جامعة بابل
كلية الطب

دراسة في التهاب المجاري البولية عند الأطفال دون سن الخامسة من العمر في مستشفى الأطفال في كربلاء

أطروحة

مقدمة الى فرع الأحياء المجهرية – كلية الطب – جامعة بابل

جزءاً من متطلبات نيل درجة الماجستير في

الأحياء المجهرية الطبية

تقدم بها

منير عبد الرسول طعمة

بكالوريوس طب وجراحة عامة

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BABYLON UNIVERSITY
COLLEGE OF MEDICINE

URINARY TRACT INFECTION IN CHILDREN UNDER FIVE YEARS IN PEDIATRIC HOSPITAL OF KARBLLA

A THESIS

Submitted to the Department of Microbiology of the College of Medicine, University of Babylon, in Partial Fulfillment of the Requirements for the Degree of Master in Medical Microbiology

By;

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بسم الله الرحمن الرحيم

(ولله غيب السموات و الأرض وإليه يرجع الأمر

كله فاعبده وتوكل عليه وما ربك بغافل عما تعملون)

صدق الله العلي العظيم

سورة هود الايه١٢٣

Abbreviations

Abbreviations	*Meaning*
WHO	**World health organization**
UTI	Urinary tract infection
CFU	Colony forming unit
rpm	Rotation per minute
IgM	Immunoglobulin m
IgG	Immunoglobulin g
WBC	White blood cell
Spp.	Species

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الخلاصـــــة

هذه الدراسة بحثت في عدوى المنطقةِ البوليةِ في الأطفالِ تحت ٥ سَنَواتِ كَانتْ قَدْ طبقت لتَمييز الأسبابِ البكتيريه ونَتائِج حسّاسيةِ المضادات الحيوية وأيضاً الاستجابة المناعية إلى تلك البكتيريا.

هذه الدراسةِ أُمتدت مِنْ ٢٠٠٤/١٢/١ إلى ٢٠٠٥/١٢/١ في مستشفى الأطفال في مدينة كربلاء، وكانت طرق البحث المستخدمة في هذه الدراسةِ كَانتْ المعاييرَ القياسيةَ لتحليلِ البول للبَحْث عن البيله القيحيه التي تَعْني حضورَ أكثر مِنْ ٥ خلايا قيحِيه في بولِ مطرود مركزيا تحت أعلى قوة للمجهرِ (منظمة الصحة العالمية، ٢٠٠١) وأيضاً اللون وضبابية وحموضة البولِ سُجّلتْ لكُلّ عيّنة وأيضا الزرع البكتيري للبولِ أجري لاكتشاف الأنواع البكتيرية المسببة لالتهاب المجاري البولية بالإضافة إلى اختبار حسّاسيةِ المضادات الحيوية وأيضا الاستجابة المناعية المحددة إلى الأنواعِ البكتيرية قُيّمتْ باختبار فحص التلازن ألشعاعي المناعي .

المعايير القياسية في هذه الدراسةِ كَانتْ مدى العُمرَ مِنْ الولادةِ إلى خمس سَنَواتِ، الجنس، التهاب المنطقةِ البوليةِ السابق في الطفلِ، السكن، نوع الرضاعة، الشكوى الرئيسية عند الطفلِ، حالات شذوذ جنينية إذا كانت موجودة ، التأريخ الطبي السابق عند الطفلِ.

وكان اختيار مجموعُ الحالاتَ بشكل عشوائي في هذه الدراسةِ كَانتْ ٣٨٥ طفلَ (١٥٦ ولد و٢٢٩ بنت)، البيله القيحيه شُخّصت في ١٠٠ حالةِ منهم هم كَانوا ٣٧ ولدَ و٦٣ بنتَ.

٥٦ زرع بكتيري للبولِ إيجابيةِ للأولادِ والبناتِ الذي منه ٢١ (١٣.٥ %) مِنْ ١٥٦ حالةِ للأولادِ والنَتائِجِ للبناتِ كَانوا ٣٥ (١٥.٣ %) مِنْ ٢٢٩ حالةِ وكانت الزروع البكتيرية للبولِ الإيجابية لـ (*E.coli*) كَانتْ ٣٧ (٦٦ %)، ولـ (*Proteus*) ١٠ (١٨ %),

وللـ (*Klebsiella*) ٣ (٥.٤ %) , وللـ (*Staphylococcus saprophyticus*) ٤(٧.١ %) , وللـ (*Pseudomonas*) ١ (١.٨%) , وللـ (*Enterobacter*) ١ (١.٨%)

أما بالنسبة إلى الزروع البكتيرية التي لم نَستطعْ أَنْ نَعْزلَ أيّ بكتيريا فيها بالرغم من أنّها حضنت لأكثر مِنْ ٤٨ ساعة، كان عددها (٤٤) لـ ٢٠ ولدَ و٢٤ بنتَ واعتبرت سالبة بسبب استعمال المضادات الحيوية.

إما بالنسبة إلى تقبل (*E.Coli*) إلى المضادات الحيوية مُيّزتْ بالمقاومةِ العاليةِ إلى الكوترايماكزازول ,الاموكسيلين، وحيث اعتبرت حساسة إلى النايتروفيورانتوين, النااليدكسيك أسد , الجنتاميسين , السيفوتوكسيم.

وكانت المقاومة العالية للـ (*Klebsiella*) أيضاً نحو الاموكسيلين و السيفوتوكسيم ، وحيث اعتبرت حساسة نحو الكوترايماكزازول و النايتروفيورانتوين.

وكانت المقاومة العالية للـ (*proteus*) نحو الاموكسيلين وحسّاسة للنااليدكسيك اسد والسيفوتوكسيم.

وكانت حساسية المضادات الحيوية للـ (*Staphylococcus saprophyticus*) نحو النااليدكسيك أسد و السيفوتوكسيم و النايتروفيورانتوين .

وكانت حساسية المضادات الحيوية للـ (*Pseudomonas*) وللـ (*Enterobacter*) نحو السيفوتوكسيم و النايتروفيورانتوين .

وكانت نَتائِج الاستجابة المناعية إلى بكتريا الـ (*E.Coli*) (٢٧٩٢.٥ ملغم/١٠٠مل) من تركيز الأجسام المضادة IgG التي تُمثّلُ العدوى المُزمنةَ و(٣١٨.٥ ملغم/١٠٠مل) من تركيز الأجسام المضادة IgM التي تُمثّلُ العدوى الحادّةَ .

وكانت إلى بكتريا الـ (*proteus*) (٢٤٣٩.٦ ملغم /١٠٠ مل) من تركيز الاجسام المضادة IgG وفقط (٦٦.٨ ملغم /١٠٠مل) من تركيز الأجسام المضادة IgM .

ولحالاتِ البيله القيحيه التي كانت زروعها البكتيرية سالبةِ (١٤٤٨ ملغم/ ١٠٠مل) من تركيز الأجسام المضادة IgG و (١٦.٨ ملغم/١٠٠مل) من تركيز الأجسام المضادة IgM ولذا عُموماً كانت الاستجابة المناعية إلى بكتريا الـ (*E.Coli*) أعلى مِنْ منها إلى (*proteus*) الذي كَان بدورها أعلى مِنْ حالاتِ البيله القيحيه التي كانت زروعها البكتيرية سالبةِ .

Abstract

A study of urinary tract infection in children under ٥years had been planed to identify the bacterial causes , antibiogram profile and also immunological response to these bacterial infection .This study extended from ١/١٢/٢٠٠٤ to ١/١٢/٢٠٠٥ in The Pediatric Hospital in Karballa .The methods of investigation used in this study were the standard criteria for urinalysis to look for pyuria which means the presence of more than ٥ pus cells in centrifuged urine in high power field (Vandepitte *et.al.,*١٩٩١) , 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 its antibiotic sensitivity test .The specific immunological response to bacterial types were assessed by immunodiffusion test .

The patients variables of this study were the age range from birth to five years ,sex ,previous urinary tract infection , circumcision in boys ,type of feeding ,chief complaint ,and past medical history of the child .

The total selected patients of this study were ٣٨٥ children (١٥٦ boys and ٢٢٩ girls) , Pyuria was diagnosed in ١٠٠ patients .They were ٣٧ boys and ٦٣ girls . Fifty six positive urine cultures for boys and girls of which ٢١(١٣.٥٪) from ١٥٦ patients for boys and the

results for girls were ٣٥(١٥.٣٪) from ٢٢٩ cases .The number of positive urine cultures for *E .coli* were ٣٧(٦٦٪),١٠(١٨٪) for *Proteus* ,٣(٥.٤٪) *Klebsiella* ,١(١.٨٪) *Pseudomonas*,٤(٧.١٪) *Staphylococcus saprophyticus* ,١(١.٨٪) for *Enterobacter* . Fourty four cultures failed to isolate any bacteria although they were incubated for more than ٤٨ hours ,they were ٢٠ boys and ٢٤ girls and considered negative due to the use of antibiotics.

Escherichia coli antibiotics susceptibility was characterized by high resistance to amoxicillin , cotrimaxazole ,while it was susceptible to nitrofurantoin , nalidixic acid , cefotaxime , gentamicin. The high resistance among *Klebsiella pneumonia* also toward amoxicillin and for cefotaxime, but it was susceptible to nitrofurantoin and cotrimaxazole . The high resistance of *proteus* to amoxicillin was noticed while it was sensitive for nalidixic acid and cefotaxime.

Staphylococcus saprophyticus isolates were sensitive to cefotaxime, nalidixic acid and nitrofurantoin .

Pseudomonas and *Enterobacter* were sensitive to cefotaxime and nitrofurantoin .

The results of immunological response to *E. coli* show (٢٧٩٢.٥ mg/dl) IgG antibodies which represent chronic infection and (٣١٨.٥ mg/dl) IgM antibodies which represent acute infection. However with *proteus* infections (٢٤٣٩.٦ mg/dl) anti proteus IgG antibodies and

only(٦٦.٨ mg/dl) IgM anti proteus antibodies were detected .

Pyuria cases with negative cultures had (١٤٤٨ mg/dl) IgG antibodies and (١٦.٨ mg/dl) IgM antibodies .So in general the immunological response for *E. coli* was higher than *proteus* which was higher than that of pyuria cases with negative cultures .

Introduction

Chapter One

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, ١٩٨٢).

The urinary tract infection is defined by the presence of pure bacterial growth > ١٠٠,٠٠٠ colony forming unit /ml (kass , ١٩٥٥).

The pyuria mean presence of more than ٥ pus cells in high power field of microscope in centrifuged urine (WHO, ٢٠٠١) .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 , ٢٠٠٢) .

Recurrent urinary tract infection are either relapse with the original infecting organism or reinfection with a different organism, In general, relapses developed within ٣ 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 ٨٠٪ of recurrent infections (stamey, ١٩٧٢)

Aims of study

١.To determine the prevalence of bacterial causes of UTI .

٢. to evaluate the efficacy of some related factors.

٣. to detect the types of antibiotics which are effective.

٤. to study the immunological response of children with UTI for specific types of bacteria.

٥.This study focus on why children have UTI and what can be done to prevent these infections.

Literature Review

Chapter Two

٢.١ Anatomy of the urinary tract

٢.١.١ Kidneys

A. Anatomy*:*

The kidneys lie along the borders of the psoas muscles and are therefore oblique placed. The adult kidney weighs about ١٥٠ 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 pyramid formed by the converging collecting tubules which drain into minor calices (Emil, ١٩٨١).

B. Histology*:*

The kidney composed of three parts

١.Nephron*:*

It is the functioning unit of the kidney which is composed of a tubules which has both secretory and excretory function.

٢.Secretory portion:

It is contained largely within the cortex and consist 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 glomerilus which projects into Bowmans 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 Henel and distal convoluted tubule.

٣.Excretory portion:

It is the collecting tubule which is continuous with the distal end of ascending limb of convoluted tubule. It empties it's content through the tip (papilla) of pyramid into a minor calix (Emil, ١٩٨١).

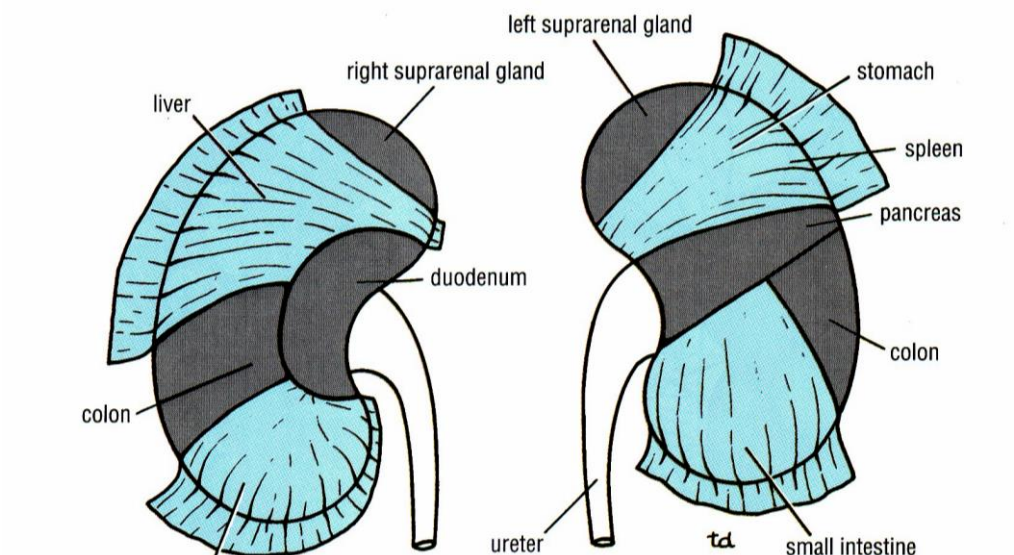


Figure ٢.١ Anterior relations of both kidneys. Visceral peritoneum covering kidneys has been left in position. Shaded areas indicate where kidney is in direct contact with adjacent viscera.

