



Journal Homepage: -www.journalijar.com
**INTERNATIONAL JOURNAL OF
 ADVANCED RESEARCH (IJAR)**

Article DOI:10.21474/IJAR01/ 9417
 DOI URL: <http://dx.doi.org/10.21474/IJAR01/9417>



RESEARCH ARTICLE

A STUDY OF URINARY TRACT INFECTION IN CHILDRENS UNDER FIVE YEARS IN KARBLA.

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Manuscript Info

Manuscript History

Received: 22 May 2019
 Final Accepted: 24 June 2019
 Published: July 2019

Abstract

A study of urinary tract infection in childrens under 5years had been planned to identify the bacterial causes ,antibiotics sensitivity results and also immunological response to these bacteria .This study extend from 1/12/2004 to 1/12/2005 in pediatric hospital in Karbala .The methods of investigation used in this study were the standard criteria for urinalysis to look for puree which mean presence of more than 5 pus cells in centrifuged urine in high power field (WHO,2001) ,Also the color, cloudiness ,acidity of urine were registered for each sample . A urine culture was performed to detect the presence of any types of bacteria as well as their antibiotic sensitivity test were performed .The specific immunological response to bacterial types were assessed by immunodiffusion test .

The standard criteria of this study were the age range from birth to five years, sex, previous urinary tract infection in the child, residence, type of feeding, chief complaint of child, congenital abnormalities if present, and past medical history of child.

The total randomly selected cases in this study were 385 childrens (156 boys and 229 girls), Pyuria were diagnosed in 100 cases of them .they were 37 boys and 63 girls . 56 positive urine cultures for boys and girls of which 21(13.5%) from 156 cases for boys and the results for girls were 35(15.3%) from 229 cases .The numbers of positive urine cultures for *E .coli* were 37(66%),10(18%) for *Proteus*, 3(5.4%) *Klebsiella*, 1(1.8%) *Pseudomonas*,4(7.1%) *Staphylococcus saprophyticus* ,1(1.8%) for *Enterobacter* . in 44 cultures we couldn't isolate any bacteria although the cultures incubated for more than 48 hours , 20 culture for boys and 24 for girls and considered negative due to the use of antibiotics.

E .coli susceptibilty to antibiotics was characterized by high resistance to amoxicillin, cotrimaxazole, and where considered susceptible to nitrofurantoin, nalidixic acid , cefotaxime, gentamicin. The high resistance among *Klebsiella pneumonia* also toward amoxicillin and for cefotaxime, where considered susceptible toward nitrofurantoin , cotrimaxazole .

The high resistance of *proteus* toward amoxicillin and sensitive for nalidixic acid and cefotaxime.

The sensitive antibiotics of *Staphylococcus saprophyticus* were for cefotaxime, nalidixic acid , nitrofurantoin .

The sensitive antibiotics of *Pseudomonas* and for *Enterobacter* were

for cefotaxime and nitrofurantoin. The results of immunological response to *E. coli* show (2792.5 mg/dl) IgG antibodies which represent chronic infection and (318.5 mg/dl) IgM antibodies which represent acute infection. However with *proteus* show (2439.6 mg/dl) anti *proteus* IgG antibodies and only (66.8 mg/dl) IgM anti *proteus* antibodies.

And for cases of pyuria with negative cultures show (1448 mg/dl) IgG antibodies and (16.8 mg/dl) IgM antibodies. So in general the immunological response for *E. coli* was higher than *Proteus* which was higher than pyuria cases with negative cultures.

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Introduction:-

The Urinary tract infection (UTI) are bacterial infection of Urinary tract (kidney, ureter, bladder, and urethra). UTI are usually associated with congenital abnormalities of urinary tract. These infection can become serious if undetected, and can some time lead to permanent kidney damage, however, they are generally treated effectively with antibiotics (Ginsburg et.al, 1982).

The urinary tract infection is defined by the presence of pure bacterial growth > 100,000 colony forming unit /ml (Kass, 1955).

The Pyuria mean presence of more than 5 pus cells in high power field of microscope in centrifuged urine (WHO, 2001). So white blood cells (leukocytes) are counted.

The Pyuria is sufficient for diagnosis of Urinary tract infection in non hospitalized patient if standard symptoms or just fever in small children are also present (Harvey, 2002).

Recurrent urinary tract infection are either relapse with the original infecting organism or reinfection with a different organism. In general, relapses developed within 3 weeks after cessation of therapy for previous infection.

Occasionally, reinfection occur with the same organism that has persisted in the vagina or feces and may be mistaken for relapse. Reinfection account for 80% of recurrent infections (Stamey, 1972).

Aims of study

1. To determine the prevalence of bacterial causes of UTI.
2. to evaluate the efficacy of some related factors.
3. to detect the types of antibiotics which are effective.
4. to study the immunological response of children with UTI for specific types of bacteria.
5. This study focus on why children have UTI and what can be done to prevent these infections.

Chapter Two Review Of Literatures

Anatomy of the urinary tract

Kidneys

Anatomy:

The kidneys lie along the borders of the psoas muscles and are therefore oblique placed. The adult kidney weighs about 150 gram. The kidneys are supported by the perineal fat (which is enclosed in the perineal fascia), the renal vascular pedicle, abdominal muscle tone, and the general bulk of the abdominal viscera. On longitudinal section the kidney is seen to be made up of an outer cortex, central medulla, and the internal calices and pelvis. The cortex is homogenous in appearance, portions of it project toward the pelvis between papillae and fornices and are called the columns of Bertin. The medulla consists of numerous pyramids formed by the converging collecting tubules which drain into minor calices (Emil, 1981).

Histology:

The kidney composed of three parts

Nephron: It is the functioning unit of the kidney which is composed of a tubules which has both secretory and

excretory function.

Secretory portion: It is contained largely within the cortex and consists of a renal corpuscle and the secretory part of the renal tubule. The excretory portion of this duct lies in the medulla.

The renal corpuscle is composed of the vascular glomerulus which projects into Bowman's capsule which in turn is continuous with the epithelium of the proximal convoluted tubule.

The secretory renal tubule is made up of the proximal convoluted tubule, the loop of Henle and distal convoluted tubule.

Excretory portion:

It is the collecting tubule which is continuous with the distal end of ascending limb of convoluted tubule. It empties its content through the tip (papilla) of pyramid into a minor calyx (Emil, 1981).

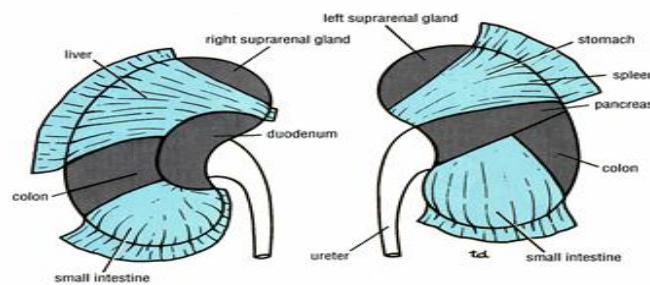


Figure 2.1 Anterior relations of both kidneys. Visceral peritoneum covering kidneys has been left in position. Shaded areas indicate where kidney is in direct contact with adjacent viscera.

