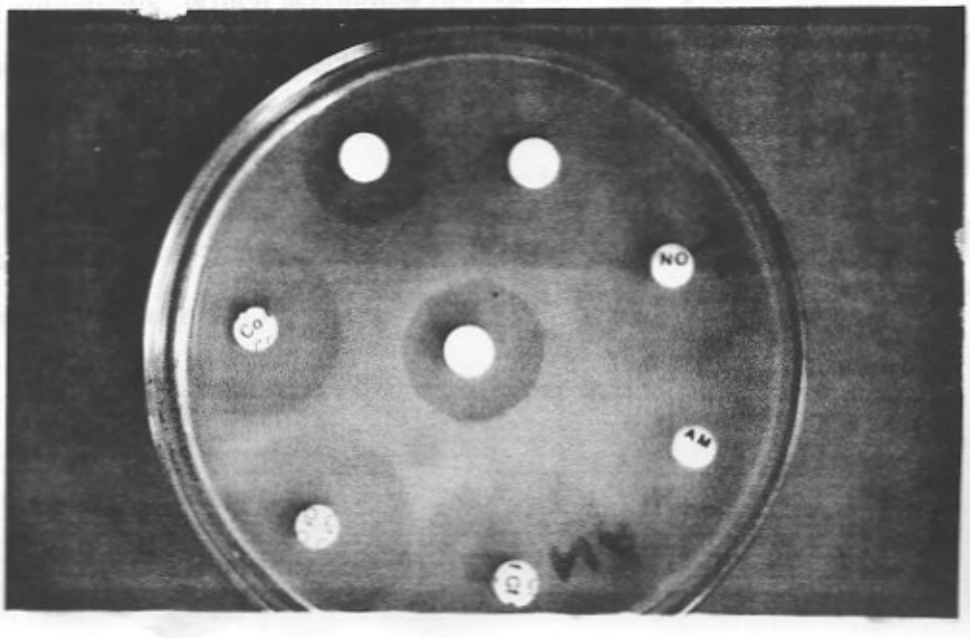
3. Biochemical test of *E. coli*.
(MR: +ve, VP: -ve, Citrate: not utilized, Indole: +ve, TSI. A/A, gas, Urease: -ve)



4. Biochemical test of *Klebsiella pneumoniae*
(TSI: A/A, Urease: +ve, Citrate: utilized, Indole: -ve)



5. Antibiotic sensitivity test by disc diffusion method on MHA plate.



CHAPTER SIX

DISCUSSION

This study was done on 205 paediatrics patients. Their age ranged from 10 days to 14 years with suspected UTI.

Table no. 3 shows the patterns of culture results, where 28% of samples showed the positive growth and remaining 33% showed negative growths. Mixed and not significant growth of bacteria were seen in 13% and 25% of samples respectively. Such a low growth could be prior use of antibiotics. This is one of the strong reason.

The present study found that the prevalence of UTI during childhood is less common than later adult life. In a study done by Jha and Yadav 1992 in Dhankuta Nepal, found that among 204 urine samples tested from various groups of patients including men and women both, 53.43% of urine samples gave positive result. Another study done by Sagarika Manandhar 1996 in TUTH found 38.2% culture report positive among 280 urine samples. In case of adult use of spermicide could be also contributory factor for enhancement of encourage the growth of enterobacterial organism. Using diaphragms for contraception of the vagina often invaded by enterobacterial organism rather then the normal flora.

In this study organisms predominantly grown on culture was *E.coli* in both sexes of in and outpatients. Which accounted (57%), followed by *Klebsiella pneumoniae* (24%), *Proteus* sps(10%) respectively. (Table no. 4) Such a high prevalence of *E.coli* isolated in this study was similar to many other studies done on UTI by various other investigators. In a study done by Umaran-k in Saudi Arabia in 1994, found that organisms predominantly grown on culture were *E.coli* followed by *Klebsiella pneumonia*. Gh Hashemi 1985 in Iran carried out a study on "Recurrent

urinary tract infection", found that the predominant organism was *E.coli* (78.8%) followed by *Klebsiella pneumonia* (18.8%).