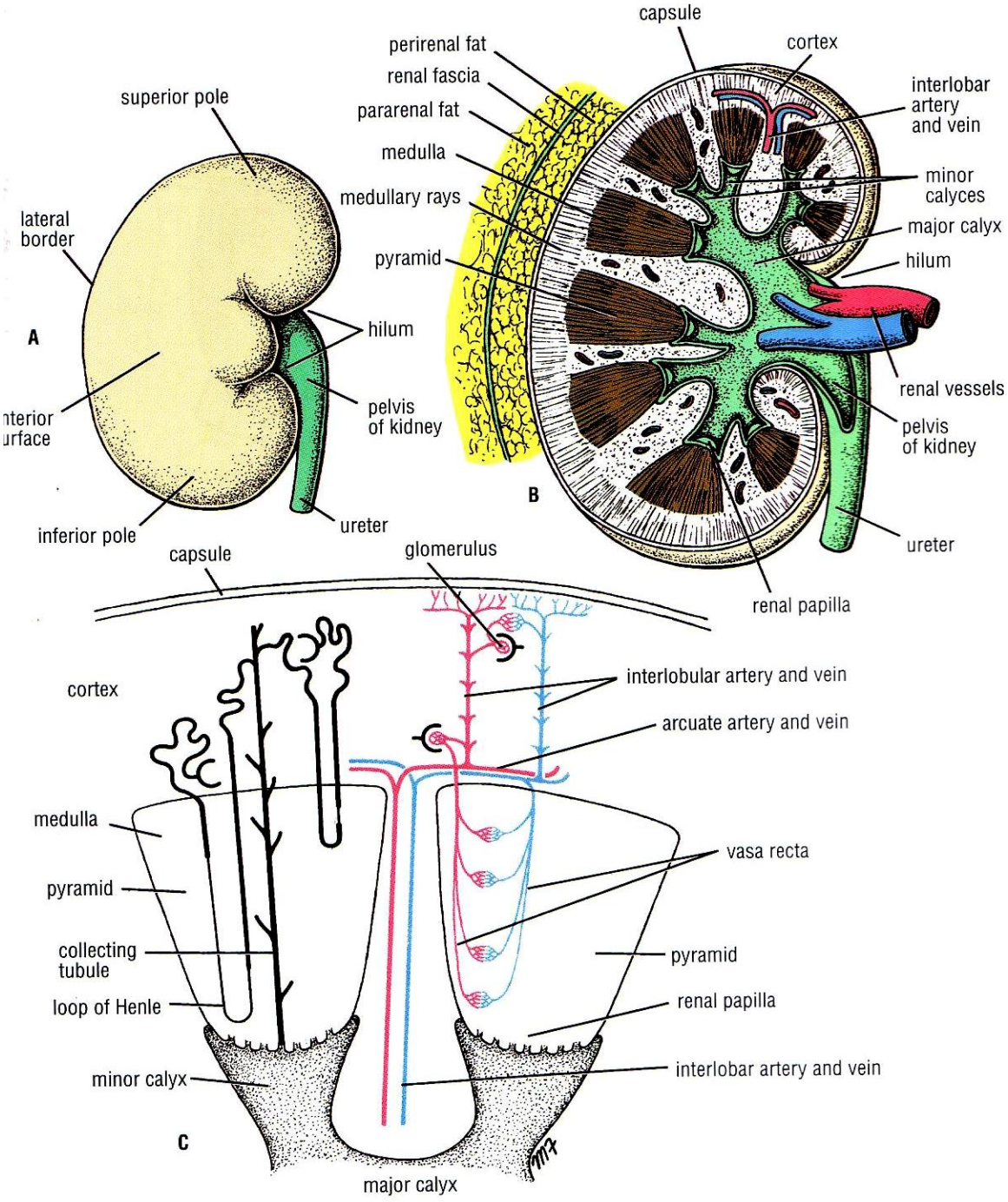


Figure ٢.٢ (A) right kidney ,anterior surface.(B) Right kidney , coronal section , showing cortex, medulla, pyramids, renal papillae, and calyces. (C) Section of

٢.١.٢ Calices,. Renal pelvis and ureters

A. Anatomy:

a- Calices**:**

The tip of the minor calices ٨-١٢ in number are indented by the projecting pyramids. These calices unite to form ٢ or ٣ major calices which join the renal pelvis.

b-Renal pelvis**:**

The pelvis may be entirely intra renal or partly intra renal and partly extra renal (Emil ١٩٨١).

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, ١٩٨٦).

c- Ureters**:**

These are narrow muscular tubes ٢٥ cm long which convey urine from the kidneys to the bladder. Ureters having ٣ constrictions:

(١) Where renal pelvis join the ureter.

(٢) Where it is kinked as it cross the pelvic brim.

(٣) 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 lumber vertebras.

٢.١.٣ Bladder

A. Anatomy*:*

This muscular urine store in women it's posterior wall and dome are invaginated by the uterus. The adult bladder has capacity of ٣٥٠-٤٥٠ 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, ١٩٨٦).

B. 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,١٩٨١) .

٢.٢. Classification of urinary tract infection :

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

١-**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.

٢-**Cystitis:** indicates that there is a bladder involvement and includes dysuria, urgency, frequency, suprapubic pain, incontinence ,and malodorous urine.

٣-**Asymptomatic bacteriuria**: refer to a positive urine culture in children's without any manifestations and occurs almost exclusively in girls (Rushton,١٩٩٧).

٢.٣. 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 my 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,٢٠٠٢).

٢.٤. 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 .١٩٨٩). 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 .١٩٩١).

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.١٩٨٤). These findings have not been confirmed in children (Schlager,et,al.١٩٩٣).

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.١٩٨٩).

The pathophysiology of UTI reflects a complex interaction between virulence factors of the microorganisms and the host defense(Barnett and Stephens.١٩٩٧).

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.١٩٨٥).

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.١٩٩٥).

٢.٥. Epidemiology

•The urinary tract infection is not uncommon in childhood. about ٨٪ or more of girls and about ٢٪ 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.

•Most UTI are due to normal bowel flora. *E. coli* is the causative

organism in ٨٠٪ of cases. Other common causative organisms are *Klebsiella ,Pseudomonas* and other gram-negative organisms.

•Urinary tract infections are much more common in girls than boys (at least ١٠:١)-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 .١٩٩٩) .

٢.٦. Diagnosis

A -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 fever than ١٠٠٠٠ 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.١٩٨٢) .So the ways of collection of urine was recommended as follow.

١- Urine bag collection (not recommended

a- High incidence of contamination.

٢- Clean catch urine of first morning void.

٣- Urine catheter specimen.

a-Recommended if child under age ٢ years.

٤- Suprapubic aspirate.

a- consider for child under age ٦ months old (Bulloch.et.al,٢٠٠٠).

B- Microscopic Urinalysis

A urinalysis involve physical and chemical examination of urine. In addition, the urine is spun in a centrifuge ١٠-١٥ ml at ١٥٠٠ to ٣٠٠٠ rpm for ٥ 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:

١- Simply observing the urine for color and cloudiness can be important.

٢- Acidity is measured .

٣- White blood cells (leukocytes) are counted. A high count in the urine is referred as pyuria. (A leukocytes count of over ١٠ 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,٢٠٠٢).

C-Urine culture

A urine culture is a urine specimen observed for ٢٤ to ٤٨ hours in a laboratory for the presence of any bacterial growth. It is not routinely performed but may be conducted under certain circumstances

-If urinalysis is negative but the patient has severe UTI symptoms, particularly in hospitatalized patients who have a catheter and who develop fever or other signs infection.

-If the infection is recurrent.

-if the physician suspects complications.

-if girls younger than two years have a high fever of unknown origin that lasts two days or more(Harvey,٢٠٠٢).

-Blood sample to assess the renal function including blood urea and serum creatinine (Baum.. *et. al,* ١٩٨٥).

-Renal ultrasonography images may show size, scarring of kidney, and the calculi if present (farmor. *et. al,* ٢٠٠٢). **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 , ١٩٨٨).

-Computerized Tomographic scan **(CT scan):** It is the procedure of choice to help diagnose chronic pyelonephritis (Gerzof and Gale, ١٩٨٢).

-Voiding cystourethrogram (VCUG); The findings may document the reflux of urine to the renal pelvis and ureteral dilatation in children with gross reflux (liyas *.et.al.,* ٢٠٠٢). **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.,* ١٩٨١).

-Cystoscopy: It showes the evidence of reflux at the ureteral orifices or site of kidney stone (liyas. *et.al,* ٢٠٠٢).

٢.٧. 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,١٩٦٥). 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 ١٠-٢٠ hours at room temperature are measured. The diameter of the ring

and the logarithm (base ١٠) 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 ١٠) 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.

٢.٨. *Antibiotics for Patients with urinary tract infection:*

١) 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,*٢٠٠٣) who observed that only ٥٢٪ of bacterial UTI responded to amoxicillin. In addition, (Leblebicioglu and Esen ,٢٠٠٣) and (Aggarwal. *et. al,* ٢٠٠٣) showed that more than ٧٣٪ of *E .coli* and ٨٨.٥٪ 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,* ٢٠٠٢; Savov. *et. al,* ٢٠٠٢; Suarez. *et. al,* ٢٠٠٢).

٢) Cephalosporin's

All first three generations of cephalosporin's have oral preparations that have been used for treatment of recurrent UTI (Wilhelm and Edson , ١٩٨٧).

a- 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 cephradine) or parenteral (cephalothin, cephazolin and cephradine). Now; the usage of first generation cephalosporins is limited because of high resistance to it (Martinez *et.al.* ١٩٩٥).

b- 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,* (٢٠٠٣) showed that only ٢٦٪ of patients with hospital-acquired UTI responded to second-generation cephalosporin's.

c- 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, ceflazidime and ceftizoxime). These drugs are excreted in bile, therefore they may use for patients

with renal insufficiency (Katzung, ٢٠٠٢).

The third generation cephalosporin's have been found affected in bacterial

UTI that more than ٨٠٪ and ٧١٪ of bacteria isolated from patients with UTI were sensitive to cefotaxime and ceftriaxone respectively (Gordon and Jones, ٢٠٠٣). In addition, ٧٨٪ of * *Acinetobacter* isolated from patients with UTI were also sensitive to cefotaxime (Irgbu *et. al.,* ٢٠٠٣) but Zhou *et. al,.* (٢٠٠٣) showed that *Enterobacter* were highly resistance to cefotaxime.

٣) 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, ١٩٨٦).

Nowadays, the resistance to TMP-SMX is slightly increased that ٤٥٪ of bacterial UTI were resistant to it including ١١٪ of *E .coli* and ٤٢٪ *of S.epidermidis* isolated from patients with UTI (Ghiro *et. al,*٢٠٠٢ and Jureen *et. al,*٢٠٠٣).

٤) Nitrofurantoin

It is synthetic nitrofuran 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,*١٩٨٧). The risk of adverse reaction increases with age and long-term therapy; therefore, should be monitored (Holmberg . *et. al,*١٩٨٠).

Nitrofurantoin has lower resistant rate than other old antibiotics that

only ٤٪ of bacteria isolated from patients with recurrent UTI were resistant to it (Leblebicioglu and Esen, ٢٠٠٣).

٥)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, ١٩٩٩). Gentamycin is cost-effective parenteral therapy because only once-daily dosing needed and has a good sensitivity against gram-negative uropathogens (٩٠٪) and it can be in combination with TMP-SMX against gram-positive uropathogens (٧٠٪) (Ghiro. *et. al,* ٢٠٠٢).

٦) 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, ciprofloxacillin, levofloxacillin, ofloxacillin, norfloxacillin and others used for empirical treatment of UT١. 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 parentefally, and then they can easily switch to oral administration and have limited use in patients with renal insufficiency (Dalkin and Schaeffer, ١٩٨٨).

٢.٩. Bacteriological Agents

٢.٩.١. 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 , ٢٠٠٤).

Table (٢.١) Biochemical tests of Enterobacteriaceae modified from (Brooks.*et. al*, ٢٠٠١).

Test	*E .coli*	*K .pneumonia*	Proteus	*Enterobacter*	*Serratia*

EMB	**metallic**	**Centrally dark**	**pale**	Centrally dark	**Centrally dark**
LF	**+**	**+**	**—**	**+**	**+**
Catalase	**+**	**+**	**+**	**+**	**+**
Oxidase	**—**	**—**	**—**	**—**	**—**
Indol	**+**	**—**	**±**	**—**	**—**
MR	**+**	**—**	**±**	**—**	**—**
VP	**—**	**+**		**+**	**+**
Urease	**—**	**+**	**+**	**—**	**—**
Citrate	**—**	**+**	**±**	**+**	**+**
Motility	**±**	**—**	**+**	**+**	**+**
TSI	**A/Alk± G**	**A±G**	**Alk±G**	**A±G**	**Alk±G**
H_2S	**—**	**—**	**+**	**—**	**—**

A acid

Alk alkalin

G gas

٢.٩.١.١ . *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,*١٩٩٠). 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, ٢٠٠٠). Some strains are urease-producing *E .coli* and they are commonly presented in complicated UTI (Collins and Falkow, ١٩٩٠. 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 ٤٧٪ of patients with UTI have *E .coli* in their cultures (Gordon and Jones, ٢٠٠٣). 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, ١٩٩٦).

٢.٩.١.٢. ***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 librations 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,٢٠٠٣) .

٢.٩.١.٣. ***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, ١٩٩٩) 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, ١٩٩٥). *Enterobacter* can produce antibacterial substance that has antagonistic activity against a wide range of bacteria except *Acinetobacter* and *Pseudomonas* (Yaping . *et. al,*٢٠٠٣).

٢.٩.١.٤. ***Serratia* spp.**

They are lactose-fermenter gram-negative short rods *Enterob-*

acteriaceae 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,*١٩٩٠). 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,* ١٩٩٠).

٢.٩.٢. ***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, ٢٠٠٤).

Table (٢.٢): Biochemical tests of Staphylococcal spp modified from

(Brooks .et.al, ٢٠٠١).

Test	***S. aureus***	S.epidermidis	***S. saprophyticus***
Catalase	**+**	**+**	**+**
Oxidase	**—**	**—**	**—**
Coagulase	**+**	**—**	**—**
Mannitol ferm.	**+**	**—**	**—**
Resist to Novobiocin	**+**	**—**	**+**
Urease	**—**	**±**	**±**
Hemolysin	**+**	**—**	**±**

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

S.saprophyticus.

٢.٩.٢.١. ***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., ١٩٩٦). 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 ٢٠٪ as a nosocomial infection (Guirguilzova . et. al, ٢٠٠٢).

٢.٩.٢.٢. ***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, ٢٠٠١). 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., ١٩٩٦).

٢.٩.٢.٣. ***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, ٢٠٠١). *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(Takahaski.et.al,١٩٩٩).

٢.٩.٣. *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-compramized patients (Bodey, ١٩٨٣).

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 Iglewski, ١٩٨٣).