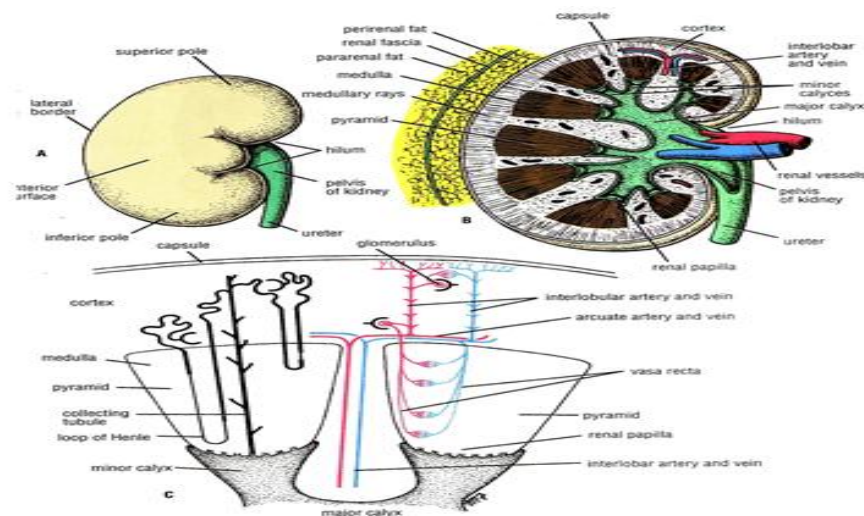


Figure 2.2 (A) right kidney, anterior surface. (B) Right kidney, coronal section, showing cortex, medulla, pyramids, renal papillae, and calyces. (C) Section of kidney showing positions of nephrons and arrangement of blood vessels within kidney. These figures taken from (Snell, 1993).

Calices, Renal pelvis and ureters

Anatomy:

Calices:

The tip of the minor calices 8-12 in number are indented by the projecting pyramids. These calices unite to form 2 or 3 major calices which join the renal pelvis.

Renal pelvis:

The pelvis may be entirely intra renal or partly intra renal and partly extra renal (Emil 1981).

The pelvis emerges through the lower part of hilus and tapering downward on psoas major, join the ureter near the inferior extremity of the kidney (Romanes, 1986).

Ureters:

These are narrow muscular tubes 25 cm long which convey urine from the kidneys to the bladder. Ureters having 3 constrictions:

1. Where renal pelvis join the ureter.
2. Where it is kinked as it cross the pelvic brim.
3. Where it pierce the bladder wall.

The ureters emerges from the hilus of kidneys and runs vertically downward behind the parietal peritoneum on the psoas muscles which separates it from the tips of the transverse processes of the lumbar vertebrae.

Bladder**Anatomy:**

This muscular urine store in women its posterior wall and dome are invaginated by the uterus. The adult bladder has capacity of 350-450 ml. The empty bladder is pyramidal in shape having an apex, a base and a superior and two inferolateral surface. The apex of the bladder lies behind the upper margin of the symphysis pubis. The base or posterior surface of the bladder triangular in shape, the superolateral angles are joined by the ureters and the inferior angle gives rise to the urethra (Richard, 1986).

Histology:

The bladder has mucous, submucous, muscular, subserous and serous (peritoneal) coats. The muscular coat is composed of smooth muscle fibers (detrusor muscle) which are thickest around the internal urethral orifice and form the vesicle sphincter (Lumely et al, 1981).

Classification of urinary tract infection :

There are three basic forms of UTI: pyelonephritis, cystitis, and asymptomatic bacteriuria.

Clinical pyelonephritis:

is characterized by any or all of the following: abdominal or flank pain, fever, malaise, nausea, vomiting, jaundice in neonates, and occasionally diarrhea. Some newborns and infants may show nonspecific symptoms such as poor feeding, irritability, and weight loss.

Cystitis:

indicates that there is a bladder involvement and includes dysuria, urgency, frequency, suprapubic pain, incontinence, and malodorous urine.

Asymptomatic bacteriuria:

refer to a positive urine culture in children's without any manifestations and occurs almost exclusively in girls (Rushton, 1997).

Normal flora of the Urogenital Tract.

Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms have problems gaining access and becoming established. The flora of the anterior urethra, as indicated principally by urine cultures, suggests that the area may be inhabited by a relatively consistent normal flora consisting of *Staphylococcus epidermidis*, *Enterococcus faecalis* and some alpha-hemolytic streptococci. Their numbers are not plentiful, however. In addition, some enteric bacteria (e.g. *E. coli*, *Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.

The vagina becomes colonized soon after birth with corynebacteria, staphylococci, non pyogenic streptococci, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). During reproductive life, from puberty to menopause, the vaginal epithelium contains glycogen due to the actions of circulating estrogens. Doderlein's bacillus predominates, being able to metabolize the glycogen to lactic acid. The lactic acid and other products of metabolism inhibit colonization by all except Doderlein's bacillus and a select

number of lactic acid bacteria. The resulting low pH of the vaginal epithelium prevents establishment of most bacteria as well as the potentially-pathogenic yeast, *Candida albicans*. This is a striking example of the protective effect of the normal bacterial flora for their human host (Todar,2002).

Pathophysiology

Almost all UTIs are ascending in origin and are caused by bacteria in the GI tract that have colonized the periurethral area. After birth, the periurethral area, including the distal urethra, becomes colonized with aerobic and anaerobic microorganisms (Bollgren and Winberg .1989). These organisms appear to function as a defense barrier against colonization by potential pathogens. Disturbance of the normal periurethral flora, such as may occur when an upper respiratory tract infection is treated with a broad-spectrum antibiotic, predisposes to colonization of the periurethral area by potential uropathogens (Lidefelt .1991).

Data from studies of women with recurrent UTIs support the concept that periurethral colonization with a uropathogen plays an important role in the pathogenesis of recurrent infections (Stamm.1984). These findings have not been confirmed in children (Schlager,et,al.1993).

However, children recently treated with antibiotics do have an increased risk of a febrile UTI compared with children with a non-UTI febrile illness who did not have recent antibiotic exposure (Mårild,et,al.1989).

The pathophysiology of UTI reflects a complex interaction between virulence factors of the microorganisms and the host defense(Barnett and Stephens.1997).

The perineal flora are normal inhabitants of the distal urethra. Urine in the proximal urethra, the urinary bladder, and more proximal sites within the urinary tract is normally sterile. Uropathogens must gain access to the urinary bladder and proliferate if infection is to occur. Bacteria in the distal urethra may gain access to the bladder because of turbulent urine flow during normal voiding, as a consequence of voiding dysfunction, or as a result of the use of instrumentation. In any case, normal voiding results in essentially complete washout of contaminating bacteria. Therefore, urinary bladder colonization does not usually occur unless bladder defense mechanisms are impaired or a virulent strain of bacteria has gained access to the bladder. In the absence of normal bladder emptying, there is proliferation of bacteria in bladder urine and the risk of a UTI. Even with normal bladder emptying, adherence to uroepithelial cells by virulent organisms such as P-fimbriated *Escherichia coli* may result in a UTI.

P fimbriae (or pili) are organelles on *E coli* that mediate attachment to specific receptors on uroepithelial cells and impair washout of the bacteria (Roberts,et,al.1985).

The majority of UTIs in neurologically and anatomically intact children are caused by *E coli*. Children with intestinal carriage of P-fimbriated *E coli* are at increased risk for UTI because of colonization of the periurethral area by these pathogens (Plos,et,al.1995).

Epidemiology

1. The urinary tract infection is not uncommon in childhood. about 8% or more of girls and about 2% of boys will have a urinary tract infection at some time during childhood. The majority of urinary tract infections occur in the first year of life.
2. Most UTI are due to normal bowel flora. *E. coli* is the causative organism in 80% of cases. Other common causative organisms are *Klebsiella* ,*Pseudomonas* and other gram-negative organisms.
3. Urinary tract infections are much more common in girls than boys (at least 10:1)-this is true for all age groups except the newborn where the higher incidence of congenital abnormalities of urinary tract in the male, makes the reverse true(Larcombe .1999) .