Such a high rate of isolated *E.coli* and *Klebsiella pneumoniae* were also seen in a previous study done by Dr. Pushpa Raj Sharma et al, 1983 on "Urinary infection" of 100 children patients of age group of four days to 14 years. The higher percentage of organism isolate were *E.coli* (48%) followed by *Klebsiella* (19%), *Proteus* (16%), *Streptococcus faecalis* (13%), *Citrobacter* (4%).
in 1983 at Kanti Children's Hospital. In a study done by H.Dele Davies, E.L.Ford Jones, R.Y., A.G.Matlow and R.Glod (1992) in Canada about the "Nosocomial urinary tract infection at a paediatric hospital found that the most common organism isolated were *E.coli* (26%), *Enterococcus sps* (10%) and *coagulase negative Staphylococcus* (9%). A study done by Adeyemo, Onyemenem and Ekweozer et al in 1994 on 65 Nigerian children who had urinary tract infection was reported. The predominant isolate in and out patient was *Klebsiella sps* which accounted for 52.8% of cases likewise *E.coli*, *Pseudomonas sps* and *proteus sps* accounted for 25%, 15.31% and 5.5% in England. *Pseudomonas aeuroginosa* also the primary pathogen causing UTI, it was isolated from one patient. It was also seen that Gram +ve bacteria are also responsible for causing UTI. Among the total +ve cases *Staphylococcus aureus* and *Streptococcus faecalis* were isolated from two cases. During the study about 95% of children having fever, abdominal pain, vomiting showed the significant bacterial growth in their urine specimen. This came very similar to the studies done by Kumud Psd. Mehata.

Table no. 10 gives the idea about the relationship between the infection and pus cells. The study shows that as the number of pus cells increased, the significant pathogenic growth also increased. In this study 10 samples showed the plenty of pus cells which could not be counted 16 urine samples showed significant

bacteriuria. Which showed very few pus cells (less than 5) in HPF (high power field).

During the study, the child with signs and symptoms of UTI sometimes produced sample of urine that show pus cells but not yield a significant growth of bacteria on routine culture. The explanation may be that the patient has been taking antibiotics prescribed on a previous occasion. This finding is similar to the study carried out by Collins et al 1986. The study of age wise distribution was showed in table no. 11. According to the table, the male children of age groups of 0-1 were more infected, whereas female children of age groups 5-10 infected more. The frequency of UTI is much more common in male new born during the first three month and there after more often in girls and later in pre-school children, the bacteriuria is considerable higher in girls than boys.

According to the table no. 7 it showed that incidence is higher in female child than male child. This finding is similar to the study done by Hoghes & Graffith, they found that in neonates with UTI, boys predominant and there is shift from male to female. Study done by RHR White found that the prevalence of bacteriuria among school girls is 1 to 2% it is only 0.03% in boys of same age.

Table no. 9 gave the knowledge about the antibiotics susceptibility pattern of bacteria. Among the 58 total isolated organism, they were consider for antibiotic sensitivity pattern study.

During study period, Ampicillin resistant Gram Negative Rod (GNR) causing UTI were a significant problem. In sensitivity profile, Gram negative bacilli showed that among 33 isolates of *E.coli*, it was resistant to Ampicillin in 29 cases. Such a similar result was also found by Sharma 1992 where 93% of cases with *E.coli* were

resistant to Ampicillin. *Klebsiella pneumoniae* and *proteus sps*. second principle isolates were 100% resistant to Ampicillin.

Present study showed that based invitro sensitivity test, all isolates were moderately sensitive to the common first line drugs used in UTI in our set up namely Cotrimoxazole and Ampicillin. But exhibited good sensitivity to Nitrofurantoin, Ciprofloxacin and Nalidixic acid. Therefore these are the first drug of choice for UTI in children. This finding is similar to the study done by Umran-k 1994 in Saudi Arabia.

CHAPTER SEVEN

SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

This study was conducted for the purpose of determining the prevalence of organisms causing UTI among children and antibiotics sensitivity test profile of these organisms, attending in Kanti Children's Hospital.

The study period consist 10 months from March 1996 to December 1996. Two hundred five urine samples were collected from the children patients who attended in Kanti Children's Hospital. Those samples were then brought to department of microbiology, Institute of Medicine which is located close to the Hospital. These samples were also subjected for chemical examination especially for albumin test.

Following were the major finding obtained from the study.