٢.١٠. Management

If treatment is injected before the results of a culture and sensitivity are available , a ٣- to ٥-day course of therapy with trimethoprim – sulfamethoxazole is effective against most strains of E.coli. Nitrofuratoin (٥-٧ mg/kg/٢٤ hr in three to four divided doses) is also effective and has the advantage of being active against Klebsiella-Enterobacter organisms. Amoxicillin (٥٠ mg/kg/٢٤ hr) is also effective as initial treatment but has no clear advantages over the sulfonamides or nitrofuratoin.

In acute febrile infections suggestive of pyelonephritis, a ١٤-day course of broad-spectrum antibiotics capable of reaching significant tissue levels is preferable. If the child is acutely ill, parenteral treatment with ceftriaxone (٥٠-٧٥ mg/kg/٢٤ hr. not to exceed ٢ g) or ampicillin (١٠٠ mg/kg/٢٤ hr) with an aminoglycosides such as gentamicin (٣ to ٥ mg/kg/٢٤ 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,١٩٩٧).

Materials

&

Methods

Chapter Three

٣.١. Patients And Materials

٣.١.١ Patients

This study began from ١/١٢/٢٠٠٤ to ١/١٢/٢٠٠٥. ١٠٠ children's patient with pyuria were taken in pediatric hospital of karballa from ٣٨٥ children's who had complaints related to UTI. Their age range between birth to ٥ years. ٦٣ females and ٣٧ 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.

٣.١.٢ 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 ١, ٢, ٣ .

Table (١) :List of Biological and chemical materials

No	Materials	Company
١	**Pepton powder**	**Rashmi Dignostics, India**
٢	**Blood agar ,Muller-Hinton agar**	**Mast lab, uk**
٣	**MacConcky agar**	**Biomark lab, India**
٤	**Nutrient agar ,Nutrient broth**	**Bio life, India**
٥	**Antibiotic disc**	**Razi, Iraq**
٦	**Urea agar base**	**Oxid Ltd, England**
٧	**Simon citrate agar, kliglar Iron agar, MR-VP broth**	**Difco , Michigen**
٨	**Immunodiffusion kit**	**Kent , USA**
٩	**Methyl red,a- Naphthol ,Tetramethyl-p-paraphenylene**	**B.D.H.**

	diamine dihydrochloride.	

Table (٢) : list of instruments used

No	Instrument	Company
١	Sensitive electronic balance	A and O , Japan
٢	Incubator	Termaks, Stockholm
٣	Ditillatur	C.fL, Germany
٤	Benson Burner	Germany
٥	Light microscope	Olympus, Japan
٦	centrifuge	NF ٨١٥-Ankra, Turkey
٧	micropipette	Oxford, USA
٨	refrigerator	General , Japan
٩	Inoculating loop	Japan
١٠	Inoculating needle	Japan
١١	oven	Memert, Germany

Table(٣) The potency of antibiotics according to antibiotic source(Iraq, Razi)

Cefotaxime	١٠ mcg
Gentamycin	١٠ mcg
Amikacin	٣٠ mcg
Amoxicillin	١٠ mcg
Cotrimaxazole	٢٥ mcg
Nitrofuratoin	٣٠٠ mcg
Nalidixic acid	٣٠٠ mcg

٣. ٢. *Diagnosis*

٣. ٢. ١ ***Urinalysis***

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

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

- **Pus cells (WBC).**
- RBC
- Crystals.
- Casts (Massey, ٢٠٠٤).

٣.٢.٢ PREAPRATION OF MEDIA

These media were prepared as manufactures recommendations

١-Blood agar base .

a -suspend by swirling ٣٧.٥ g of powder in liter of distilled or deionised water

b- autoclave at١٢١ c for ١٥ minute

c-mix well before pouring. For blood agar add ٧٪ blood at ٥٠ c .

٢- Nutrient agar.

suspend ٢٣ g in ١٠٠٠ml of cold distilled water .heat to boiling ,adjust the pH to ٧.٤ .distribute and sterilize at ١٢١ c for ١٥ minute.

٣- Macconkey agar.

suspend ٥١.٥ g in ١٠٠٠ ml distilled water . boil to dissolve the medium completely ,adjust the pH to ٧.٤ .sterilize by autoclaving at ١٢١ c for ١٥ minutes.

٤- 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 esoin-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., ١٩٩٦).

٣.٢.٣. *Biochemical Tests*

٣.٢.٣.١. ***Catalase Test :***

It was prepared by dissolving of ٠.١ gm of Tetra-P-fJ paraphenylene diamine dihydrochloride in ١٠ ml of distill water and stored in a dark container (Baron *et.al,* ١٩٩٦). A colony of the organism was transferred to a drop of ٣٪ 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.,* l٩٩٦).

٣.٢.٣.٢. ***Oxidase Test:***

It was prepared by dissolving ٣ gm of H_2O_2 to ١٠٠ml of distill water and was stored it in dark container (Baron *et.al.,* ١٩٩٦).

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,* ١٩٩٦).

٣.٢.٣.٣. ***Coagulase Test:***

Several colonies of bacteria were transferred with a loop to a tube containing ٠.٥ ml of plasma. The tube was covered to prevent evaporation and incubated at ٣٧°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.,* ١٩٩٦).

٣.٢.٣.٤. ***Methyl red reagent :***

٠.١ gm of Methyl red was dissolved in ٣٠٠ ml of ٩٩٪ ethanol and then the volume was completed to ٥٠٠ ml by distill water (Macfaddin, ٢٠٠٠).

Methyl Red Test:

The test was performed on ٥ ml of MR-VP

broth cultured by the organism and then it was incubated for ٢٤ hours at ٣٧°C. After that ٦-٨ drops of Methyl Red reagent were added to culture. The change of color to orange-red was a positive reaction (Collee et.al.,١٩٩٦).

٣.٢.٣.٥. *Voges -Proskauer reagent*

Reagent A) ٥ gm of a-naphthol was dissolved in ١٠٠ ml of ٩٩٪ ethanol.

Reagent B) ٤٠ gm of KOH was dissolved in ١٠٠ ml of distill water (Collee, et.al., ١٩٩٦).

Voges-Proskaur Test:

The test was performed on ٥ ml of MR-VP broth cultured by the organism and then it was incubated for ٢٤ hours at ٣٧°C. After that ١٥ drops of ٥٪ alpha naphthol (reagent A) were added followed by ١٠ drops of ٤٠٪ KOH (reagent B) and shaken well and allowed to stand for up to ٣٠ 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., ١٩٩٦).

٣.٢.٣.٦. ***Indol Test:***

A ١٪ solution of tryptone broth was prepared in the tubes then it was sterilized into the autoclave at ١٢١ C for ١٥ minutes. After that the broth inoculated with bacterial colonies and it was incubated for ٤٨-٧٢ hours at ٣٧°C. Testing for indole production was done by adding ٦-٨ 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 , ٢٠٠٠).

well and allowed standing for up to ٣٠ 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.,* ١٩٩٦).

٣.٢.٣.٧. ***Simon Citrate Test:***

After the sterilization of Simon citrate slants by autoclave at ١٢١ C for ١٥ minutes then cooled to ٥٠ C and inoculated with the bacterial cultures and incubated for ٢٤-٤٨ hours at ٣٧°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, ١٩٩٨).

٣.٢.٣.٨. ***Urease Test:***

The urea base agar was sterilized by autoclave at ١٢١ C for ١٥ minutes. After cools it to ٥٠°C, the urea substrate was added to it and was poured in sterile tubes; then inoculated by bacterial cultures and it incubated them for ٢٤-٤٨ hours at ٣٧°C. The positive result was a deep pink color. Failure of deep pink color to develop was a negative reaction (Benson, ١٩٩٨).

٣.٢.٣.٩. 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 ٣٧°C" for ٢٤-٤٨ 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, ٢٠٠٠).

٣.٢.٣.١٠ Motility test by using semisolid media:

According to the method described by (Macfaddin,٢٠٠٠).١٠ 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 ٣٧°C and examine at ٦ hours, ٢٤ and ٤٨ 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 .

٣.٣. *The modified Kirby-Bauer method*

١-Mueller-Hinton agar

١. Mueller-Hinton agar should be prepared from a dehydrated base according to the manufacturer's(Mast lab ,uk) recommendations.

٢. the medium was cooled to ٤٥-٥٠ ْC and pour into the plates. Allow to set on a level surface, to a depth of approximately ٤ mm. A ٩-cm plate requires approximately ٢٥ ml of medium.

٣. after agar had solidified, the plates placed in the upright position in the incubator with the lids tilted at ٣٥c for ٣٠ minute to dry..

٤. 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 ٢ weeks.

٢-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 - ٢٠ °C; the freezer.

Antibiotics discs can be kept in the refrigerator for up to ١ month. On removal from the refrigerator, the containers should be left at room temperature for about ١ hour to allow the temperature to equilibrate.

٣-*Procedure*

As the way of (Macfaddin,٢٠٠٠).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 ٥ isolated colonies grown on a blood agar plate to ٥ml of broth. This culture was then incubated for ٢ 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 ١٤ hours at ٣٧c .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.

٣.٤. RADIAL IMMUNODIFFUSION TEST

١-Materials

١-١- Serum samples

٢١ blood samples were collected from children's under five years with pyuria ,٧ cases with E .coli ,٧ cases with proteus and ٧ cases with negative urine culture in pediatric hospital in karballa . these samples were then used to obtain its serum by centrifugation at

٣٠٠٠ rpm.(Fahey,et,al.١٩٦٥).

١- ٢- *Materials used.*

١. Three ٢٤ (٣x٨) well radial immunodiffusion plates(Kent,USA).

٢. Blood collection tubes.

٣.syringes and needles.

٤. Centrifuge (٢٠٠rcf)

٥. Microliter dispenser (٥ microliters)

٦. Human Reference Sera: ٣ x ٠.٢ml vials(in kits only).

٧. Measuring device-calibrated in ٠.١mm increments available separately.

٨. Two cycle semi-logarithmic graph paper or linear graph paper

٢. procedure

according to the methods of (fahey,et,al.١٩٦٥) the radial immunodiffusion kit used to determine the levels of antibodies

(IgG.IgM) in the serum of children's with pyuria and as follow.

١. The blood were collected without anticoagulant and allow to clot at room temperature.

٢.The serum were Separated by centrifugation at about ٢٠٠ rcf within ٢-٣ hours after collection.

٣.The plates were removed from refrigerator to room temperature approximately ٣٠ minutes before filling wells. Do not open bag until ready for use.

٤ 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.

٥. 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.

٦. 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.

٧. More consistent results may be obtained when wells are filled with a ٥ microliter pipette.

٨. Mark time of completion on plate cover and replace cover.

٩. Replace plate in bag and reseal carefully.

١٠ Incubate plates upright on a flat surface at room temperature (٢٠° to ٢٤° C) for ١٦-٢٠ hours. Overnight readings and over ٤٨ hours for end point readings

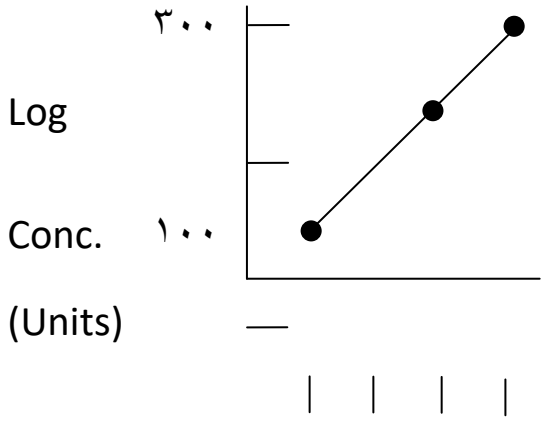
١١. The diameters of precipitin zones were measured to within ٠.١mm.

١٢ 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 ٠.١ mm.

١٣. ٢ or ٣ 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 ٣.١)

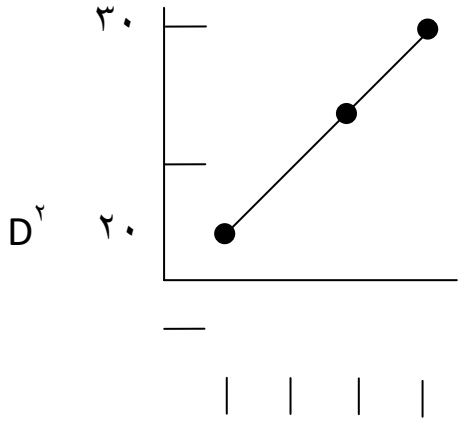
١٤. regular graph paper were used plot the concentration on the X axis and the zone diameters squared on the Y axis for each protein for End point readings as in (figure ٣.٢)

١٥. A straight line were drawed of "best fit" between the three points.



D(mm)

Figure (٣.١)Show the relation between diameter of IgG,IgM and logarithm of concentration.



Concentration

Figure (٣.٢)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

Chapter four

٤.١ Results

The study included ٣٨٥ children's who had complaints related to UTI (٢٥٩ girls and ١٢٦ boys) from birth to ٥ years old age came to pediatric hospital of karballa and general urine examination were done for them and found the pyuria in ١٠٠ children's (٦٣ girls and ٣٧ boys) as show in table (١) .we found that ٥٦ of them with positive urine culture (٣٥ girls and ٢١ boys) as show in table (٢) .

٤.٢ Analysis of some relate factors Showed the following result

٤.٢.١ Sex distribution

Table ٢

demonstrated the distribution of cases according to their sexes. It show the prevalence of urinary tract infection among girl higher than boys (٦٢.٥ % VS ٣٧.٥ %)

٤.٢.٢ Age distribution

Table ٣

Show distribution of cases according ٥ age groups.

(group ١ from birth to ١ year) ٢٠

(group ٢ from ١ to ٢ year) ١٨

(group ٣ from ٢ to ٣ year) ٧

(group ٤ from ٣ to ٤ year) ٤

(group ٥ from ٤ to ٥ year) ٧

٤.٢.٣ Brest feeding effect.

Table ٤

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

٤.٢.٤ Circumcision affect on UTI in boys under ٢ years .

Table ٥

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

٤.٢.٥ Recurrent urinary tract infection in children's under ٥ years .

Table ٦

Show Distribution of cases of recurrent urinary tract infection in the children's under ٥ years according to the sex..

٤.٢.٦ Bacterial cases of urinary tract infection in the children's.

Table ٧

Show the numbers and percentage of bacterial cases of urinary tract infection in the children's under ٥ years .

Table ٨

Show the numbers of bacterial cases of urinary tract infection in children's under ٥ years according to sex and age groups .