Diagnosis

Urine sample collection

the specimen for urinalysis and culture should be obtained by catheter or suprapubic aspiration in the infant or child unable to void on request .suprapubic aspiration is the method of choice in the uncircumcised male .a midstream clean catch specimen may be obtained from the child with urinary control. A bagged specimen of urine that show no growth or fewer than 10000 colony-forming units(CFU) per ml is evidence of the absence of a UTI .if the child who not yet achieved urinary control has symptoms that mandate immediate treatment ,and analysis of the urine specimen obtained by bag shows pyuria ,a urine sample should be obtained by suprapubic aspiration or catheter

before stating antibiotic therapy because of high incidence of false positive urine culture (Ginsburg, et al, 1982). So the ways of collection of urine was recommended as follow.

1. Urine bag collection (not recommended)
2. High incidence of contamination.
3. Clean catch urine of first morning void.
4. Urine catheter specimen.
5. Recommended if child under age 2 years.
6. Suprapubic aspirate.
7. consider for child under age 6 months old (Bullock, et al, 2000).

Microscopic Urinalysis

A urinalysis involve physical and chemical examination of urine. In addition, the urine is spun in a centrifuge 10-15 ml at 1500 to 3000 rpm for 5 minutes to allow sediments containing blood cells, bacteria, and other particles to collect. This sediment is then examined under a microscope. A urinalysis then, offers a number of valuable clues for an accurate diagnosis:

1. Simply observing the urine for color and cloudiness can be important.
2. Acidity is measured.
3. White blood cells (leukocytes) are counted. A high count in the urine is referred as pyuria. (A leukocytes count of over 10 per micro liter of urine indicates pyuria).

Pyuria is usually sufficient for a diagnosis of UTI in non hospitalized patient if standard symptoms (or just fever in small children) are also present (Harvey, 2002).

Urine culture

A urine culture is a urine specimen observed for 24 to 48 hours in a laboratory for the presence of any bacterial growth. It is not routinely performed but may be conducted under certain circumstances

1. If urinalysis is negative but the patient has severe UTI symptoms, particularly in hospitalized patients who have a catheter and who develop fever or other signs infection.
2. If the infection is recurrent.
3. if the physician suspects complications.
4. if girls younger than two years have a high fever of unknown origin that lasts two days or more (Harvey, 2002).

Blood sample

to assess the renal function including blood urea and serum creatinine (Baum, et al, 1985).

Renal ultrasonography images

may show size, scarring of kidney, and the calculi if present (farmor, et al, 2002).

Intravenous Urography (IVU)

It helps to establish the diagnosis of pyelonephritis because they reveal caliceal dilatation and blunting with cortical scars. Ureteral dilatation and reduced renal size also may be evident. There: may be also cortical thinning over pelvo-calyceal lesions (Teplick, 1988).

Computerized Tomographic

scan (CT scan): It is the procedure of choice to help diagnose chronic pyelonephritis (Gerzof and Gale, 1982).

Voiding cystourethrogram (VCUG);

The findings may document the reflux of urine to the renal pelvis and ureteral dilatation in children with gross reflux (Ilyas, et al., 2002). **Radio isotopic scanning with technetium dimercaptosuccinic acid:** It is more sensitive than intravenous urography for helping detect renal scars. This is the preferred test for many pediatric nephrologists and radiologists because it is sensitive and easy to perform and can detect VUR and renal scarring (Carroll, et al., 1981).

Cystoscopy:

It shows the evidence of reflux at the ureteral orifices or site of kidney stone (Ilyas, et al, 2002).

Radial immunodiffusion test

Intended use

The quantitation of serum proteins as an aid in diagnosing deficiency disorders.

Summary

Single radial immunodiffusion tests have evolved from the work of (Fahey .et.al,1965). They are specific for the various proteins in serum or other fluids and depend on the reaction of each protein with its specific antibody.

When the wells in antibody containing gels are completely filled with the antigen, the precipitin rings which develop after 10-20 hours at room temperature are measured. The diameter of the ring and the logarithm (base 10) of the protein concentration are related in a linear fashion. Using appropriate reference standards, the concentration of unknown samples may be measured.

Principle

Radial immunodiffusion is based on the diffusion of antigen from a circular well radial into a homogeneous gel containing specific antiserum for each particular antigen. A circle of precipitated antigen and antibody forms, and continues to grow until equilibrium is reached. The diameters of the rings are a function of antigen concentration. After overnight incubation, the zone diameters of reference sera are plotted against the logarithm (base 10) of the antigen concentration. If equilibrium is reached, the reference sera zone diameters are squared and plotted against their concentration (linear). At intervals in between, a linear relationship does not occur. Unknown concentrations are measured by reference to the standard curve.

Antibiotics for Patients with urinary tract infection:

Amoxicillin :

It is semi synthetic antibiotic from penicillin group that interferes with synthesis of cell wall mucopeptides during active multiplication of bacteria, resulting in bactericidal activity against gram-positive and gram-negative bacteria. In past, it has been used frequently for treatment of UTI. Now, most bacteria presented in UTI have high resistance to amoxicillin as shown by (Sakran.et.al,2003) who observed that only 52% of bacterial UTI responded to amoxicillin. In addition, (Leblebicioglu and Esen ,2003) and (Aggarwal. *et. al*, 2003) showed that more than 73% of *E.coli* and 88.5% of *K.pneumonia* isolated from patients with UTI were resistant to amoxicillin. *Enterobacter*, *Acinetobacter* and *Corynebacterium spp.* have a high resistance to amoxicillin (Zhou. *et. al*, 2002; Savov. *et. al*, 2002; Suarez. *et. al*, 2002).

Cephalosporin's

All first three generations of cephalosporin's have oral preparations that have been used for treatment of recurrent UTI (Wilhelm and Edson , 1987).

First generation cephalosporin's:

They arrest bacterial growth by inhibiting bacterial cell wall synthesis. The bactericidal activity is against gram-positive bacteria and the administration is either oral (cefadroxil, cephalexin and cephadrine) or parenteral (cephalothin, cephalozin and cephradine). Now; the usage of first generation cephalosporins is limited because of high resistance to it (Martinez et.al. 1995).

Second generation cephalosporin's:

They have bactericidal activity that inhibits bacterial cell wall synthesis. It has greater activity against anaerobic bacteria and the administration is either oral (cefaclor and cefuroxime axetile) or parenteral (cefamandole, cefmetazole, cefotetan, cefoxitin and cefuroxime). Second generation cephalosporins has limited use that Dumpis. *et. al*, (2003) showed that only 26% of patients with hospital-acquired UTI responded to second-generation cephalosporin's.

Third generation cephalosporin's:

They have bactericidal action that inhibits cell wall synthesis. They are highly stable in the presence of B-lactamase enzyme and they are effective in wide range of hospital-acquired and nosocomial bacterial infections. The administration is either oral (cefixime, cefpodoxime and ceftibuten) or parenteral (cefotaxime, ceftriaxone, ceftazidime and ceftizoxime). These drugs are excreted in bile, therefore they may use for patients with renal insufficiency (Katzung, 2002).The third generation cephalosporin's have been found affected in bacterial

UTI that more than 80% and 71% of bacteria isolated from patients with UTI were sensitive to cefotaxime and ceftriaxone respectively (Gordon and Jones, 2003). In addition, 78% of * *Acinetobacter* isolated from patients with

UTI were also sensitive to cefotaxime (Irgbu et. al., 2003) but Zhou et. al., (2003) showed that *Enterobacter* were highly resistance to cefotaxime.

Trimethoprim-sulfamethoxazole (TMP-SMX)

A combination of trimethoprim and sulfamethoxazole inhibits bacterial growth by inhibiting synthesis of dihydrofolic acid. It is represented the essential co-factor of purine, pyrimidine and amino acid synthesis. Its antibacterial activity includes most common urinary tract pathogens except *Pseudomonas aeruginosa*. The combination is contributed to the efficacy in treatment of upper UTI via synergistic bactericidal effect and may diminish the emergence of resistance (Burman, 1986).