1. The commonest organism causing UTI among children were *E.coli* 57% followed by *Klebesiella pneumoniae* 24% and *Proteus species* 10%.
2. With regards Gram +ve bacteria only *Staph. aureus* and *Streptococcus faecalis* were isolated separately into urine samples.
3. All the samples showing more than 5 pus cells per high power field while examining wet preparation of urinary deposit showed culture positive.
4. The most effective antibiotics were according to invitro experiment were Nitrofurantoin (88%) followed by Ciprofloxacin (81%), Nalidixic acid (69%) respectively. Whereas least effective antibiotic were Amoxycillin and Cotrimoxazole.

To get the information about seasonal variation of the organisms causing UTI this type of study should be conducted throughout the year. In order to have a

knowledge on UTI causing organism in different geographical region of Nepal included Terai, Mountain and Himalaya region of Nepal.

7.2 RECOMMENDATIONS :

1. If more than 5 WBC/ HPF are seen in the urinary sediment the patient should be advised for urine culture and sensitivity test.
2. Urine culture and direct microscopy is mandatory for appropriate diagnosis and treatment of disease.
3. This study should be conducted throughout the year to have information regarding seasonal variation of the organisms causing UTI.
4. It would be much better if this type of study could be extended to other part of country such as Terai, Mountain and Himalaya region of Nepal to get information about geographical variation of organisms causing UTI.

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APPENDIX ONE

QUESTIONNAIRE MODEL OF THE PATIENT

CLINICAL AND MICROBIOLOGICAL PROFILE

CLINICAL PROFILE

Patient's Name :
I.P. No. :
Ward :

Age :
O.P.D. No :

Sex :
Cabin:

- Brief clinical history :

- Any underlying condition :

- Provisional Diagnosis :

- Date of collection of specimen :
- Method of collection of specimen :
- Receiving time :
- Processing time :

II. MICROBIOLOGICAL PROFILE

(A) MACROSCOPIC EXAMINATION OF SPECIMEN :

Colour :
Whether (I) Clear (ii) Cloudy

(B) MICROSCOPIC EXAMINATION OF SPECIMEN FROM CENTRIFUGED URIN

i. White cells (Pus cells):
ii. Red cells (R.B.C) :
iii. Yeast cells :
iv. Epithelial cells :
v. Casts :
vi. Crystals :
vii. Others if any :

Chemical examination of Urine

i. Albumin :
ii. Sugar :

DAY ONE

C. **CULTURE OF SPECIMEN BY SEMIQUANTITATIVE METHOD WITH USING STANDARD LOOP :**

Media used :

Blood Agar (BA) :

Mac Conkey Agar (MA) :

DAY TWO

Blood Agar (BA) :
Mac Conkey Agar (MA) :

- Interpretation of Result :

Whether,

i. No Growth
ii. Not significant
iii. Low count significant bacteriuria
iv. Significant bacteriuria
    a. Gram's Stain
    b. Culture of single colony into Nutrient broth/ Peptone water for 4-6 hours at 37 ^{0}C & subculture into NA & MA/BA

DAY THREE

D. **ENZYMATIC TEST**

Result

I. Catalase
ii. Oxidase
iii. Coagulase

E. BIOCHEMICAL TEST

Result

I. TSI :
ii. Oxidation fermentation (O/F) :
iii. SIM :
iv. Methyl Red (MR) :
v. Voges- Proskauer (VP) :
vi. Citrate :
vii. Ureas :
viii. Decarboxylase test

Antibiotic Used	Result

H. **SEROTYPING IF NECESSARY**

- Organism Typed as :

I. **FINAL REPORT :**

APPENDIX TWO

DETAIL ABOUT COMPOSITION AND PREPARATION OF MEDIA USED IN ISOLATION IDENTIFICATION AND SENSITIVITY TESTING OF BACTERIA IN URINE SAMPLES

1. Culture media :

a. Nutrient agar (Hi - Media)

Ingredients	Grams/litre
Beef extract	10.0
Peptone	10.0
Sodium chloride	10.0
Agar	12.0
Final pH (at 25° C)	7.4 + 0.2

Directions :
37 grams was suspended in 100 ml D/W, boiled to dissolve the medium completely & and sterilized by autoclaving at 15 lbs pressure at 121^0 C for 15 minutes. It was mix well before pouring.

b. Blood agar base + 5% blood (Hi - Media).