٤.٢.٦ Antibiotic sensitivity Result .

Table ٩

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

٤.٢.٧ Immune response for urinary tract infection

We took in this study ٢١ children's with pyuria ٧ cases with E .coli,٧ cases of proteus and ٧ 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 (٢) .

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

Table ١

Sex distribution of Pyuria cases.

Sex	Children's number	**Pyuria present**	**Percentage of pyuria**
Female	٢٥٩	٦٣	٢٤.٣ %
Male	١٢٦	٣٧	٢٩.٤ %
Total	٣٨٥	١٠٠	٢٦ %

Table ٢

Sex distribution of UTI cases (pyuria with positive urine culture).

Sex	**number**	**percentage**
Female	٣٥	٦٢.٥ %
Male	٢١	٣٧.٥ %

Total	٥٦	١٠٠ %

Table ٣

Distribution of cases of UTI according to age groups

Age group	**Number of cases**	**percentage**

Group ١ (birth to ١ years)	٢٠	٣٥.٧ %
group ٢ (١-٢ years)	١٨	٢٣.١ %
Group ٣ (٢-٣ years)	٧	١٢.٥ %
Group ٤ (٣-٤ years)	٤	٧.١ %
Group ٥ (٤-٥ years)	٧	١٢.٥ %
Total	٥٦	١٠٠ %

Table ٤

Number and percentage of types of feeding for child with UTI

Types of feeding	Numbers	percentage
Breast feeding	٢٠	٣٥.٧ %
Mixed feeding	٩	١٦.١ %
Bottle feeding	٢٧	٤٨.٢ %
Total	٥٦	١٠٠ %

Recurrent UTI	Boys	percentage	Girls	percentage	Total	percentage
Present	١٢	٥٧٪	٢٧	٧٧٪	٣٩	٧٠ %
Not present	٩	٤٣٪	٨	٢٣٪	١٧	٣٠٪
Total	٢١	١٠٠٪	٣٥	١٠٠٪	٥٦	١٠٠٪

Table ٦

Distribution of cases of recurrent UTI in the children's according to the sex .

Table ٥ Distribution of cases of UTI in boys in the first two years of age according to the circumcision affect

Age groups	Circumcised boys	Percentage	Uncircumcised boys	Percentage	Total
٠-١ year	١	١٢.٥٪	٧	٨٧.٥٪	٨
١-٢ year	٢	٣٣.٣٪	٤	٦٦.٦٪	٦

Total	٤	٢٨.٦٪	١٠	٧١.٤٪	١٤

Table ٧

Number and percentage of bacteria isolates in urine culture in UTI patient.

Type of bacteria	**Numbers**	**percentage**
E. coli	٣٧	٦٦ %

Gram

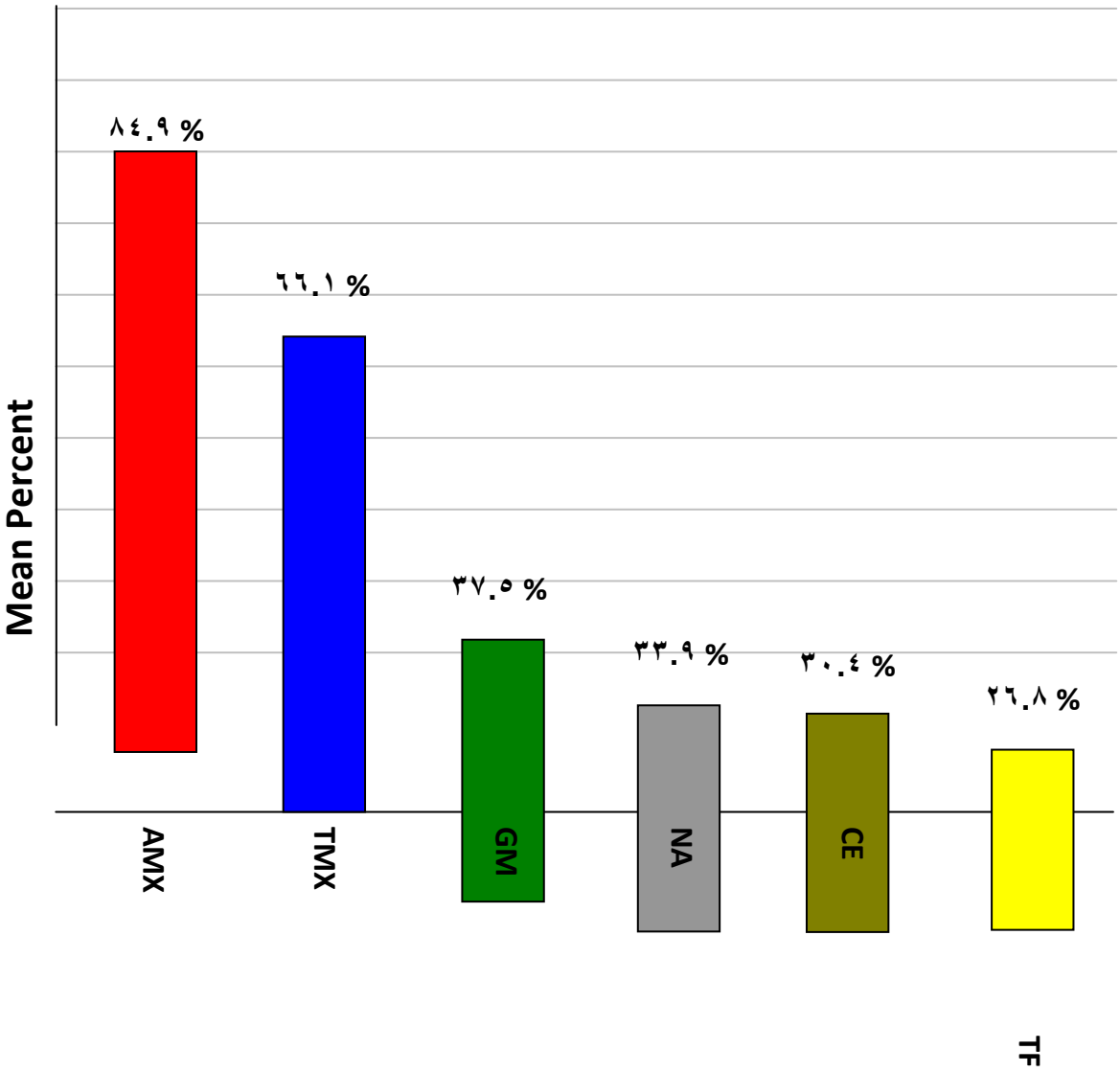
	P. Mirabilis	١٠	١٧.٩ %
	K. pneumonia	٣	٥.٤ %
	P. aeruginosa	١	١.٨ %
	Enterobacter	١	١.٨ %
Gram	*Staph.saprophytic*	٤	٧.١ %
	Total	٥٦	١٠٠ %

Table ٨

Distribution of cases of UTI according to age groups and types of bacteria

The bacteria	٠-١		١-٢		٢-٣		٣-٤		٤-٥	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
E. coli ٣٧	٤	١٠	٢	٩	٢	٣		٢	١	٤
proteus. mirabilis ١٠	٣	١	٢	١	١		١		١	
Staph. saprophytic ٤	١		١	١						١
pseudomonas ١		١								
Klebsiella ٣			١	١		١				
Enterobacter							١			

١										
Total	٨	١٢	٦	١٢	٣	٤	٢	٢	٢	٥



AMX Amoxicillin TMX Trimethoprim Sulphamethaxazole

GM Gentamicin NA Nalidixic acid

CE Cefotaxime TF Nitrofurantoin

Figure ١

Show the percentage of resistance of bacterial isolates to antibiotics.

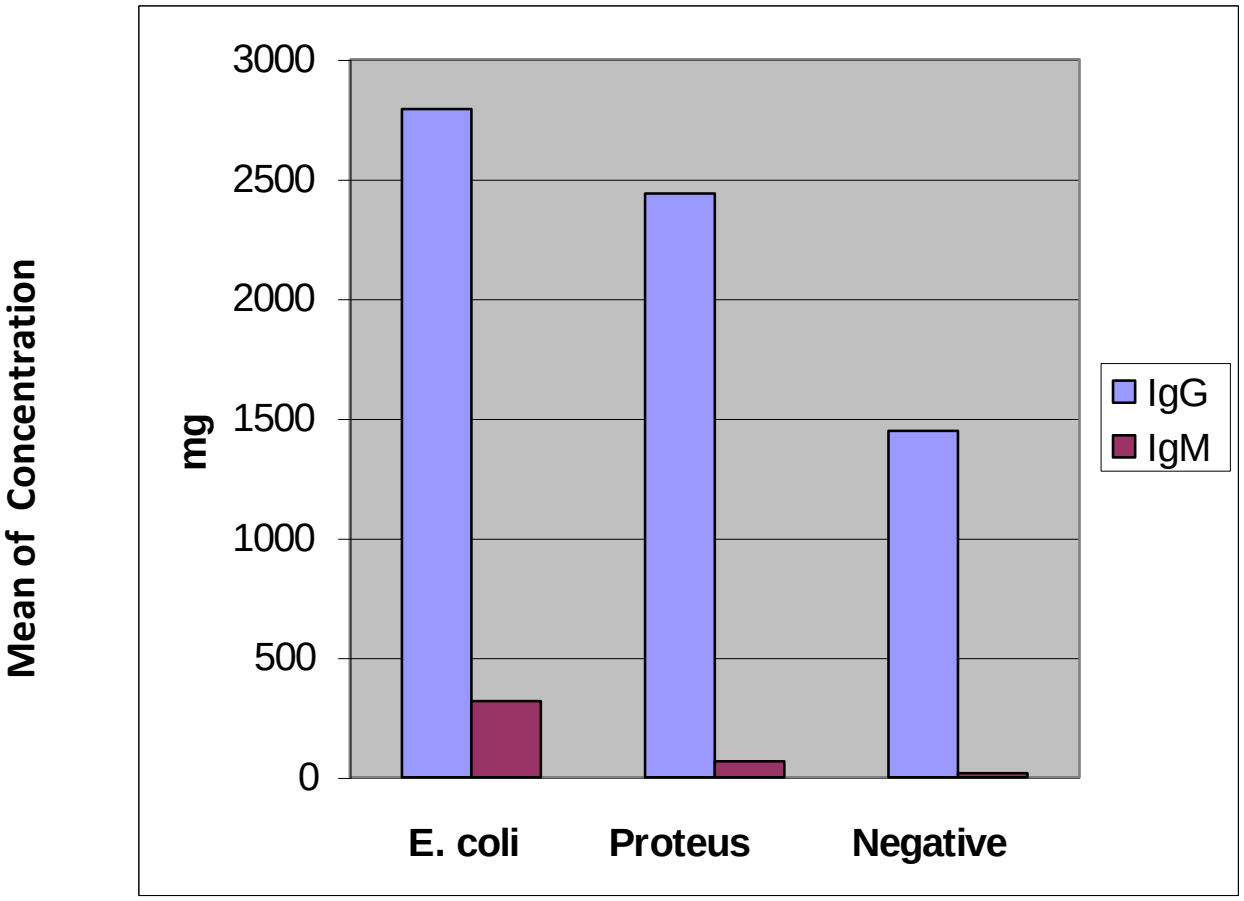


Figure ٢

Show the means of concentrations of IgG,IgM antibodies according to type of bacteria

Certification

I certify that this thesis was prepared under my supervision at the college of medicine .University of Babylon ,as partial fulfillment of the requirement for the master degree of science in Medical Microbiology.

Advisor

Signature

Ass. prof. Dr. Mohammed Aboud AL-Qaraguli

Department of Microbiology

College of medicine

University of Babylon

Date: / /٢٠٠٦

In view of the available recommendations, I forward this thesis for debate by the examination committee .

Signature

Ass. prof . Dr . Mohammed Sabri

Head of

Department of microbiology

College of Medicine

University of Babylon

Date: / /٢٠٠٦

Acknowledgement

A simple statement of thanks to Dr. Mohamed abood , my supervisor would be inadequate in expressing my deep gratitude to him for his advice and readiness to discuss the problems I came across in my work.

Special thanks to all members in the department of microbiology in college of medicine in Babylon university for their advise ,support and encouragement.

Thanks also are presented to the staff of microbiology laboratory in pediatric hospital in karballa city for their help and support during my study..

Dedication

To My parents

To my wife and daughter

List of Tables

٤. ٦	**Number and percentage of bacteria isolates in urine culture in UTI patient.**	
٤.٧	**Show the types and numbers of bacteria according to age groups and sex.**	
٤.٨	**Show the resistance of bacterial causes of urinary tract infection to specific types of bacteria**	

List of Figures

Abbreviations

WHO	World health organizasion
UTI	Urinary tract infection
CFU	Colont forming unit

Summary

Epidemiological study in children's had been planed to identify the bacterial causes of urinary tract infection under ٥years ,antibiotics sensitivity results and also immunological response to these bacteria .This study extend from ١/١٢/٢٠٠٤ to ١/١٢/٢٠٠٥ in pediatric hospital in karbala .The methods of investigation used in this study were the standard criteria for urinalysis to look for any pus cells present in the urine (pyuria is more than ٥ pus cells in centrifuged urine in high power field) (Who ٢٠٠١) , also the color, cloudiness ,acidity of urine were registered for each sample . A urine culture were performed to detect the presence and types of bacteria as well as their Antibiotic sensitivity test were performed .The specific immunological response to bacterial types were assessed by immunodiffusion .The standard criteria of this study were the age range from birth to five years ,sex ,previous UTI in the child ,residence ,type of feeding ,chief complaint of child ,congenital abnormalities if present ,past medical history of child . The total cases for children's in this study were ٣٨٥ and the pyuria were founded in ١٠٠ of them .they were ٣٧ for boys and ٦٣ for girls . The results for positive urine cultures for boys were ٢١(٣٧.٥٪) from ٣٧ and the results for girls were ٣٥(٦٢.٥٪) from ٦٣ .The numbers of positive urine cultures for E .coli were ٣٧(٦٦.١٪),١٠(١٧.٩٪) for Proteus ,٣(٥.٤٪) Klebsiella ,١(١.٨٪)

Pseudomonas,٤(٧.١٪) Staphylococcus saprophytic ,١(١.٨٪) for Enterobacter .

E .coli susceptibility to antibiotics was characterized by high resistance to amoxicillin , cotrimaxazole ,and may be considered susceptible to nitrofuratoin ,nalidixic acid , claforan , gentamycin. the high resistance among Klebsiella pneumonia also toward amoxicillin and for claforan, and very low resistance toward nitrofuratoin ,amikacin , cotrimaxazole .the high resistance of proteus toward amoxicillin and low resistance for nalidixic acid ,claforan ,amikacin.