Nowadays, the resistance to TMP-SMX is slightly increased that 45% of bacterial UTI were resistant to it including 11% of *E. coli* and 42% of *S. epidermidis* isolated from patients with UTI (Ghiro et. al, 2002 and Jureen et. al, 2003).

Nitrofurantoin

It is synthetic nitrofurantoin that interferes with bacterial carbohydrate metabolism by inhibiting acetyl coenzyme A. It is bacteriostatic at low concentrations, bactericidal at higher concentrations, and effective against most uropathogens but not *Pseudomonas* and *Proteus* species. It is presented for brief periods at high concentrations in the urine and leads to repeated elimination of bacteria from urine (Stamey et. al, 1987). The risk of adverse reaction increases with age and long-term therapy; therefore, should be monitored (Holmberg . et. al, 1980).

Nitrofurantoin has lower resistant rate than other old antibiotics that only 4% of bacteria isolated from patients with recurrent UTI were resistant to it (Leblebicioglu and Esen, 2003).

Aminoglycosides

It is bacteriostatic action such as streptomycin, gentamicin, amikacin, netilmicin and tobramycin. Gentamicin is effective but it is associated with a risk of nephrotoxicity and ototoxicity, making tobramycin possible alternatives. Because gentamicin is stored in renal tissues, it can prevent acute retrograde pyelonephritis. Since different aminoglycosides accumulate and persist to various degrees in the kidney parenchyma, they have protective activity of aminoglycosides against renal scarring (chronic pyelonephritis). These results suggest that renal accumulation and persistence of aminoglycosides may be used to advantage in the prophylaxis or in the treatment of kidney infections (Robert, 1999). Gentamycin is cost-effective parenteral therapy because only once-daily dosing needed and has a good sensitivity against gram-negative uropathogens (90%) and it can be in combination with TMP-SMX against gram-positive uropathogens (70%) (Ghiro. et. al, 2002).

Fluoroquinolones

They are bactericidal drugs that act as inhibitors of bacterial DNA gyrase enzyme (which responsible for supercoiling of bacterial DNA). They affected against gram-positive and gram-negative bacteria. Recently, the oral administration of fluoroquinolones like nalidixic acid, ciprofloxacin, levofloxacin, ofloxacin, norfloxacin and others used for empirical treatment of UTI. They increased considerably for managing of complicated UTI particularly chronic pyelonephritis due to the ability to treat difficult pathogens with high antibiotic resistance like *Pseudomonas*. They can be administered parenterally, and then they can easily switch to oral administration and have limited use in patients with renal insufficiency (Dalkin and Schaeffer, 1988).

Bacteriological Agents

Enterobacteriaceae

They are gram-negative bacilli; normally habituate in the intestinal tract of human being. Some of them act as a part of a normal flora and incidentally causes the diseases while others are pathogenic for humans. They possessed a complex antigenic structure and produced a variety of enzymes and toxins with other virulence factors (Mims, 2004).

Table 2.1:- Biochemical tests of Enterobacteriaceae modified from (Brooks. et. al, 2001).

Test	<i>E. coli</i>	<i>K. pneumonia</i>	<i>A. Proteus</i>	<i>Enterobacter</i>	<i>Serratia</i>
EMB	metallic	II. CENTRALLY DARK	pale	a) Centrally dark	Centrally dark
LF	+	+	—	+	+
Catalase	+	+	+	+	+
Oxidase	—	—	—	—	—

Indol	+	—	±	—	—
MR	+	—	±	—	—
VP	—	+		+	+
Urease	—	+	+	—	—
Citrate	—	+	±	+	+
Motility	±	—	+	+	+
TSI	A/Alk± G	A±G	2) Alk±G	A±G	Alk±G
H2S	—	—	+	—	—

A acid
Alk alkaline
G gas

E .coli

It is a member of *Enterobacteriaceae* and it is the most common cause of urinary tract infections arising outside of a hospital setting. These strains have PAP pili as well as CFA's (CFA/I, CFA/II, CFA/III). The pili are responsible for adherence in the urinary tract epithelium that the adhesin has important role in pathogenesis of chronic pyelonephritis and associated with severity of disease (Malsumoto. *et. al*, 1990). The capsule of *E .coli* represented the antigenic structure (K antigen) and it is highly associated with the pathogenicity of pyelonephritis, so that K antigen of *E .coli* help in the attachment of bacteria to the epithelial cells prior to the urinary tract invasion. Nephropathogenic *E .coli* may produce hemolysin as a part of the virulence factors in the complicated UTI and this is not well clear on blood agar (Eisenstein and Azaleznik, 2000). Some strains are urease-producing *E .coli* and they are commonly presented in complicated UTI (Collins and Falkow, 1990). The antibiotic resistance of *E .coli* isolated from UTI is highly increased due to the abuse of antibiotics from the patients in addition to the toxins and enzymes like endotoxin and p-lactamase that play an important role in the virulence of bacteria.

The recent studies showed that 47% of patients with UTI have *E .coli* in their cultures (Gordon and Jones, 2003). The incidence of urinary tract infection with *E .coli* is decreased due to the increase of the nosocomial infection of urinary tract (Schrier and Gottschalk, 1996).

proteus spp.

proteus species produce infections in humans only when the bacteria leave the intestinal tract .they are found in urinary tract infections and produce bacteremia ,pneumonia ,and focal lesions in debilitated patients or those receiving intravenous infusions .P mirabilis cause urinary tract infection and occasionally other infection. P vulgaris and Morgaella morgnii are important nosocomial infection.

Proteus species produce urease resulting in rapid hydrolysis of urea with libration of ammonia .thus , in urinary tract infections with proteus ,the urine becomes alkaline promoting stone formation and making acidification virtually impossible .the rapid motility of proteus may contribute to its invasion of urinary tract .

Strains of proteus vary greatly in antibiotic sensitivity .P mirabilis is often inhibited by penicillin's ,the most active antibiotics for other members of the group are aminoglycosides and cephalosporin's(Abbott,2003) .

Klebsiella pneumonia

Klebsiella is a member of the family *Enterobacteriaceae* . Colonies are large and highly mucoid. It is most common cause of hospital-acquired urinary tract infections or burn wound infections. The autoimmune disease (ankylosing spondylitis/ is thought to be a possible sequel of Klebsiella infection (Abbott, 1999) but the virulence of Klebsiella is not well understood, but its antiphagocytic capsule plays a role in the infections by preventing phagocytosis. It is thought that" aerobactin, an iron-binding protein, and the production of B-lactamase enzyme contribute to pathogenicity and antibiotic resistance of bacteria. Some strains of Klebsiella produced hemagglutinins (may be a mannose-sensitive phenotype) and they may be associated with the pathogenicity endotoxin has an important role in virulence and antibiotic resistance of bacteria (Gilchrist, 1995). Enterobacter can produce antibacterial substance that has antagonistic activity against a wide range of bacteria except Acinetobacter and Pseudomonas (Yaping . et. al,2003).

Serratia spp.

They are lactose-fermenter gram-negative short rods *Enterobacteriaceae* with one to two flagella. They represented

as opportunistic pathogens with wide ranges of infectivity in nosocomial infections like respiratory or urinary tracts infections. The virulence of bacteria commonly associated with the production of urease enzyme, hemolysin enzyme, siderophore and extracellular protease like gelatinase enzyme with presence of fimbriae help in adhesion of bacteria (either mannose-sensitive or mannose-resistant fimbriae) (Marumo. et. al,1990). Swarming motility characterized to the bacteria on solid or viscous media due to presence of flagella. The production of B-lactamase enzyme has given to bacteria high resistance to several antibiotics like that in *Pseudomonas* bacteria (Kouda. et.al, 1990).

Staphylococcal spp.