Ingredients	Gram /litre
Beef heart	500
Infusion from tryptase	10
Sodium chloride	5
Agar	15
Final pH (at 25 °)	7.3 ±0.2

Directions :
40 gms. was suspended in 1000 ml distilled water. It was boiled to dissolved the medium completely and sterilized by autoclaving at 15 lbs pressure at 121^0 C for sterile defibrinated blood was added aseptically.

c. Mac Conkey agar (Hi- media) :

Ingredients	Grams/litre
Peptone	20
Lactose	10
Sodium Taurocholate	5
Agar	20
Neutral Red	0.04
Final pH (at 25^0 C)	7.4 ± 0.2

Directions :
55 grams was suspended in 1000 ml distilled water, then medium was boiled to dissolved completely. It was then sterilized by autoclaving at 15 lbs pressure at 121^0 C for 15 minutes.

2. Biochemical Media :

The biochemical media were weight out accurately. It was then dissolved to required volume of distilled water. Each medium was dispensed 5 ml. To each test tubes. At 121^0 C for 15 minutes at 15 lbs pressure media were sterilized and stored for further use.

a. MR-VP medium

Ingredients	Grams/litre
Buffered	7.0
Peptone	5.0
Dextrose Dipottassium Phosphate	5.0
Final pH (at 25^0 C)	6.9 ± 0.2

Directions :
17 grams was suspended in 1000 ml of distilled water. It was distributed in 10 ml amounts in test tubes and was sterilized by autoclaving at 15 lbs pressure at 121^0 C for 15 minutes.

b. Chritensen Urea Agar-Base :

Ingredients	Grams/ litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Disodium Phosphate	1.2
Monopottassium phosphate	0.8
Phenol Red	0.012
Agar	15.0
Final (at 25^0 C)	6.9 ± 0.2

Directions :
24 grams was suspended in 950 ml distilled water. It was boiled to dissolved the medium completely. Then it was sterilized by autoclaving at 10 lbs pressure at 115^0 C for 20 minutes. Medium was cooled to 45^0 C and 5 ml of sterilized 40 % urea solution (FD 048) was added for each 95 ml base. It was dispensed in tubes and cooled in a slant position.

c. SIM medium :

Ingredients	Grams/litre
Beef extracts	3
Peptone	30
Peptonized iron	0.2
Sodium thiosulphate	0.025
Agar	3
pH	7.3 ± 0.2

Directions :
36 grams was suspended in 1000 ml D/W. It was heated to boiling to dissolved the medium completely. After dissolving the medium, 5 ml medium was dispensed to each test tubes, then sterilized at 121^0 C for 15 minutes at 15 lbs.

d. Nutrient broth :

Ingredients	Grams/litre
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 25^0 C)	7.4 ± 0.2

Directions :
13 grams was suspended in 1000 ml distilled water. It was heated to boiling to dissolved the medium completely. Then 5 ml of medium was dispensed in each test tubes. After that sterilized in autoclave for 15 minutes at 15 lbs at 121^0 C.

e. Triple Sugar Iron agar :

Ingredients	Grams/ litre
peptone	10
Tryptone	10
yeas extract	3
Beef extract	3
Lactose	10
Saccharose	10
Dextrose	1
Ferrous Sulphate	0.2
Sodium Thiosulphate	5
Phenol Red	0.3
Agar	0.24
Final pH (at 25^0 C)	7.4 ± 0.2

Directions :
65 grams was suspended in 1000 ml of distilled water. It was boiled to dissolved the medium completely. It was mixed well and distributed into test tubes 5 ml each. Finally was sterilized by autoclaving at 15 lbs pressure (121^0 C) for 15 minutes and medium was allowed to set in sloped form with a butt about 1 inch long.

f. Peptone water :

Ingredients	Grams/litre
Peptone	10
Sodium chloride	5.0
Final pH (at 25 ^{0} C)	7.2 $\pm$ 0.2

Directions :
15 grams was dissolved in 1000 ml distilled water and sterilized at 15 lbs pressure at 121^0 C for 15 minutes.

g. Simmons citrate Agar (Hi - media)

Compositions	Grams/litre
Magnesium Sulphate	0.2
Monoammonia phosphate	1.0
Dipotassium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Agar	15.0
Bromothymal blue	0.08
Final pH (at 25 ^{0} C)	6.8 $\pm$ 0.2