The low resistance antibiotics for staphylococcus saprophytic were claforan , nalidixic acid , nitrofuratoin .

The low resistance antibiotics of pseudomonas were claforan ,amikacin and for Enterobacter to claforan and nitrofuratoin . The results of immunological response to E. coli was higher than proteus which was higher than in normal cases .So in general the immune response for child with UTI was higher than in normal cases.

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f bacteria	AMX	TF	GM	TMX	CE	
mirabilis	٣١ (٨٣.٨ %)	٧(٢٢.٥ %)	١٤(٣٧.٨)	٢٣(٤١.١٪)	٩(١٦.١٪)	١٣ (
monia	١٠(١٠٠٪)	٦ (٦٠ %)	٤(٤٠%)	٨ (٨٠٪)	٤(٤٠٪)	٢ (
aprophytic	٢(٦٦.٧٪)	١ (٣٣.٣ %)	١(٣٣.٣%)	٣(١٠٠٪)	٢(٦٦.٧٪)	١(٣
inosa	٢(٥٠٪)	٠	٢(٥٠ %)	١(٢٥ %)	١(٢٥ %)	١ (

acter	١ (١٠٠٪) ١ (١٠٠٪) ٤٧(٨٣.٩ %)	١ (١٠٠٪) ٠ ١٥(٢٦.٨ %)	٠ ٠ ٢١(٣٧.٥ %)	١(١٠٠٪) ١(١٠٠٪) ٣٧(٦٦.١٪)	١(١٠٠٪) ٠ ١٧ (٣٠.٤%)	١ (١ (١٩ (

Table (٤.٩) Show the resistance of bacterial causes of urinary tract infection to specific types of antibiotics.

We, the Examining Committee, after reading this thesis and examining, the student in its content, find it adequate as a thesis for degree of Master of Science in Medical Microbiology.

Signature :

Name : Prof Dr. Abd almajed A.AL-Saadi

Member :Chief of the committee

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Approved for the Council of College

Signature :

Name : Assist. Prof Dr Ali k. AL-Shaaeli

Dean of the College of Medicine

Introduction

Chapter One

Introduction

The Urinary tract infections (UTI) are bacterial infection of Urinary tract in most of cases (kidney , ureter , bladder , and urethra) . The UTI is defined by the presence of pure bacterial growth > ١٠٠,٠٠٠ colony forming unit(CFU) /ml (Baron *et.al.,*١٩٩٦). These infection can become serious if undetected early, and can some times lead to permanent kidney damage, however , they are generally treated effectively with antibiotics (Ginsburg *et.al.*, ١٩٨٢). The pyuria mean presence of more than ٥ pus cells in high power field of microscope in centrifuged urine (Vandepitte *et.al.,*١٩٩١), so white blood cells (leukocytes) should be counted. The pyuria is sufficient for diagnosis of UTI in non hospitalized patient if standard symptoms or just fever in small children is also present (Harvey , ٢٠٠٢) .

Recurrent urinary tract infection is either relapse with the original infecting organism or reinfection with a different organism (Panaretto,١٩٩٩) .In general, A reinfection is a UTI that occurs more than ٢ weeks after antibiotic treatment of the original UTI is completed; it may be caused by the same bacteria as the original infection or a different one. Reinfection account for ٨٠٪ of recurrent infections. A relapse is a UTI caused by the same bacteria as the original UTI that occurs within ٢ weeks after the individual has completed antibiotic treatment (Coyle, ٢٠٠٢).

Aim of the study :

To evaluate UTI of children under ٥ years in pediatric hospital of Karballa.

Objectives :

١.To determine the bacterial etiology of UTI .

٢. To evaluate the roles of some related factors as sex , age, breast feeding and circumcision to UTI .

٣. To detect the types of antibiotics which are effective to be used in UTI cases .

٤. To evaluate the immunological response of children with UTI for specific causative types of bacteria.

Literature review

Chapter Two

٢.١ Anatomy of the urinary tract

٢.١.١ Kidneys

A . Anatomy:

The kidneys lie along the borders of the psoas muscles and are therefore oblique placed. The adult kidney weighs about ١٥٠ 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 as seen in (Fig ٢- ١) (Richard ,١٩٩٥) .

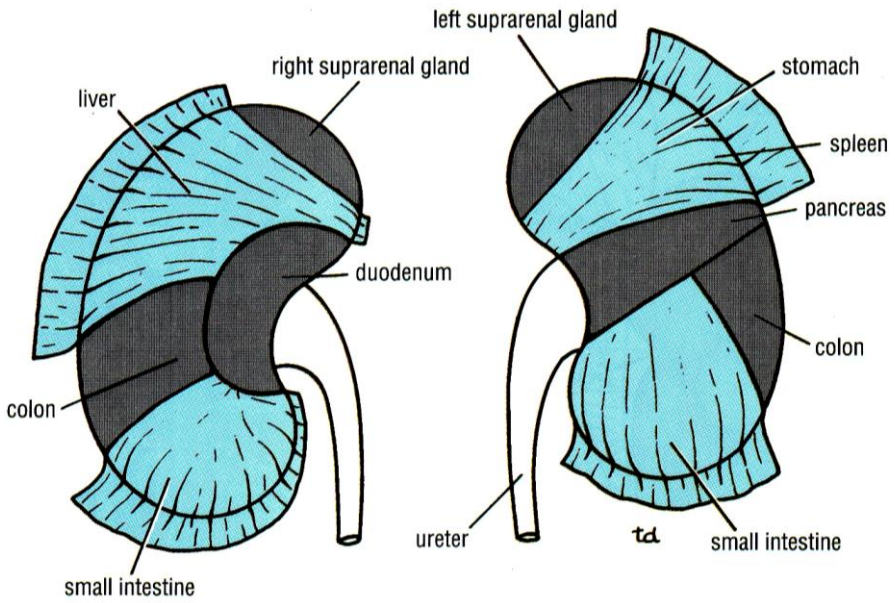


Figure ٢.١ Anterior relations of both kidneys. Visceral peritoneum covering kidneys has been left in position. Shaded areas indicate where kidney is in direct contact with adjacent viscera(Richard,١٩٩٥).

B. Histology*:*

The kidney is composed of three parts as seen in (Fig ٢-٢) . 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 pyramid formed by the converging collecting tubules which drain into minor calices (Emil, ١٩٨١).

١.Nephron*:*

It is the functioning unit of the kidney which is composed of a tubules which has both secretory and excretory function(Emil, ١٩٨١).

٢.Secretory portion:

The secretory portion is contained largely within the cortex and consist 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 glomerilus which projects into Bowmans 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(Emil , ١٩٨١).

٣.Excretory portion:

Excretory portion is the collecting tubule which is continuous with the distal end of

the ascending limb of a convoluted tubule. It empties it's content through the tip (papilla) of pyramid into a minor calix (Emil, ١٩٨١).

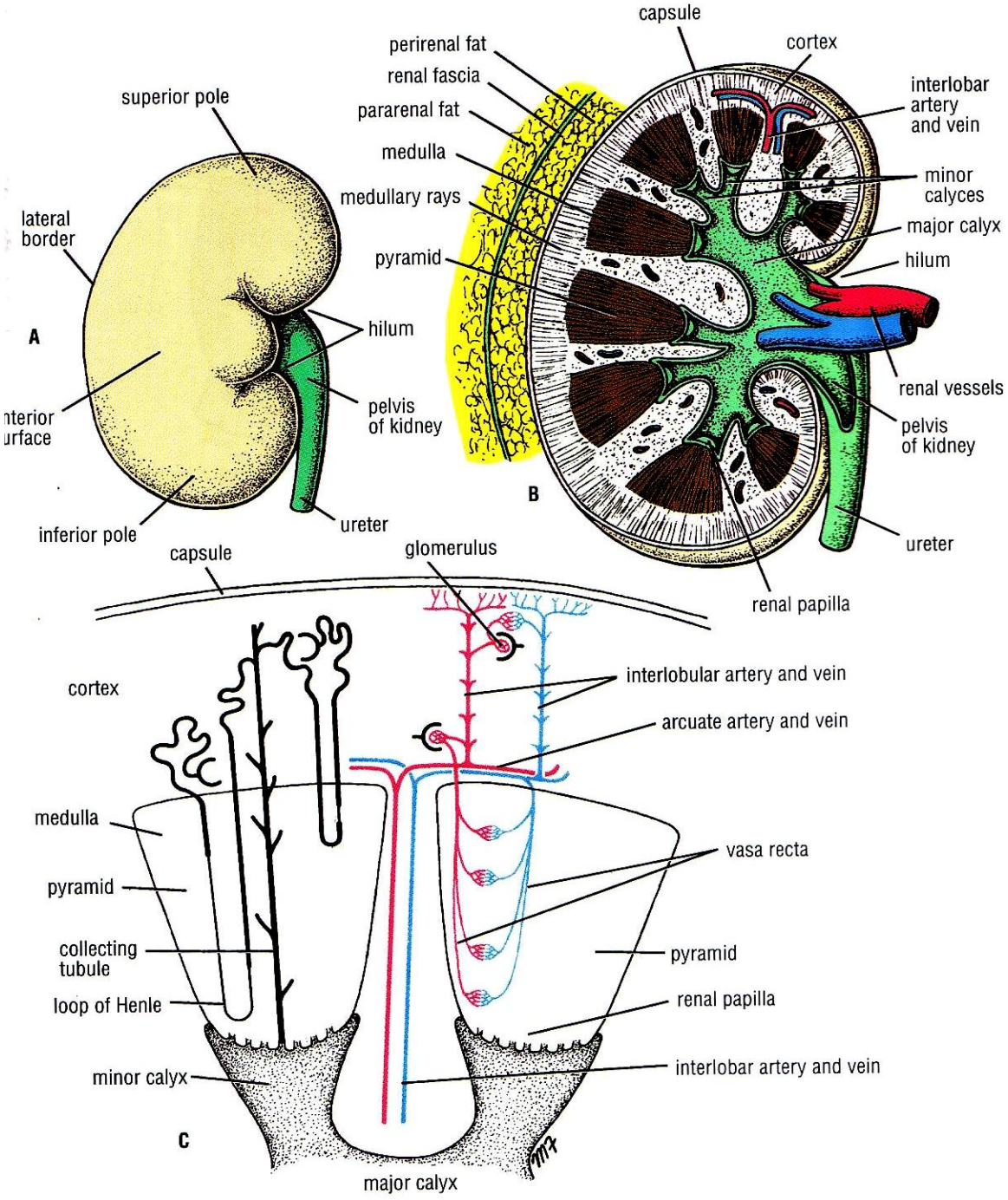


Figure ٢.٢ (A) right kidney ,anterior surface.(B) Right kidney , coronal section , showing cortex, medulla, pyramids, renal papillae, and calyces. (C) Section of kidney showing positions of nephrons and arrangement of blood vessels within kidney.these figures taken from (Richard,١٩٩٥).

٢.١.٢ Calices,. Renal Pelvis and Ureters

١- Calices:

The tip of the minor calices ٨-١٢ in number are indented by the projecting pyramids. These calices unite to form ٢ or ٣ major calices which join the renal pelvis(Emil ,١٩٨١).

٢-Renal pelvis:

The pelvis may be entirely intra renal or partly intra renal and partly extra renal (Emil ١٩٨١). 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, ١٩٨٦).

٣- Ureters:

These are narrow muscular tubes ٢٥ cm long which convey urine from the kidneys to the bladder. Ureters having ٣ constrictions:

(١) Where renal pelvis join the ureter.

(٢) Where it is kinked as it cross the pelvic brim.

(٣) 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 lumber vertebras.(Richard,١٩٩٥).

٢.١.٣ Bladder

This is a muscular urine store. In women it's posterior wall and dome are invaginated by the uterus. The adult bladder has capacity of ٣٥٠-٤٥٠ 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, ١٩٩٥).

B. 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 forming the vesicle sphincter(Lumely

*et.al.,*١٩٨١) .

٢.٢. Classification of urinary tract infection :

There are three basic forms of UTI: pyelonephritis, cystitis, and asymptomatic bacteriuria(Rushton,١٩٩٧).

١-**Clinical pyelonephritis**: is characterized by any or all of the followings: 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(Rushton,١٩٩٧).

٢-**Cystitis:** indicates that there is a bladder involvement and includes dysuria, urgency, frequency, suprapubic pain, incontinence ,and malodorous urine(Rushton,١٩٩٧).

٣-**Asymptomatic bacteriuria**: refer to a positive urine culture in children's without any manifestations and occurs almost exclusively in girls (Rushton,١٩٩٧).

٢.٣. 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 my 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(Todar,٢٠٠٢).

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). (Todar,٢٠٠٢).

٢.٤. Pathophysiology

Almost all UTIs are ascending in origin and are caused by bacteria in the gastrointestinal 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 ,١٩٨٩). 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 ,١٩٩١).

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.١٩٨٤). These findings have not been confirmed in children (Schlager *et.al.*,١٩٩٣).

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.*,١٩٨٩).

The pathophysiology of UTI reflects a complex interaction between virulence factors of the microorganism and the host defense(Barnett and Stephens,١٩٩٧). 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 are 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*.,١٩٨٥). 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*.,١٩٩٥).

٢.٥. Epidemiology

•The urinary tract infection is not uncommon in childhood. About ٨٪ or more of girls and about ٢٪ 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 (Larcombe .١٩٩٩) .

•Most UTI are due to normal bowel flora. *E. coli* is the causative organism in ٨٠٪ of cases. Other common causative organisms are *Klebsiella ,Pseudomonas* and other gram-negative organisms(Larcombe ,١٩٩٩) .

•Urinary tract infections are much more common in girls than boys (at least ١٠:١)-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 ,١٩٩٩) .

٢.٦. Diagnosis

A -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 fever or growth of more than ١٠٠٠٠ colony-forming units(CFU) per ml is evidence of the absence of a UTI .If the

child who had 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.*,١٩٨٢) .So the ways of collection of urine as recommended by (Bulloch *et.al.*,٢٠٠٠) were as follow:

١- Urine bag collection (not recommended because of high incidence of contamination).

٢- Clean catch urine of first morning void.

٣- Urine catheter specimen is recommended if the child is under ٢ years of age.

٤- Suprapubic aspirate is considered for child under ٦ months old .