They are gram-positive spherical bacteria usually arranged in grap like irregular clusters. They are a normal flora of human skin and mucous membranes and their spread is either endogenously or from infected skin. They included many species but the main three species are *S.aureus*, *S.epidermidis* and *S.saprophyticus*. The pathogenicity of Staphylococci is contributed to hemolysis of the blood, coagulation of the plasma and production of extracellular enzymes and toxins (Mims et.al, 2004).

Table 2.2:-Biochemical tests of Staphylococcal spp modified from (Brooks .et.al, 2001).

Test	<i>S. aureus</i>	<i>S.epidermidis</i>	<i>S. saprophyticus</i>
Catalase	+	+	+
Oxidase	—	—	—
Coagulase	+	—	—
Mannitol ferm.	+	—	—
Resist to Novobiocin	+	—	+
Urease	—	±	±
Hemolysin	+	—	±

* Novobiocin used only to distinguish between *S.epidermidis* and *S.saprophyticus*.

S.epidermidis

It is a coagulase negative Staphylococci and a common member of the normal flora of skin and mucous membranes. Its large numbers and ubiquitous distribution make it one of the most commonly isolated organisms in the clinical laboratory. The first appearance of *S.epidermidis* in clinical material could dismiss as contamination; it is now one of the most important agents of hospital-acquired infections. Immunosuppressed patients are particularly at risk, as are individuals with indwelling catheters or prosthetic devices (Baron et.al., 1996). The hydrophobic nature of the organism's cell surface facilitates its adherence to synthetic devices. Following initial colonization, a copious amount of extracellular polysaccharide or slime is synthesized that forming a protective biofilm around the colony. Because many isolates are multiple antibiotic resistant, these infections are very serious and can even be fatal. In complicated UTI, *S.epidermidis* is represented more than 20% as a nosocomial infection (Guirguilzova . et. al, 2002).

S.saprophyticus

It is coagulase negative Staphylococci, commonly isolated from uncomplicated urinary tract infection in nonhospitalized patients, notably sexually active woman. *S.saprophyticus* may also be involved in recurrent infection and in stone formation that the incidence of *S.saprophyticus* in urinary tract infection varies according to the institutions and the geographical areas (Todar, 2001). It is resistant to several antibiotics such as novobiocin and nalidixic acid. Although the species do not produce number of extracellular products but it may produce hemolysin and the antiphagocytic capsule that the production of the slime may correlate with pathogenicity and bacterial adherence (Baron et.al., 1996).

S.aureus

It is coagulase positive Staphylococci, presented significantly in greater percentage of people in the hospital setting that the carrier state serves as reservoir for infection of hospitalized patients (Todar, 2001). S.aureus has a polysaccharide capsule to protect it from phagocytosis and the cell wall composed of peptidoglycan and teichoic acid moieties that protect it from lyses by osmotic condition and aid the bacteria to attach to mucosal surfaces. The virulence of the bacteria occurred by secretion of toxins and enzymes which act on host cell membrane and mediated the cell destruction. It is penicillin-resistance bacteria due to production of B-lactamase enzyme that is chromosomally resistance (Takahashi et al., 1999).

Pseudomonas aeruginosa

It is non-fermenter aerobic gram-negative bacilli. It has one of the broadest ranges of infectivity among all pathogenic microorganisms as opportunistic pathogens. It is a significant cause of burn wound infection and nosocomial infection in human body like respiratory tract infection in patients with cystic fibrosis, eye infection and genitourinary tract infection in immuno-compromised patients (Bodey, 1983).

The pathogenicity of the bacteria contributed to the virulence factors of it. The capsule or slime layer is associated in adherence and effectively protected the bacteria from phagocytosis. The productions of extracellular protease, cytotoxins and hemolysin have an important role in virulence; in addition, the siderophore production under low iron condition helps the growth of pathogen (Woods and Igleski, 1983).

Management

If treatment is injected before the results of a culture and sensitivity are available, a 3- to 5-day course of therapy with trimethoprim-sulfamethoxazole is effective against most strains of E.coli. Nitrofurantoin (5-7 mg/kg/24 hr in three to four divided doses) is also effective and has the advantage of being active against Klebsiella-Enterobacter organisms. Amoxicillin (50 mg/kg/24 hr) is also effective as initial treatment but has no clear advantages over the sulfonamides or nitrofurantoin.

In acute febrile infections suggestive of pyelonephritis, a 14-day course of broad-spectrum antibiotics capable of reaching significant tissue levels is preferable. If the child is acutely ill, parenteral treatment with ceftriaxone (50-75 mg/kg/24 hr. not to exceed 2 g) or ampicillin (100 mg/kg/24 hr) with an aminoglycosides such as gentamicin (3 to 5 mg/kg/24 hr in three divided doses) is preferable. The potential ototoxicity and nephrotoxicity of aminoglycosides should be considered and serum creatinine levels must be obtained prior to initiating treatment as well as daily thereafter as long as treatment continues. Treatment with aminoglycosides is particularly against pseudomonas (Rushton, 1997).

Materials And Methods:-

Patients And Materials

Patients

This study began from 1/12/2004 to 1/12/2005. 100 children's patient with pyuria were taken in pediatric hospital of Karballa from 385 children's who had complaints related to UTI. Their age range between birth to 5 years. 63 females and 37 males. The investigations were made to children's, GUE. urine culture and antibiotic sensitivity results and also the immunodiffusion test to some patients serum with specific types of bacteria.

Materials

Many types of instruments and chemical materials in addition to biological materials were used in this thesis to complete the research. The materials were taken from different sources and companies which listed in tables 1, 2, 3

Table 1:-List of Biological and chemical materials

No	III. MATERIALS	Company
1	Pepton powder	Rashmi Dignostics, India
2	Blood agar ,Muller-Hinton agar	Mast lab, uk
3	MacConcky agar	Biomark lab, India
4	Nutrient agar ,Nutrient broth	Bio life, India
5	Antibiotic disc	Razi, Iraq
6	Urea agar base	Oxid Ltd, England

7	Simon citrate agar, kliglar Iron agar, MR-VP broth	Difco , Michigen
8	Immunodiffusion kit	Kent , USA
9	Methyl red,a- Naphthol ,Tetramethyl-p-paraphenylene diamine dihydrochloride.	B.D.H.

Table 2:-list of instruments used

No	IV. INSTRUMENT	Company
1	Sensitive electronic balance	A and O , Japan
2	Incubator	Termaks, Stockholm
3	Ditillatur	C.fL, Germany
4	Benson Burner	Germany
5	Light microscope	Olympus, Japan
6	centrifuge	NF 815-Ankra, Turkey
7	micropipette	Oxford, USA
8	refrigerator	General , Japan
9	Inoculating loop	Japan
10	Inoculating needle	Japan
11	oven	Memert, Germany

Table 3:-The potency of antibiotics according to antibiotic source(Iraq, Razi)

Cefotaxime	10 mcg
Gentamycin	10 mcg
Amikacin	30 mcg
Amoxicillin	10 mcg
Cotrimaxazole	25 mcg
Nitrofuratoin	300 mcg
Nalidixic acid	300 mcg

3.2. Diagnosis

Urinalysis

A urinalysis involved a physical and chemical examination of urine (color, reaction and albumin).

The urine was spun in a centrifuge to allow sediments containing:

1. Pus cells (WBC).
2. RBC
3. Crystals.
4. Casts (Massey, 2004).

Preparation Of Media

These media were prepared as manufactures recommendations

Blood agar base .

1. suspend by swirling 37.5 g of powder in liter of distilled or deionised water
2. autoclave at 121 c for 15 minute
3. mix well before pouring. For blood agar add 7% blood at 50 c .

Nutrient agar.

suspend 23 g in 1000ml of cold distilled water .heat to boiling ,adjust the pH to 7.4 .distribute and sterilize at 121 c for 15 minute.