Preparation :
About 24.2 gm was suspended in 1000 ml distilled water. The medium was boiled to dissolved completely. It was then distributed in tubes and sterilized by autoclaving at 15 lbs pressure at 121 ^{0}C for 15 minutes.

h. Hugh - Leifson Media (O/F Medium) :

Ingredients	Gram/ litre
Peptone	2.0
Sodium chloride	5.0
Dipotassium phosphate	0.3
Dextrose	10 .
Bromothymal blue	0.03
Agar	2.5
Final pH(at 25 ^{0} C)	7.1

Directions :
0.5 gm of dry ingredients was mixed in 50 ml of distilled water. Boiled to dissolved the ingredients completely dispensed in test tubes and sterilized by autoclaving.

3. Sensitivity testing media :

Muller Hinton Agar (Hi - media) :

Ingredients	Grams/litre
Beef infusion form	300
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25 ^{0} C)	7.4 $\pm$ 0.2

Directions :
Every constituent was added together. It was boiled to dissolve the medium completely. It was sterilize by autoclaving at 15 lbs pressure at 121 ^{0} C for 15 minutes.

4. Test Reagents :

a. Kovac's reagent (Indole test) :

n- Amyl alcohol	75.0 ml
Hydrochloric acid (conc.)	25.0 ml
P- dimethylamino- benzylaldehyde	5.0 gm

b. Methyl Red indicator solution :

Methyl red	0.1 gm
Ethanol	300 ml
Distilled water	200 ml

c. Baritt's reagent (Voges - Praskauer test) :

Solution A :

α - napthanol	5.0 gm
Ethyl alcohol (95 %	1000 ml

Solution B :

Potassium hydroxide	40 gm
Distilled water	100 ml

d. Oxidase test reagent :

About 1 gm of tetra- methyl dihydrochloride was mixed on 100 ml of distilled water.

E. Catalase reagent (3 % H_2O_2)

conc. Hydrogen peroxide	3.0 ml
Distilled water	9.7 ml

f. Normal Saline

0.85 % Sodium chloride in distilled water.

5. Staining reagents :

Gram staining reagents :

i. Crystal violet

Solution A :

Crystal violet	2.0 gm
Ethyl alcohol (95 %)	20 ml

Solution B :

Ammonium oxalate	0.8 gm
Distilled water	80 ml

ii. Grams iodine :

Potassium iodide	2 gm
Iodine	1 gm
Distilled water	100

iii. Decolorizer :

Acetone	75 ml
Distilled water	25 ml

APPENDIX THREE

A. Morphology & cultural Characteristics of Bacteria Commonly Isolated from Urine Samples.

Bacteria	Morphological Characteristics	Cultural Characteristics
1. E.coli	Gm -ve rod, 0.5 X 1.0 to 3.0 micron Non-spore forming, not encapsulated & motile.	On EBM agar circular, smooth convex colonies having a striking blue-purple or deep purple - black metallic seen on MA; LF colonies with smooth, glossy & translucent. On BA, convex shiny opaque showing haemolysis. Most urine produce a fetid odour.
2. Klebseilla pneumoniae	Plump, Gm -ve rods, 0.3 to 0.3X5.0 micron; non-motile & encapsulated	On EMB, blood & MA it give rise to large glistening mucoid colonies which produce mucoid string when growth is touched with needle.
3. Pseudomonas aeruginosa	Gm -ve rod, 0.5 to 0.5X1.5 micron, motile, occur singly in pairs of short chains.	It is characterized by the large, irregular, spreading lime-green or blue green fluorescent colonies with translucent periphery on NA & distinctive aromatic grape like odour. On BA, moist & shiny colonies.
4. Proteus species	Gm -ve rod, 0.5 to 1.0X1.0 to 3.0 micron, occurs singly, pairs of frequently in chains	The pleomorphic motile rod of the Proteus group is demonstrated by a characteristic swarming on NA. On BA, colonies swarm & produce an ammonical odour and at times, haemolysis. On EMB, colonies are colourless with fuzzy edges.
5. Aerobacter aerogenes	Gm -ve plump rod, 0.5 to 0.8X1.0 to 2.0 micron, occurs singly.	On MA red, glistening mucoid colonies having translucent border. On EMB, light blue with metallic sheen & occasionally have depressed centers.
6. Streptococcus faecalis (Enterococci)	Gm +ve, 0.5 to 1.0 micron in diameter non motile, non spore forming.	On BA, alpha or beta haemolysis.
7. Staphylococcus aureus	Gm +ve cocci, 0.1 - 0.5 micron in size, non spore forming, non capsulated, non motile, grape like cluster.	Facultative anaerobic,non lactose fermenting appearance, cream buff colour in BA.
8. Staphylococcus albus	Gm +ve cocci, non spore forming, non capsulated, non motile, grape like cluster.	Facultative anaerobec colonies are 1-3 micron in diameter with smooth glistening surface entire edge, opaque BA - pink colour colonies, beta haemolytic.