B- Microscopic Urinalysis

A urinalysis involve physical and chemical examination of urine. In addition, the urine is spun in a centrifuge ١٠-١٥ ml at ١٥٠٠ to ٣٠٠٠ rpm for ٥ minutes to allow sediments containing blood cells, bacteria, and other particles . This sediment is then examined under a microscope. A urinalysis then, offers a number of valuable clues for an accurate diagnosis:

١- Simply observing the urine for color and cloudiness can be important.

٢- Acidity is measured .

٣- White blood cells (leukocytes) are counted. A high count in the urine is referred as 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,٢٠٠٢).

C-Urine culture

A calibrated loop designed to deliver a known volume either ٠.٠٠١ or ٠.٠٠٠١ ml of urine on to agar plates, incubating at ٣٧ C for ٢٤ hours and then counting the number of bacterial colonies ***(***Hoeprich , I٩٦٠***).***

A urine culture is a urine specimen observed for ٢٤ to ٤٨ hours in a laboratory for the presence of any bacterial growth . Harvey, ٢٠٠٢ had mentioned that urine culture is not routinely performed but may be conducted under certain circumstances;-

-If urinalysis is negative but the patient has severe UTI symptoms, particularly in hospitatalized patients who have a catheter and who develop fever or other signs of infection.

-If the infection is recurrent.

-if the physician suspects complications.

-if girls younger than two years have a high fever of unknown origin

that lasts ten days or more .

-Blood sample to assess the renal function including blood urea and serum creatinine and specific humeral or cellular response(Baum *et.al.,* ١٩٨٥).

-Renal ultrasonography images may show size, scarring of kidney, and the calculi if present (farmor *et.al.,* ٢٠٠٢).

Intravenous Urography (IVU) It helps to establish the diagnosis of pyelonephritis because it 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 , ١٩٨٨).

-Computerized Tomographic scan **(CT scan):** It is the procedure of choice to help diagnose chronic pyelonephritis (Gerzof and Gale, ١٩٨٢).

-Voiding cystourethrogram (VCUG); The findings may document the reflux of urine to the renal pelvis and ureteral dilatation in children with gross reflux (liyas *et.al.,* ٢٠٠٢).

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.,* ١٩٨١).

-Cystoscopy: It showes the evidence of reflux at the ureteral orifices or site of kidney stone (liyas *et.al.,* ٢٠٠٢).

٢.٧. RADIAL IMMUNODIFFUSION TEST.

Single radial immunodiffusion test has evolved from the work of (Fahey *et.al.*,١٩٩٥).

This test is used for quantitation of serum proteins as an aid in diagnosing immune deficiency disorders. 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 ١٠-٢٠ hours at room temperature are measured. The diameter of the ring and the logarithm (base ١٠) of the protein concentration are related

in a linear fashion. Using appropriate reference standards, the concentration of unknown samples may be measured.

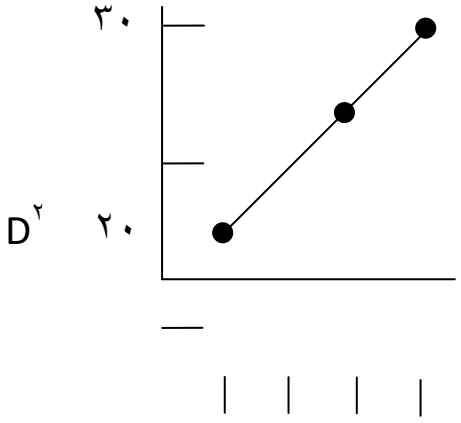
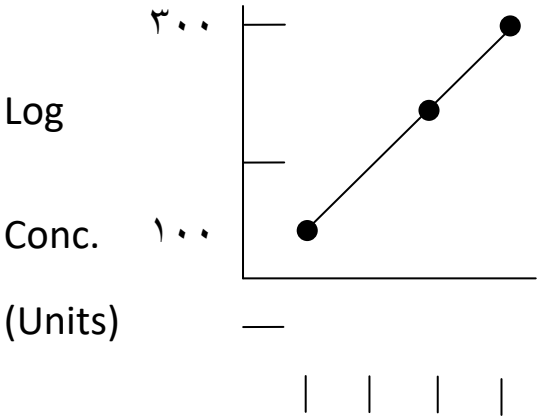


Figure (٢.٣)Show the relation between concentration of IgG,IgM and square of diameter(Fahey *et.al.*,١٩٩٥).



D(mm)

Figure (٢.٤)Show the relation between diameter of IgG,IgM and logarithm of concentration(Fahey *et.al.*,١٩٩٥).

Principle

:

Radial immunodiffusion is based on the diffusion of antigen from a circular well radially into a homogeneous gel containing specific antiserum for each particular antigen. A circle of precipitated antigen and antibody complex 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 ١٠) 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.

٢.٨. *Antibiotics for Patients with urinary tract infection:*

١-Amoxicillin :

It is semi synthetic antibiotic from the 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 the past, it has been used frequently for the treatment of UTI. Now, most bacteria presented in UTI have high resistance to amoxicillin as shown by (Sakran *et.al.,*٢٠٠٣) who observed that only ٥٢٪ of bacterial UTI responded to amoxicillin. In addition, (Leblebicioglu and Esen ,٢٠٠٣) and (Aggarwal *et.al.,* ٢٠٠٣) reported that more than ٧٣٪ of *E .coli* and ٨٨.٥٪ 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.,* ٢٠٠٢; Savov *et.al.,* ٢٠٠٢; Suarez *et.al.,* ٢٠٠٢).

٢- Cephalosporins

All first three generations of cephalosporins have oral preparations that have been used for the treatment of recurrent UTI

(Wilhelm and Edson , ١٩٨٧).

a- 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 cephradine) or parenteral (cephalothin, cephazolin and cephradine). Now; the usage of first generation cephalosporins is limited because of high resistance to it (Martinez *et.al.,* ١٩٩٥).

b- 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.,* (٢٠٠٣) reported that only ٢٦٪ of patients with hospital-acquired UTI responded to second-generation cephalosporin's.

c- 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, ceflazidime and ceftizoxime).

These drugs are excreted in bile, therefore they may use for patients with renal insufficiency (Katzung, ٢٠٠٢).

The third generation cephalosporin's have been found affective in bacterial UTI that more than ٨٠٪ and ٧١٪ of bacteria isolated from patients with UTI were sensitive to cefotaxime and ceftriaxone respectively (Gordon and Jones, ٢٠٠٣). In addition, ٧٨٪ of *Acinetobacter* isolated from patients with UTI were also sensitive to cefotaxime (Irgbu *et.al.,* ٢٠٠٣) but Zhou *et.al.,* (٢٠٠٣) found that *Enterobacter* were highly resistance to cefotaxime.

٣- Trimethoprim-sulfamethoxazole (TMP-SMX)

A combination of trimethoprim and sulfamethoxazole inhibits bacterial growth by inhibiting synthesis of dihydrofolic acid. It representes 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, ١٩٨٦). Nowadays, the resistance to TMP-SMX has slightly increased that ٤٥٪ of bacterial UTI were resistant to it including ١١٪ of *E .coli* and ٤٢٪

of S.epidermidis isolated from patients with UTI (Jureen *et.al.,*٢٠٠٣).

٤- Nitrofurantoin:

It is synthetic nitrofuran 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.,*١٩٨٧). The risk of adverse reaction increases with age and long-term therapy; therefore, should be monitored (Holmberg *et. al.,*١٩٨٠).

Nitrofurantoin has lower resistant rate than other old antibiotics that only ٤٪ of bacteria isolated from patients with recurrent UTI were resistant to it (Leblebicioglu and Esen, ٢٠٠٣).

٥- Aminoglycosides

It has a bacteriostatic action such as streptomycin, gentamicin,

amikacin, netilmicin and tobramycin.

Gentamicin is effective but it is associated with a risk of nephrotoxicity and ototoxicity, but it can be used if blood levels of creatinine and urea checked .

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, ١٩٩٩). Gentamycin is cost-effective parenteral therapy because only once-daily dosing needed and has a good sensitivity against gram-negative uropathogens (٩٠٪) and it can be in combination with TMP-SMX against gram-positive uropathogens (٧٠٪) (Jureen *et.al.,* ٢٠٠٣).

٦- Fluoroquinolones:

They are bactericidal drugs that act as inhibitors of bacterial DNA gyrase enzyme (which is responsible for supercoiling of bacterial DNA). They are affective against gram-positive and gram-negative bacteria. Recently, the oral administration of fluoroquinolones like nalidixic acid, ciprofloxacillin, levofloxacillin, ofloxacillin, norfloxacillin and others are used for empirical treatment of UT١. 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 parentefally, and then they can easily switch to oral administration and have limited use in patients with renal insufficiency (Dalkin and Schaeffer, ١٩٨٨).

٢.٩. Bacteriological Agents

٢.٩.١. 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 possess a complex antigenic structure and produce a variety of enzymes and toxins with other virulence factors (Mims , ٢٠٠٤).

Table (٢.١) Biochemical tests of Enterobacteriaceae modified from (Brooks *et.al.*, ٢٠٠١).

Test	*E .coli*	*K .pneumonia*	Proteus	*Enterobacter*	*Serratia*
EMB	Metallic sheen	Centrally dark	pale	Centrally dark	Centrally dark

Catalase	**+**	**+**	**+**	**+**	**+**
Oxidase	**—**	**—**	**—**	**—**	**—**
Indol	**+**	**—**	**±**	**—**	**—**
MR	**+**	**—**	**±**	**—**	**—**
VP	**—**	**+**		**+**	**+**
Urease	**—**	**+**	**+**	**—**	**—**
Citrate	**—**	**+**	**±**	**+**	**+**
Motility	**+**	**—**	**+**	**+**	**+**
TSI	**A/Alk± G**	**A±G**	**Alk±G**	**A±G**	**Alk±G**
H٢S	**—**	**—**	**+**	**—**	**—**

A acid

Alk alkaline

G gas

٢.٩.١.١ . ***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 (Matsumoto *et.al.,*١٩٩٠). The capsule of *E .coli* representes 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, ٢٠٠٠). Some strains are urease-producing *E .coli* and they are commonly presented in complicated UTI (Collins and Falkow, ١٩٩٠). The antibiotic resistance of *E .coli* isolated from UTI has been highly increased due to the abuse of antibiotics by the patients in addition to the toxins and enzymes like endotoxin and B-lactamase that play an important role in the virulence of bacteria.

The recent studies indicate that ٤٧٪ of patients with UTI have *E .coli* in their cultures (Gordon and Jones, ٢٠٠٣). The incidence of urinary tract infection with *E .coli* has decreased due to the increase of the nosocomial infection of urinary tract (Schrier and Gottschalk, ١٩٩٦).

٢.٩.١.٢. ***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 .*Proteus mirabilis cause* urinary tract infection and occasionally other infection. *Proteus vulgaris* and *Morganella morgnii* are important nosocomial infection agents .

Proteus species produce urease resulting in rapid hydrolysis of urea with librations 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 .*Proteus mirabilis* is often inhibited by penicillins ,the most active antibiotic for other members of the groups are aminoglycosides and cephalosporin's (Abbott,٢٠٠٣) .

٢.٩.١.٣. ***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, ٢٠٠٣) but the virulence of *Klebsiella* is not well understood, however its antiphagocytic capsule plays a role in the infections by resisting intracellular phagocytic killing. It is thought that" aerobactin , an iron-binding protein, and the production of B-lactamase enzyme contribute to pathogenicity and antibiotic resistance of the bacteria. Some strains of *Klebsiella* produce 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, ١٩٩٥).

٢.٩.١.٤. ***Serratia spp*** :

They are lactose-fermenter gram-negative short rods *Enterob-acteriaceae* with one or two flagella. They representes 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.,*١٩٩٠). Swarming motility is characteristic to the bacteria on solid or viscous media due to presence of flagella. The production of B-lactamase enzyme has given the bacteria high

resistance to several antibiotics like that in *Pseudomonas* bacteria (Kouda *et.al.,* ١٩٩٠).

٢.٩.٢. ***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.,* ٢٠٠٤).

Table (٢.٢): Biochemical tests of Staphylococcal spp modified from (Brooks *et.al.,* ٢٠٠١).

Test	***S. aureus***	S.epidermidis	***S. saprophyticus***
Catalase	***+***	***+***	***+***

Oxidase	**—**	**—**	**—**
Coagulase	**+**	**—**	**—**
Mannitol fermenter.	**+**	**—**	**—**
Resist to Novobiocin	**+**	**—**	**+**
Urease	**—**	**±**	**±**
Hemolysin	**+**	**—**	**±**

Novobiocin is only used to distinguish between *S.epidermidis* and *S.saprophyticus* (Mims *et.al.,* ٢٠٠٤).

٢.٩.٢.١. ***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 be dismissed 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.,* ١٩٩٦). 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 form 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* represent more than ٢٠٪ as a nosocomial infection (Guirguitzova . *et.al.,* ٢٠٠٢).

٢.٩.٢.٢. *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, ٢٠٠١). 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.,* ١٩٩٦).

٢.٩.٢.٣. ***S. aureus*** :

It is coagulase positive *Staphylococci,* present significantly in greater percentage of people in the hospital setting that the carrier state serves as reservoir for infection of hospitalized patients (Todar, ٢٠٠١). *Staphylococcus aureus* has a polysaccharide capsule to protect it from phagocytosis and the cell wall is 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 the production of B-lactamase enzyme that is chromosomally resistance(Takahaski *et.al.,*١٩٩٩).

٢.٩.٣. ***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, ١٩٨٣).

The pathogenicity of the organism is contributed to its virulence factors . The capsule or slime layer is associated to adherence and effectively protect 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 Iglewski, ١٩٨٣).

٢.١٠. Management

If treatment has been started before the results of a culture and sensitivity are available , then a ٣- to ٥-day course of therapy with trimethoprim –sulfamethoxazole is effective against most strains of *E.coli* . Nitrofurantoin (٥-٧ mg/kg/٢٤ hr in three to four divided doses) is also effective and has the advantage of being active against

Klebsiella- Enterobacter organisms. Amoxicillin (٥٠ mg/kg/٢٤ hr) is also effective as initial treatment but has no clear advantages over the sulfonamides or nitrofurantoin (Rushton,١٩٩٧).

In acute febrile infections suggestive of pyelonephritis, a ١٤-day course of broad-spectrum antibiotic capable of reaching significant tissue levels is preferable. If the child is acutely ill, parenteral treatment with ceftriaxone (٥٠-٧٥ mg/kg/٢٤ hr. not to exceed ٢ g) or ampicillin (١٠٠ mg /kg / ٢٤ hr) with an aminoglycosides such as

gentamicin (٣ to ٥ mg/kg/٢٤ 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,١٩٩٧).