MacConkey agar.

suspend 51.5 g in 1000 ml distilled water . boil to dissolve the medium completely ,adjust the pH to 7.4 .sterilize by autoclaving at 121 c for 15 minutes.

Eosin Methylene Blue (EMB) Agar.

Lactose fermenting colonies were either dark or possess dark centers with transparent colorless peripheries, while

organisms that did not ferment lactose remain uncolored. This purple color was due to the absorption of the eosin-methylene blue complex, which formed in the presence of acid. Certain members of the coliform group, especially *Escherichia coli*, exhibited a greenish metallic sheen in the reflected light (Collee et.al., 1996).

Biochemical Tests

Catalase Test :

It was prepared by dissolving of 0.1 gm of Tetra-P-FJ paraphenylene diamine dihydrochloride in 10 ml of distill water and stored in a dark container (Baron et.al, 1996). A colony of the organism was transferred to a drop of 3% H_2O_2 on a microscope slide. The presence of catalase was meant that the formation of gas bubbles has occurred which indicated the positive result (Collee et.al., 1996).

Oxidase Test:

It was prepared by dissolving 3 gm of H_2O_2 to 100ml of distill water and was stored it in dark container (Baron et.al., 1996).

A piece of filter paper was saturated in a petri dish with oxidase reagent then a colony of organism was spread onto the filter paper. When the color around the smear turned from rose to purple, the oxidase test was positive (Collee et.al, 1996).

Coagulase Test:

Several colonies of bacteria were transferred with a loop to a tube containing 0.5 ml of plasma. The tube was covered to prevent evaporation and incubated at 37°C overnight. The test was read by tilting the tube and observing for clot formation in the plasma. Negative test results in the plasma remained free-flowing with no evidence of a clot (Collee et.al., 1996).

Methyl red reagent :

0.1 gm of Methyl red was dissolved in 300 ml of 99% ethanol and then the volume was completed to 500 ml by distill water (Macfaddin, 2000).

Methyl Red Test:

The test was performed on 5 ml of MR-VP

broth cultured by the organism and then it was incubated for 24 hours at 37°C. After that 6-8 drops of Methyl Red reagent were added to culture. The change of color to orange-red was a positive reaction (Collee et.al.,1996).

Voges -Proskauer reagent

Reagent A) 5 gm of α -naphthol was dissolved in 100 ml of 99% ethanol.

Reagent B) 40 gm of KOH was dissolved in 100 ml of distill water (Collee, et.al., 1996).

Voges-Proskaur Test:

The test was performed on 5 ml of MR-VP broth cultured by the organism and then it was incubated for 24 hours at 37°C. After that 15 drops of 5% α naphthol (reagent A) were added followed by 10 drops of 40% KOH (reagent B) and shaken well and allowed to stand for up to 30 minutes before calling a reaction negative. The positive culture was turning to red at the surface of the liquid, and the color was spread gradually throughout the tube (Baron et.al., 1996).

Indol Test:

A 1% solution of tryptone broth was prepared in the tubes then it was sterilized into the autoclave at 121 C for 15 minutes. After that the broth inoculated with bacterial colonies and it was incubated for 48-72 hours at 37°C. Testing for indole production was done by adding 6-8 drops of Kovac's Reagent (p-dimethylaminobenzaldehyde in amyl alcohol). The formation of red color ring at top of broth was a positive reaction.

A yellow color ring was a negative result (Macfaddin , 2000).

well and allowed standing for up to 30 minutes before calling a reaction negative. The positive culture was turning to red at the surface of the liquid, and the color was spread gradually throughout the tube (Baron et.al., 1996).

Simon Citrate Test:

After the sterilization of Simon citrate slants by autoclave at 121 C for 15 minutes then cooled to 50 C and inoculated with the bacterial cultures and incubated for 24-48 hours at 37°C. The positive result was a change of the color of media from green to blue. The unchanging of the color was a negative reaction (Benson, 1998).

Urease Test:

The urea base agar was sterilized by autoclave at 121 C for 15 minutes. After cools it to 50°C, the urea substrate was added to it and was poured in sterile tubes; then inoculated by bacterial cultures and it incubated them for 24-48 hours at 37°C. The positive result was a deep pink color. Failure of deep pink color to develop was a negative reaction (Benson, 1998).

Kliglar Iron Agar (KIA) Test:

The aim is to differentiate the Enterobacteriaceae according to carbohydrate fermentation and hydrogen sulfide production. The organism was grown on KIA slant by stab and streak and then it was inoculated at 37°C for 24-48 hours. The changing of the color of the media from orange-red to yellow was due to carbohydrate fermentation with or without gas formation at butt of slant. In addition, the formation of Hydrogen sulfide was given a black color precipitation at butt (Macfaddin, 2000).

Motility test by using semisolid media:

According to the method described by (Macfaddin,2000).10 mls of semisolid media were dispensed in the tubes and left to set at the vertical position, I had inoculated with a straight wire, making a single stab down the center of the tube to about half the depth of the medium. The tubes were incubated at 37°C and examine at 6 hours, 24 and 48 hours. Non-motile bacteria had generally confined to the stab-line and given sharply defined margins with leaving the surrounding medium clearly transparent. Motile bacteria were typically given diffuse hazy growths that spread through out the medium rendering it slightly opaque.

The modified Kirby-Bauer method**Mueller-Hinton agar**

1. Mueller-Hinton agar should be prepared from a dehydrated base according to the manufacturer's(Mast lab ,uk) recommendations.
2. the medium was cooled to 45-50 °C and pour into the plates. Allow to set on a level surface, to a depth of approximately 4 mm. A 9-cm plate requires approximately 25 ml of medium.
3. after agar had solidified, the plates placed in the upright position in the incubator with the lids tilted at 35c for 30 minute to dry..
4. Any unused plates may be stored in a plastic bag as the recommendation of (vandepitte,et.al), which should be sealed and placed in the refrigerator. Plates stored in this way will keep for 2 weeks.

Antibiotic discs

Antibiotics discs of (Iraq,Razi) with the proper diameter and potency which had be used. Stocks of antibiotic discs should preferably be kept at - 20 °C; the freezer.

Antibiotics discs can be kept in the refrigerator for up to 1 month. On removal from the refrigerator, the containers should be left at room temperature for about 1 hour to allow the temperature to equilibrate.

Procedure

As the way of (Macfaddin,2000).The modified Kirby-Bauer method was performed by using a pure culture of previously identified bacterial organism .the inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on a blood agar plate to 5ml of broth. This culture was then incubated for 2 hours to produce a bacterial suspension of moderate turbidity. A sterile swab used to obtain an inoculum from the standardized culture .this inoculum was then streaked on a Mueller-Hinton plate.

The antibiotics discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator. incubation was usually overnight with an optimal time being 14 hours at 37c .antibiotics inhibition zones were measured using a caliber. Zone size was compared to standard zones to determine the susceptibility or resistance of the organism to each antibiotic.

Radial Immunodiffusion Test

Materials

Serum samples

21 blood samples were collected from children's under five years with pyuria ,7 cases with E .coli ,7 cases with proteus and 7 cases with negative urine culture in pediatric hospital in karballa . these samples were then used to obtain its serum by centrifugation at 3000 rpm.(Fahey,et,al.1965).

Materials used.

1. Three 24 (3x8) well radial immunodiffusion plates(Kent,USA).
2. Blood collection tubes.
3. 3.syringes and needles.
4. Centrifuge (200 rcf)
5. Microliter dispenser (5 microliters)
6. Human Reference Sera: 3 x 0.2ml vials(in kits only).
7. Measuring device-calibrated in 0.1mm increments available separately.
8. Two cycle semi-logarithmic graph paper or linear graph paper

procedure

according to the methods of (fahey,et,al.1965) the radial immunodiffusion kit used to determine the levels of antibodies (IgG.IgM) in the serum of children's with pyuria and as follow.