9. Salmonella typhi	Gm -ve rod, motile, non capsulated, non sporing, 1-3 micron in diameter.	On MA: NLF, pale smooth shining & translucent colony. On SS agar, colonies were NLF.
10. Staphylococcus hemoluticus	Gm +ve cocci in grape like clusters, non sporing, non motile, non capsulated.	Pale coloured opaque colonies in NA no haemolysis on BA colonies were surrounded by yellow zone due to acid production by fermentation of Mannitol.
11. Staphylococcus	Gm +ve cocci in short chain clusters, non motile, non capsulated and non spore forming.	Whitish colony on NA . Dark pink colony in MA. No haemolysis on BA colonies were circular entire raised and smooth.

B. Features use to assist in the identification of bacteria isolated from urine samples.

Bacteria	Indole Production	Methyl Red	Voges-Praskauer	Citrate Utilization	Hydrogen sulphide TSI	Urea Hydrolysis	Motility	Lactose fermentation	Coaguiase	O/F
E.coli	+ve	+ve	-ve	-ve	A/A	-ve	Motile	LF	-ve	Oxidative
Klebsiella pneumoniae	-ve	+ve	-ve	+ve	A/A,gas	+ve	Non motile	LF	-ve	Oxidative
Proteus mirabilis	-ve	+ve	-ve	+ve	Alk/A,H2S	+ve	Motile	NLF	-ve	Oxidative
Proteus vulgaris	+ve	+ve	-ve	+ve	Alk/A,H2S	+ve	Motile	NLF	-ve	Oxidative
Pseudomonas aeuroginosa	-ve	-ve	-ve	-ve	Alk/A	+ve	Motile	NLF	-ve	Oxidative
Salmonella typhi	-ve	+ve	-ve	-ve	Alk/A,H2S	-ve	Motile		-ve	Oxidative
Streptococcus faecalis							Non motile			Oxidative
Shigella boydii	-ve	+ve	-ve	-ve	Alk/A	-ve	Non motile	NLF		Oxidative
Staphylococcus aureus	-ve	-ve	+ve		A/A		Non motile		-ve	Oxidative

APPENDIX FOUR

DIFFERENT CULTURES BIOCHEMICAL AND SENSITIVITY TESTING MEDIA USED FOR ISOLATION AND IDENTIFICATION OF UTI CAUSING PATHOGENS

CULTURE MEDIA USED:

1. Nutrient agar
2. Blood agar
3. Mac Conkey agar

Biochemical Media Used :

All media used were from Hi Media Company India.

1. Simmon's citrate agar
2. Nutrient broth
3. Peptone water
4. Triple sugar Iron agar
5. SIM (Sulphide indol and mortility) media
6. Amino acid decarboxylase basal media
7. Urease agar
8. Hugh-Leifson media
9. MR-VP medium

REAGENT AND CHEMICALS USED :

1. MR reagent
2. VP reagent
3. Catalase reagent (i.e. 3% H_2O_2 solution)
4. Oxidase test reagent in filter paper strip
5. Kovac's indole test reagent
6. Gram's staining reagent

Antibiotic Disc Used :

For sensitivity tests different antibiotic discs were used. All of which were from Span Diagonostic Limited, India.

EQUIPMENT USED :

1. Autoclave- Sakura
2. Drying Oven - Sakura
3. Microscope - Olympus
4. Incubator - Sanyo
5. Weighing Machine
6. Freeze - Toshiba
7. Centrifuge - Kubota 8100