Materials & Methods

Chapter Three

٣.١. Patients And Materials

٣.١.١ Patients

This study began from ١/١٢/٢٠٠٤ to ١/١٢/٢٠٠٥. one hundred patients with pyuria were selected attending the Pediatric Hospital of Karballa from ٣٨٥ children who had complaints related to UTI. Their age range between birth to ٥ years (٦٣ females and ٣٧ males). The investigations included : general urine examination, urine culture and antibiotic sensitivity test and also the immunodiffusion test to some patients serum with the corresponding types of bacteria isolates.

٣.١.٢ Materials

Many types of instruments and chemical materials in addition to biological materials were used in this study to complete the research. The materials were taken from different sources and companies which listed in tables (٣-١) ,(٣-٢) and (٣-٣) .

Table (٣- ١) :List of Biological and chemical materials

No	Materials	Origin
١	Pepton powder	Rashmi Dignostics, India
٢	Blood agar ,Muller-Hinton agar	Mast lab, uk
٣	MacConcky agar	Biomark lab, India
٤	Nutrient agar ,Nutrient broth	Bio life, India
٥	Antibiotic disc	Razi, Iraq
٦	Urea agar base	Oxid Ltd, England
٧	Simon citrate agar, kliglar Iron agar, MR-VP broth	Difco , Michigen
٨	Immunodiffusion kit	Kent , USA
٩	Methyl red,a- Naphthol ,Tetramethyl-p-paraphenylene diamine dihydrochloride.	B.D.H.

Table (٣- ٢) : list of instruments used

No	Instrument	Origin
١	Sensitive electronic balance	A and O , Japan
٢	Incubator	Termaks, Stockholm
٣	Distillatur	C.fL, Germany
٤	Benson Burner	Germany
٥	Light microscope	Olympus, Japan
٦	Centrifuge	NF ٨١٥-Ankra, Turkey
٧	Micropipette	Oxford, USA
٨	Refrigerator	General , Japan
٩	Inoculating loop	Japan
١٠	Inoculating needle	Japan
١١	Oven	Memert, Germany

Table(٣- ٣) The potency of antibiotics according to antibiotic source (Iraq, Razi)

Cefotaxime	١٠ mcg

Gentamicin	١٠ mcg
Amoxicillin	١٠ mcg
Cotrimaxazole	٢٥ mcg
Nitrofurantoin	٣٠٠ mcg
Nalidixic acid	٣٠٠ mcg

٣.٢. Method of Diagnosis

٣.٢.١ Urinalysis

A urinates involved a physical and chemical examination of urine (color, reaction and albumin). **According to Massey-(٢٠٠٤) , the urine was spun in a centrifuge at ٣٠٠٠ rpm for ٥ minutes to allow sediments containing:**

- **Pus cells (WBC).**
- RBC
- Crystals.
- Casts .

٣.٢.٢ Preparation Of Media

These media were prepared after manufactures recommendations

٣.٢.٢.١-Blood agar base .

a -suspend by swirling ٣٧.٥ g of powder of blood agar base in ١٠٠٠ ml of distilled water

b- autoclave at١٢١ c for ١٥ minute

c-mixed well before pouring. For blood agar ,٧٪ blood at ٥٠ c was added .

d-PH was readjusted after autoclaving to be ٧.٤ .

٣.٢.٢.٢- Nutrient agar.

A quantity of ٢٣ g of powder of nutrient agar was suspended in ١٠٠٠ ml of cold distilled water .heated to boiling ,distributed and sterilize at ١٢١ c for ١٥ minute. The PH readjusted after autoclaving to be ٧.٤

٣.٢.٢.٣- MacConkey agar.

As recommended by manufacture , ٥١.٥ g of powder of macConkey agar was suspended in ١٠٠٠ ml distilled water .then boiled to dissolve the medium completely , sterilize by autoclaving at ١٢١ c for ١٥ minutes. The pH was readjusted to

٧.٤ .

٣.٢.٢.٤- Eosin Methylene Blue (EMB) Agar.

This medium is used to identify lactose fermenting bacteria. 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 is due to the absorption of the esoin-methylene blue complex, 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., ١٩٩٦).

٣.٢ .٣. Biochemical Tests

٣.٢.٣.١. <u>Catalase Test :</u>

It was prepared by dissolving ٣ gm of H_2O_2 into ١٠٠ml of distill water and was stored in a dark container (Baron *et.al.,* ١٩٩٦) .

A colony the organism was transferred to a drop of ٣٪ H_2O_2 on a microscope slide. The presence of catalase meant that the formation of gas bubbles has occurred which indicated a positive result (Collee *et.al.,* I٩٩٦).

٣.٢.٣.٢. Oxidase Test:

It was prepared by dissolving ٠.١ gm of Tetra-P- paraphenylene diamine dihydrochloride in ١٠ ml of distilled water and stored in a dark container (Baron *et.al.,* ١٩٩٦). A piece of filter paper was saturated in a petri dish with oxidase reagent then a colony of tested 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.,* ١٩٩٦).

٣.٢.٣.٣. Coagulase Test:

Several colonies of bacteria were transferred with a loop to a tube containing ٠.٥ ml of human plasma. The tube was covered to prevent evaporation and incubated at ٣٧°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.,* ١٩٩٦).

٣.٢.٣.٤. Methyl red reagent :

Methyl red in a ٠.١ gm quantity was dissolved in ٣٠٠ ml of ٩٩٪ ethanol and then the volume was completed to ٥٠٠ ml by distill

water (Macfaddin, ٢٠٠٠).

Methyl Red Test:

The test was performed on ٥ ml of MR-VP broth cultured by the examined organism and then it was incubated for ٢٤ hours at ٣٧°C. After that ٦-٨ drops of Methyl Red reagent were added to culture. The change of color to orange-red indicated a positive reaction (Collee *et.al.,*١٩٩٦).

٣.٢.٣.٥. Voges -Proskauer reagent

Reagent A) ٥ gm of a-naphthol was dissolved in ١٠٠ ml of ٩٩٪ ethanol.

Reagent B) ٤٠ gm of KOH was dissolved in ١٠٠ ml of distilled water (Collee *et.al.,* ١٩٩٦).

Voges-Proskaur Test:

The test was performed on ٥ ml of MR-VP broth cultured by the organism and then it was incubated for ٢٤ hours at ٣٧°C. After that ١٥ drops of ٥٪ alpha naphthol (reagent A) were added followed by ١٠ drops of ٤٠٪ KOH (reagent B) and shaken well and allowed to stand for up to ٣٠ minutes before calling a reaction negative. for positive culture at the surface of the liquid turned to red, and the color spread gradually throughout the tube (Baron *et.al.,* ١٩٩٦).

٣.٢.٣.٦. **Indol Test**:

A ١٪ solution of tryptone broth was prepared then distribute in culture tubes then it was sterilized into the autoclave at ١٢١ C for ١٥ minutes. The pH was readjusted after autoclaving to be ٧.٤ . After that the broth was inoculated with bacterial isolate and it was incubated for ٤٨-٧٢ hours at ٣٧°C. Testing for indole production was made by adding ٦-٨ drops of Kovac's Reagent (p-dimethylaminobenzaldehyde in amyl alcohol). The formation of red color ring at the top of broth means a positive reaction.

A yellow color ring indicates a negative result (Macfaddin , ٢٠٠٠). Tubes were allowed standing for up to ٣٠ minutes before calling a reaction negative. In positive reactions the color turnes red at the surface of the liquid, and the color spread gradually throughout the tube later (Baron *et.al.,* ١٩٩٦).

٣.٢.٣.٧. **Simon Citrate Test:**

This test was carried out according to (Benson,١٩٩٨).

Simon citrate slants were sterilized by autoclave at ١٢١ C for ١٥ minutes then cooled to ٥٠ C and the pH was readjusted to ٧.٤ and then inoculated with the bacterial culture and incubated for ٢٤-٤٨ hours at ٣٧°C. The positive result was a change of the color of media

from green to blue. A no change of the color indicates a negative reaction .

٣.٢.٣.٨. Urease Test:

This test was carried out according to (Benson,١٩٩٨).The urea base agar was sterilized by autoclaving at ١٢١ C for ١٥ minutes . After it cools to ٥٠°C the pH was readjusted to ٧.٤ . The urea substrate was added and was poured in sterile tubes; then inoculated with the bacterial culture and incubated for ٢٤-٤٨ hours at ٣٧°C. The positive result was a deep pink color. Failure of deep pink color to develop meant a negative reaction .

٣.٢.٣.٩. Kliglar Iron Agar (KIA) Test:

This test was applied according to(Macfaddin,٢٠٠٠). The test is used 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 incubated at ٣٧°C" for ٢٤-٤٨ hours. The change color of the media from orange-red to yellow is due to carbohydrate fermentation with or without gas formation at butt of slant. In addition, the formation of hydrogen sulfide results in a black color precipitation at the butt .

٣.٢.٣.١٠ Motility test by using semisolid media:

According to the method described by (Macfaddin,٢٠٠٠), ١٠ ml of semisolid media was dispensed in culture tubes and left to stand at a vertical position ,it was 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 ٣٧°C and examine at ٦ hours, ٢٤ and ٤٨ hours. Non-motile bacterial growth is generally confined to the stab-line and gives sharply defined margins leaving the surrounding medium clearly transparent. Motile bacteria typically gives diffuse hazy growths that spread through out the medium rendering it slightly opaque .

٣.٣. The modified Kirby-Bauer method

١-Mueller-Hinton agar

١. Mueller-Hinton agar was prepared from a dehydrated base according to the manufacturer's(Mast lab ,uk) recommendations.

٢. The medium has to be autoclaved for ١٢١ c for ١٥ minutes then cooled to ٤٥-٥٠ ْC and pour into the plates. Allow to set on a level surface, to a depth of approximately ٤ mm. A ٩-cm plate requires approximately ٢٥ ml of medium.

٣. After agar had solidified, the plates were placed in the upright position in the incubator with the lids tilted at ٣٥c for ٣٠ minute to

dry..

٤. Any unused plates were stored in a plastic bag as the recommendation of (Vandepitte *et.al.,*١٩٩١),which were sealed and placed in the refrigerator. Plates stored in this way can be kept for ٢ weeks.

٢-Antibiotic discs

Antibiotic discs of (Razi, Iraq) with the proper diameter and potency were used. Stocks of antibiotic discs were kept at - ٢٠ °C; the freezer. Antibiotics discs can be kept in the refrigerator for up to ١ month. On removal from the refrigerator, the containers were left at room temperature for about ١ hour to allow the temperature to equilibrate before use .

٣-Procedure

As the method described by (Macfaddin,٢٠٠٠).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 ٥ isolated colonies grown on a blood agar plate to ٥ml of broth. This culture was then incubated for ٢ hours to produce a bacterial suspension of moderate turbidity. A sterile swab was used to obtain an inoculum from the

standardized culture .This inoculum was then streaked on a Mueller-Hinton plate.

The antibiotic 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 ١٤ hours at ٣٧c .Antibiotic 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.

٣.٤. RADIAL IMMUNODIFFUSION TEST

١-Materials

١-١- Serum samples

Fifty seven blood samples were collected from children under five years with pyuria ,٣٧ cases with E .coli ,١٠ cases with proteus and ١٠ cases with negative urine culture in the Pediatric Hospital in Karballa . These samples were then used to obtain serum by centrifugation at ٣٠٠٠ rpm.(Fahey *et.al.,*١٩٩٥).

١-٢- Materials used.

١. Three (٣x٨) well radial immunodiffusion plates(Kent,USA).

٢. Blood collection tubes.

٣. Syringes and needles.

٤. Centrifuge (٣٠٠٠ rpm)

٥. Microliter dispenser (٥ microliters)

٦. Human Reference Sera: ٣ x ٠.٢ml vials(in kits only).

٧. Measuring device-calibrated in ٠.١mm increments available separately.

٨. linear graph paper between the concentration and squire of diameter

٢. Procedure

according to the method of (Fahey *et.al.*,١٩٩٥) the radial immunodiffusion kit was used to determine the levels of antibodies (IgG.IgM) in the serum of children with pyuria and as follows**:**

١. The blood was collected without anticoagulant and allowed to clot at room temperature.

٢. The serum was separated by centrifugation at about ٣٠٠٠ rpm within ٢-٣ hours after collection.

٣. The plates were removed from refrigerator to room temperature

approximately ٣٠ minutes before filling wells.

٤. Excess moisture was removed from the plate and the cover was removed until evaporation had dried the surface and wells. the cover were replaced until use .

٥. Three wells were filled with reference sera for each plate. Location of each was noted. Each vial of reference serum was mixed thoroughly.

٦. The specimen was delivered to the well by placing the pipette tip at the bottom of the well. The bubbles were avoided to ensure proper volume and diffusion of sample. Visualization was aided by placing the plate on a dark background.

٧. For the consistency wells were filled with a ٥ microliter pipette.

٨. The time of completion were marked on plate cover .

٩. The plate was replaced in its bag .

١٠. The plates were incubated upright on a flat surface at room temperature (٢٠° to ٢٤° C) for ١٦-٢٠ hours. Overnight readings and after ٤٨ hours for end point readings.

١١. The diameters of precipitin zones were measured to within ١mm.

١٢. The reference sera provided in kits were used , such as the College of American Pathologists reference standard, determine their ring diameters to the nearest ٠.١ mm.

١٣. Regular graph paper were used to 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 ٤.٢ – ٤.٣)

١٤. A straight line was drawn of "best fit" between the three points.

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.

٣.٥. Biostatistical analysis

The biostatistical analysis of this study was obtained by the SPSS and Excel programs . The significance association at level of P value < ٠.٥ and higher signficance association of P value < ٠.٠١ .

Results

Chapter four

٤.١ Results

The study included ٣٨٥ children who had complaints related to UTI (٢٢٩ girls and ١٥٦ boys) from birth to ٥ years age attending the Pediatric Hospital of Karbalia . General urine examination was made for all of them . pyuria was diagnosed in ١٠٠ (٦٣ girls and ٣٧ boys) as shown in table (٤.١) .It was found that ٥٦ of them children had positive urine culture (٣٥ girls and ٢١ boys) as shown in table (٤.٢) .

٤.٢ Analysis of some related factors revealed the following results

٤.٢.١ Sex distribution

The sex distribution of pyuria cases indicated that the pyuria was more common in girls (٢٧.٥٪) than boys (٢٣.٧٪) and also the pyuria with positive urine culture was more common in girls (١٥.٣٪) than boys (١٣.٥٪) .