1. The blood were collected without anticoagulant and allow to clot at room temperature.
2. The serum were Separated by centrifugation at about 200 rcf within 2-3 hours after collection.
3. The plates were removed from refrigerator to room temperature approximately 30 minutes before filling wells. Do not open bag until ready for use.
4. If excess moisture is present, the plate were removed from its bag and remove cover until evaporation has dried the surface and wells. Replace cover until used.
5. For best results, three wells should be filled with reference sera for each plate. Location of each should be noted. Mix each vial of reference serum thoroughly.
6. The specimen were delivered to well by placing the pipette tip at the bottom of the well. Allow the well to fill to the top of the agar surface. Avoid bubbles to ensure proper volume and diffusion of sample. Visualization may be aided by placing the plate on dark background. If practice is required, a used plate may be utilized.
7. More consistent results may be obtained when wells are filled with a 5 microliter pipette.
8. Mark time of completion on plate cover and replace cover.
9. Replace plate in bag and reseal carefully.
10. 10 Incubate plates upright on a flat surface at room temperature (20° to 24° C) for 16-20 hours. Overnight readings and over 48 hours for end point readings
11. The diameters of precipitin zones were measured to within 0.1mm.
12. the reference sera provided in kits were used, or other, such as the College of American Pathologists reference standard, determine their ring diameters to the nearest 0.1 mm.
13. 2 or 3 cycle semi-logarithmic graph paper were used plot the concentration on the Y axis and the zone diameters on the X axis for each protein for Overnight readings as in (figure 3.1)
14. regular graph paper were used plot the concentration on the X axis and the zone diameters squared on the Y axis for each protein for End point readings as in (figure 3.2)
15. A straight line were drawed of "best fit" between the three points.

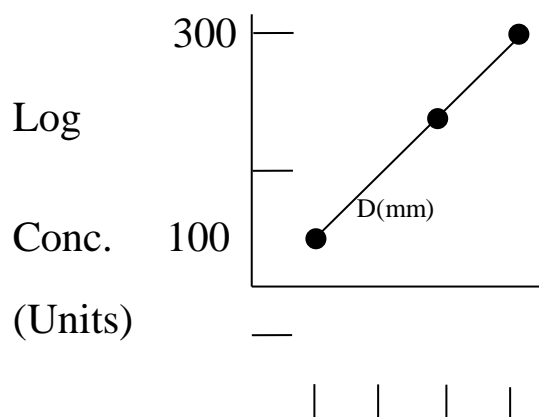
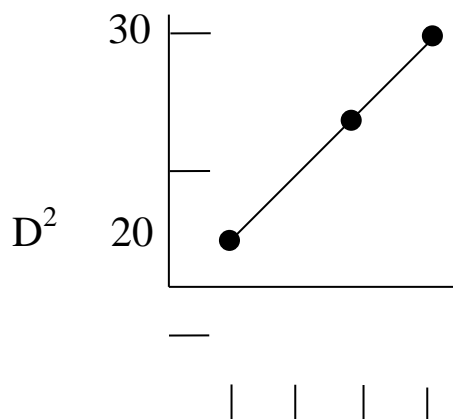


Figure 3.1:-Show the relation between diameter of IgG,IgM and logarithm of concentration.**Concentration****Figure 3.2:-**Show the relation between concentration of IgG,IgM and square of diameter .**Results:-**

Determine the concentration of each unknown or specimen protein by reading its zone diameter on the reference curve and the corresponding concentration. Zone diameter must be squared for End Point calibration.

Results

The study included 385 children's who had complaints related to UTI (259 girls and 126 boys) from birth to 5 years old age came to pediatric hospital of karballa and general urine examination were done for them and found the pyuria in 100 children's (63 girls and 37 boys) as show in table (1) .we found that 56 of them with positive urine culture (35 girls and 21 boys) as show in table (2) .

Analysis of some relate factors Showed the following result**Sex distribution****Table 2**

demonstrated the distribution of cases according to their sexes. It show the prevalence of urinary tract infection among girl higher than boys (62.5 % VS 37.5 %)

Age distribution**Table 3**

Show distribution of cases according 5 age groups.

(group 1	from birth to	1 year)	20
(group 2	from	1 to 2 year)	18
(group 3	from	2 to 3 year)	7
(group 4	from	3 to 4 year)	4
(group 5	from	4 to 5 year)	7

Brest feeding effect.**Table 4**

Show the number and percentage of cases according to breast feeling effect.

Circumcision affect on UTI in boys under 2 years .**Table 5**

Show the Distribution of cases of UTI in boys in the first two years of age according to the circumcision affect .

Recurrent urinary tract infection in children's under 5 years .**Table 6**

Show Distribution of cases of recurrent urinary tract infection in the children's under 5 years according to the sex..

Bacterial cases of urinary tract infection in the children's.**Table 7**

Show the numbers and percentage of bacterial cases of urinary tract infection in the children's under 5 years .

Table 8

Show the numbers of bacterial cases of urinary tract infection in children's under 5 years according to sex and age groups .

Antibiotic sensitivity Result .**Table 9**

Show the numbers and percentage of resistance of bacterial isolates to several antibiotic's.

Immune response for urinary tract infection We took in this study 21 children's with pyuria 7 cases with E .coli,7 cases of Proteus and 7 cases with negative urine culture and we measured for them serum IgG ,IgM antibodies and compared with means of concentrations of these three groups and we founded that the mean of serum IgG,IgM antibodies of E .coli was higher than Proteus mirabilis and for Proteus was higher than negative cases as seen in figure (2) .

So the immune response of E .coli bacteria was higher than proteus bacteria and for proteus bacteria was higher than negative cases.

Table 1:-Sex distribution of Pyuria cases.

a) Sex	b) Children's c) number	Pyuria present	Percentage of pyuria
Female	259	63	24.3 %
Male	126	37	29.4 %
Total	385	100	26 %

Table 2:-Sex distribution of UTI cases (pyuria with positive urine culture).

d) Sex	number	percentage
Female	35	62.5 %
Male	21	37.5 %
Total	56	100 %

Table 3:-Distribution of cases of UTI according to age groups

e) Age group	Number of cases	percentage
Group 1 (birth to 1 years)	20	35.7 %
group 2 (1-2 years)	18	23.1 %
Group 3 (2-3 years)	7	12.5 %
Group 4 (3-4 years)	4	7.1 %
Group 5	7	12.5 %

(4-5 years)		
f) Total	56	100 %

Table 4:-Number and percentage of types of feeding for child with UTI

g) Types of feeding	Numbers	percentage
Breast feeding	20	35.7 %
Mixed feeding	9	16.1 %
Bottle feeding	27	48.2 %
Total	56	100 %

Table 6:-Distribution of cases of recurrent UTI in the children's according to the sex .**Table 5 :-**Distribution of cases of UTI in boys in the first two years of age according to the circumcision affect**Table 7:-**Number and percentage of bacteria isolates in urine culture in UTI patient.

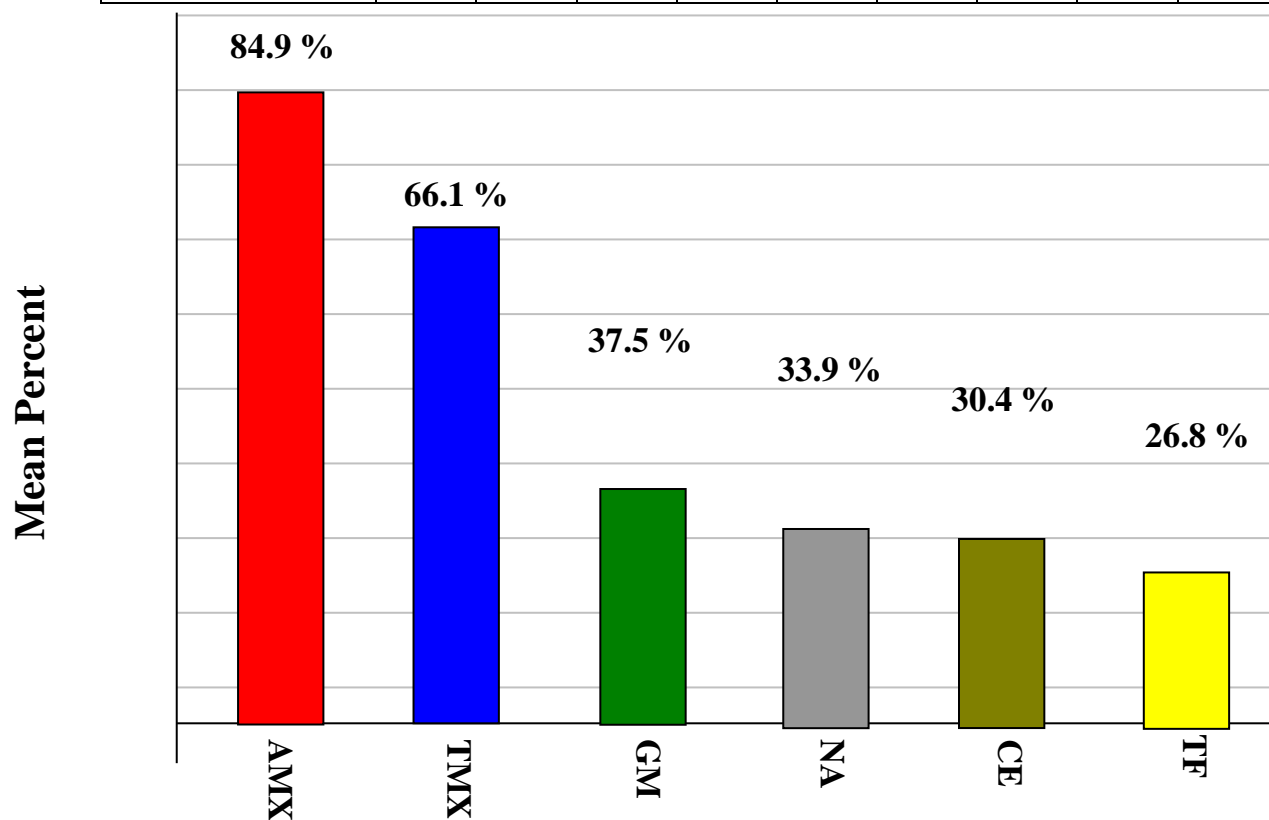
h) Type of bacteria	Numbers	percentage
E. coli	37	66 %
P. Mirabilis	10	17.9 %
K. pneumonia	3	5.4 %
P. aeruginosa	1	1.8 %
Enterobacter	1	1.8 %
Staph.saprophytic	4	7.1 %
Total	56	100 %

Table 8:-Distribution of cases of UTI according to age groups and types of bacteria

The bacteria	0-1		1-2		2-3		3-4		4-5	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀

Age groups	Circumcised boys		Percentage		Uncircumcised boys		Percentage		Total	
0-1 year	1		12.5%		7		87.5%		8	
1-2 year	2		33.3%		4		66.6%		6	
Total	4		28.6%		10		71.4%		14	
E. coli	4	10	2	9	2	3	2	1	4	
37										
Proteus. mirabilis	3	1	2	1	1		1	1		
10										

Staph. saprophytic 4	1		1	1						1
Pseudomonas 1		1								
Klebsiella 3			1	1		1				
Enterobacter 1							1			
							1			
Total	8	12	6	12	3	4	2	2	2	5



AMX Amoxicillin
 TMX Trimethoprim Sulphamethaxazole
 GM Gentamicin NA Nalidixic acid
 CE Cefotaxime TF Nitrofurantoin

Figure 1:-Show the percentage of resistance of bacterial isolates to antibiotics.

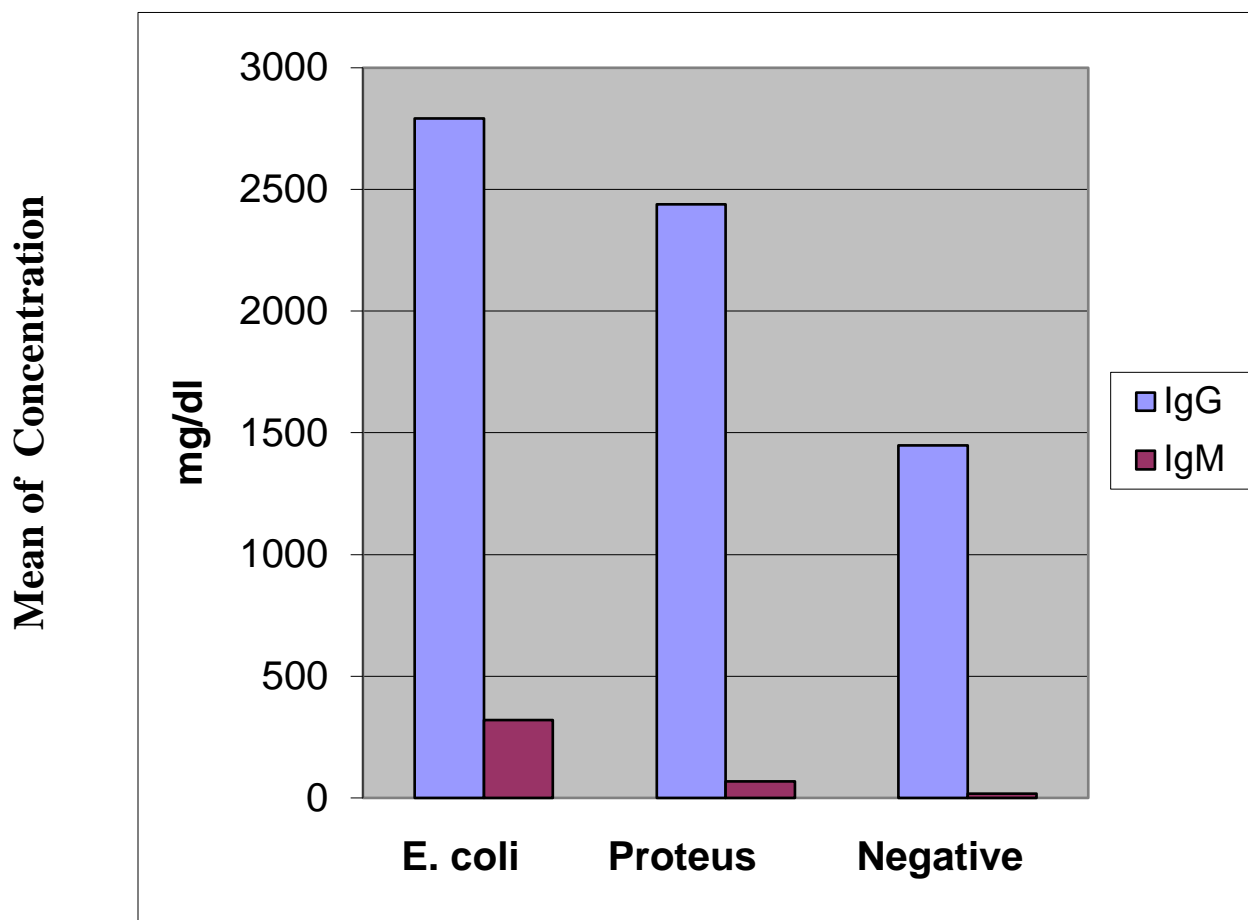


Figure 2:-Show the means of concentrations of IgG,IgM antibodies according to type of bacteria