Table (٤.١)

Sex distribution of Pyuria cases .

Sex	Children number	**Pyuria present**	**Percentage of pyuria**
Female	٢٢٩	٦٣	٢٧.٥ %
Male	١٥٦	٣٧	٢٣.٧ %
Total	٣٨٥	١٠٠	٢٦ %

Table (٤. ٢)

Sex distribution of UTI cases (pyuria with positive urine culture).

Sex	**number**	**percentage**

Female	٣٥(٢٢٩)	١٥.٣ %
Male	٢١(١٥٦)	١٣.٥ %
Total	٥٦(٣٨٥)	١٤.٥ %

٤.٢.٢ Urinary tract infection according to age.

The distribution of UTI cases according to age groups indicated that UTI was more common in first(٣٥.٧٪) and second(٢٣٪) year of life and then decrease later in the third(١٢.٥٪) ,fourth(٧.١٪) and fifth(١٢.٥٪) year of life .

Table (٤.٣)

Distribution of positive urine culture cases according to age group.

Age group	**Number of cases**	**percentage**

Group ١ **(birth to ١ years)**	٢٠	٣٥.٧ %
Group ٢ **(١-٢ years)**	١٨	٢٣.١ %
Group ٣ **(٢-٣ years)**	٧	١٢.٥ %
Group ٤ **(٣-٤ years)**	٤	٧.١ %
Group ٥ **(٤-٥ years)**	٧	١٢.٥ %
Total	**٥٦**	**١٠٠ %**

٤.٢.٣ Type of feeding.

This study observed the effect of breast feeding on occurrence of UTI under the age of two years of life. It was

found that the number of children on bottle feeding with UTI were significantly higher in comparison with those children on breast feeding with UTI at the level of significance (P value < ٠.٠٠١) .Table (٤.٤) represent children with UTI and on breast feeding were (٣٥٪) and with mixed feeding (breast and bottle) were (١٧.٩٪) and with bottle feeding were (٤٦.٣٪).

Table (٤.٤)

Number and percentage of types of feeding for children under ٢years with UTI. .

Types of feeding	**Numbers**	**percentage**
Breast feeding	٢٤	٣٥.٨ %
Mixed feeding	١٢	١٧.٩٪
Bottle feeding	٣١	٤٦.٣ %
Total	٦٧	١٠٠ %

٤.٢.٤ Circumcision effect on UTI in boys under ٥ years.

The circumcision effect was evaluated in children with UTI . The percentage of circumcised boys with UTI in the first year of life were (١٥.٤٪) and in the second year were (٣٣.٣٪)and in the third year were(٥٠٪) and in the fourth year were(٦٦.٧٪)and in the fifth year were (٦٠٪) .So it was found that the number of uncircumcised boys with UTI were significantly higher in numbers than the numbers of circumcised boys with UTI at the level of significance (P value < ٠.٠٥) as shown in table (٤.٥) .

Table (٤.٥)

Distribution of cases of UTI in boys under ٥ years according to circumcision affect.

Age groups	Circumcised boys	Percentage	Uncircumcised boys	Percentage	Total
٠-١ year	٢	١٥.٤٪	١١	٨٤.٦٪	١٣
١-٢ year	٣	٣٣.٣٪	٦	٦٦.٧٪	٩

٢-٣ years	٢	٥٠٪	٢	٥٠٪	٤
٣-٤ Years	٤	٦٦.٧٪	٢	٣٣.٧٪	٦
٤-٥ Years	٣	٦٠٪	٢	٤٠٪	٥
Total	١٤	٣٧.٨٪	٢٣	٦٢.٢٪	٣٧

٤.٢.٥ Recurrent urinary tract infection in children under ٥ years .

This study indicated that percentage of recurrent UTI in children under the ٥ years was (٧٠٪) and in boys was (٥٧٪) while in girls it was (٧٧٪).The recurrent UTI was more common in girls than boys.

Table (٤.٦)

Distribution of cases of recurrent UTI in children according to sex .

Recurrent UTI	Boys	percentage	Girls	percentage	Total	percentage
Present	١٢	٥٧٪	٢٧	٧٧٪	٣٩	٧٠ %
Not present	٩	٤٣٪	٨	٢٣٪	١٧	٣٠٪
Total	٢١	١٠٠٪	٣٥	١٠٠٪	٥٦	١٠٠٪

٤.٢.٦ Bacterial causes of urinary tract infection in children .

The most common bacterial causes of UTI isolated was *E. coli*

bacteria (٦٦٪) and then *p. mirabilis* (١٧.٩٪) , *Staph. saprophyticus* (٧.١٢٪) , *K. pneumonia*(٥.٤ %), *P. aeruginosa(*١.٨ %) and *Enterobacter* (١.٨٪).

Table (٤.٧)

Number and percentage of bacteria isolates in urine culture in UTI patient.

Type of bacteria		Numbers	percentage
Gram	***E. coli***	٣٧	٦٦ %
	P. mirabilis	١٠	١٧.٩ %
	K. pneumonia	٣	٥.٤ %
	P. aeruginosa	١	١.٨ %
	Enterobacter	١	١.٨ %
Gram	***Staph.saprophyticus***	٤	٧.١ %
Total		٥٦	١٠٠ %

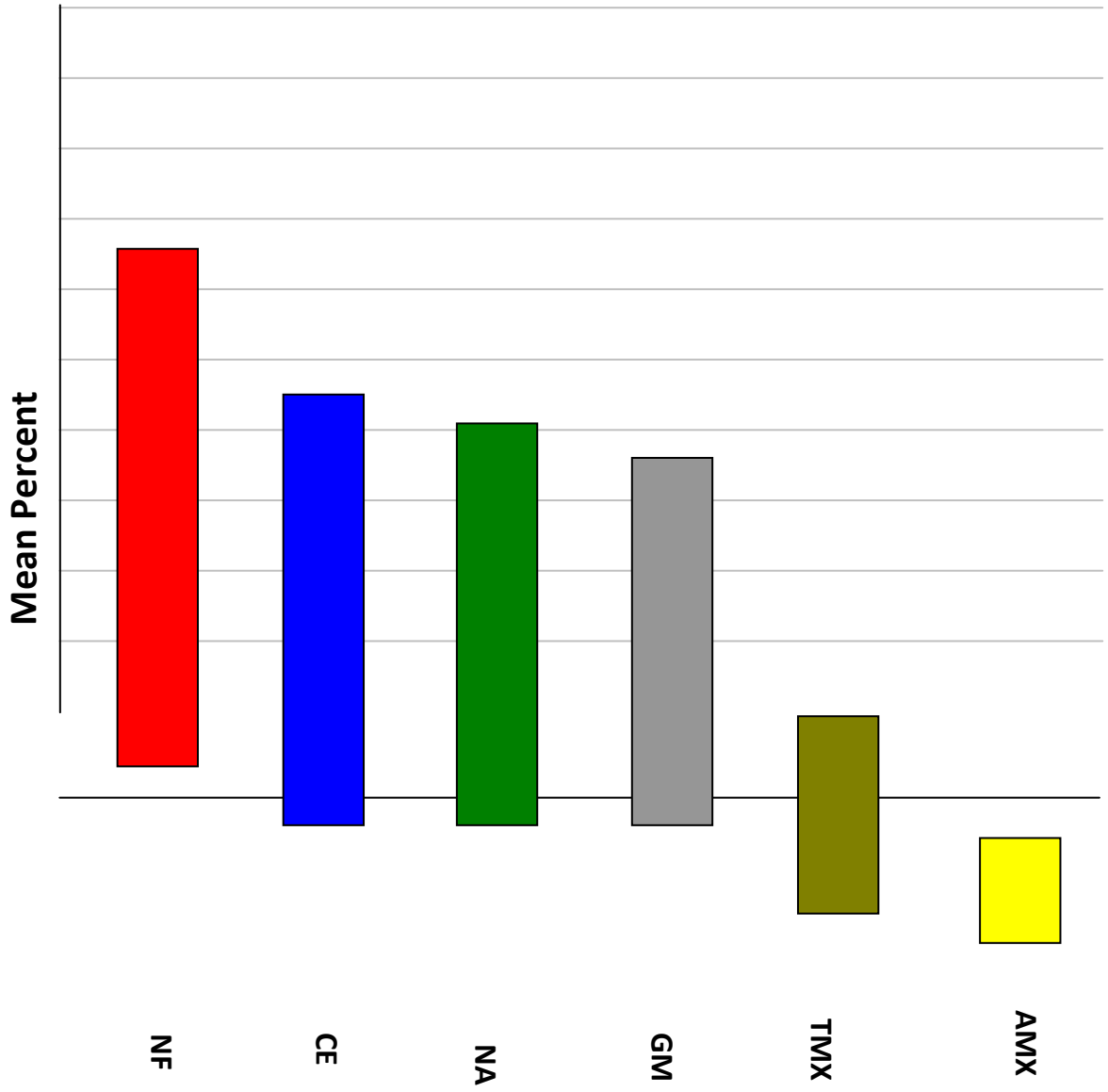
Table (٤.٨)

Distribution of cases of UTI according to age groups and types of bacteria

The bacteria	٠-١ year		١-٢ years		٢-٣ years		٣-٤ years		٤-٥ years	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
E. coli ٣٧	٤	١٠	٢	٩	٢	٣		٢	١	٤
proteus. mirabilis ١٠	٣	١	٢	١	١		١		١	
Staph. saprophyticus ٤	١		١	١						١
pseudomonas ١		١								
Klebsiella ٣			١	١		١				
Enterobacter ١							١			
Total	٨	١٢	٦	١٢	٣	٤	٢	٢	٢	٥

٤.٢.٧ Antibiotic sensitivity Result .

The antibiotic which was more sensitive on bacterial causes of UTI was Nitrofuratoin then Cefotaxime ,Nalidixic acid, gentamicin, Trimethoprim-sulphamethaxazole and Amoxicillin .



Drugs used

AMX Amoxicillin TMX Trimethoprim Sulphamethaxazole

GM Gentamicin NA Nalidixic acid

CE Cefotaxime NF Nitrofurantoin

Figure (٤.١)

The percentage of sensitivity of bacterial isolates to antibiotics.

٤.٢.٨ Immune response for urinary tract infection.

This study included ٥٧ children with pyuria ٣٧ cases with E .coli,١٠ cases with proteus and ١٠ cases had negative urine culture . The serum IgG ,IgM antibodies concentrations was measured and was compared the mean of concentrations of these three groups. It was found that the mean of serum IgG IgM concentrations of antibodies of *E .coli* were significantly higher than *Proteus mirabilis* at the level of significance (P value < ٠.٠٥) . For *proteus* it was significantly higher than negative cases of urine culture at the level of significance (P value < ٠.٥) as seen in figure (٤.٤).So the immune response to *E .coli* was higher than *proteus* and for *proteus* was higher than negative cases.

The immune response of cases of UTI according to age groups also were studied by taking the mean of concentrations of antibodies for each type of bacteria for each age group as seen in figure (٤.٥-٤.٦)

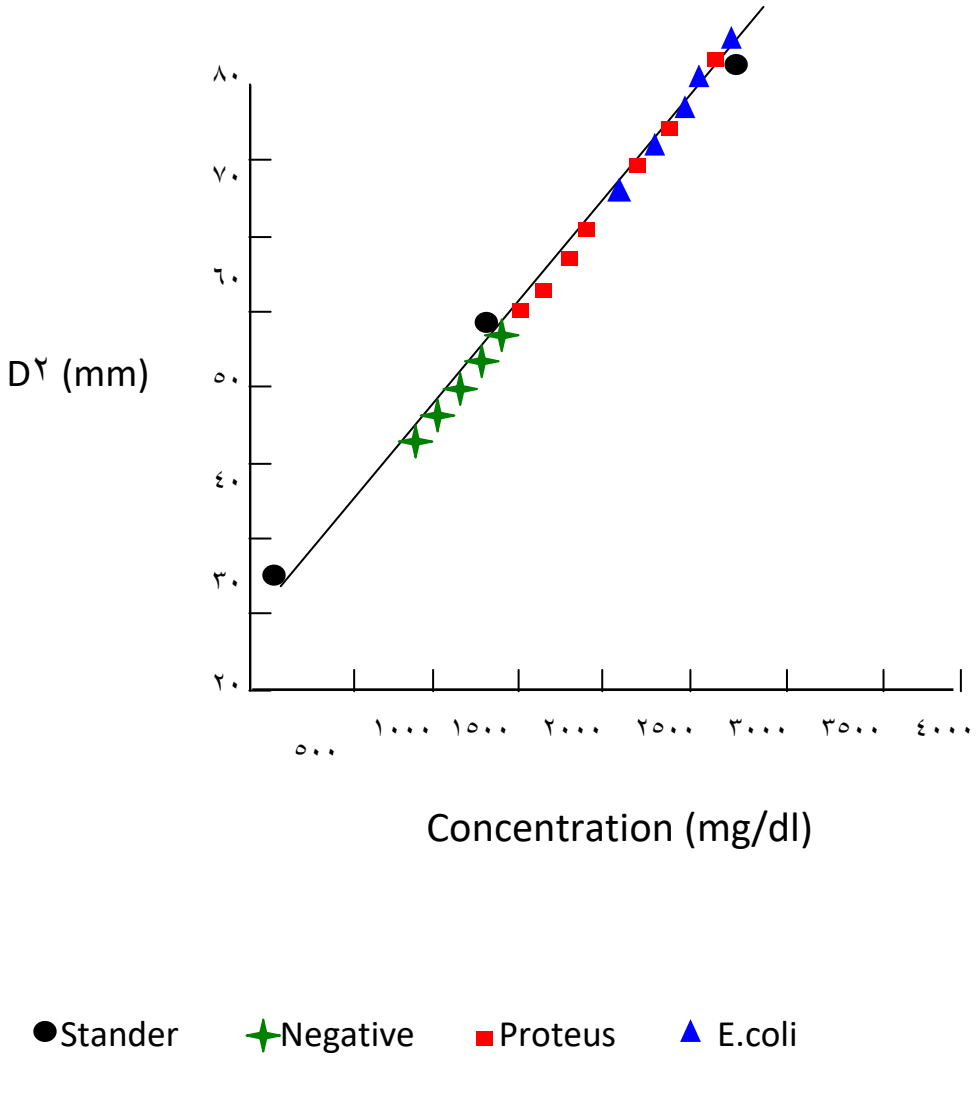


Figure (٤.٢) The relation between concentration of IgG and square of diameter of three standers of radial immunodiffusion kit.

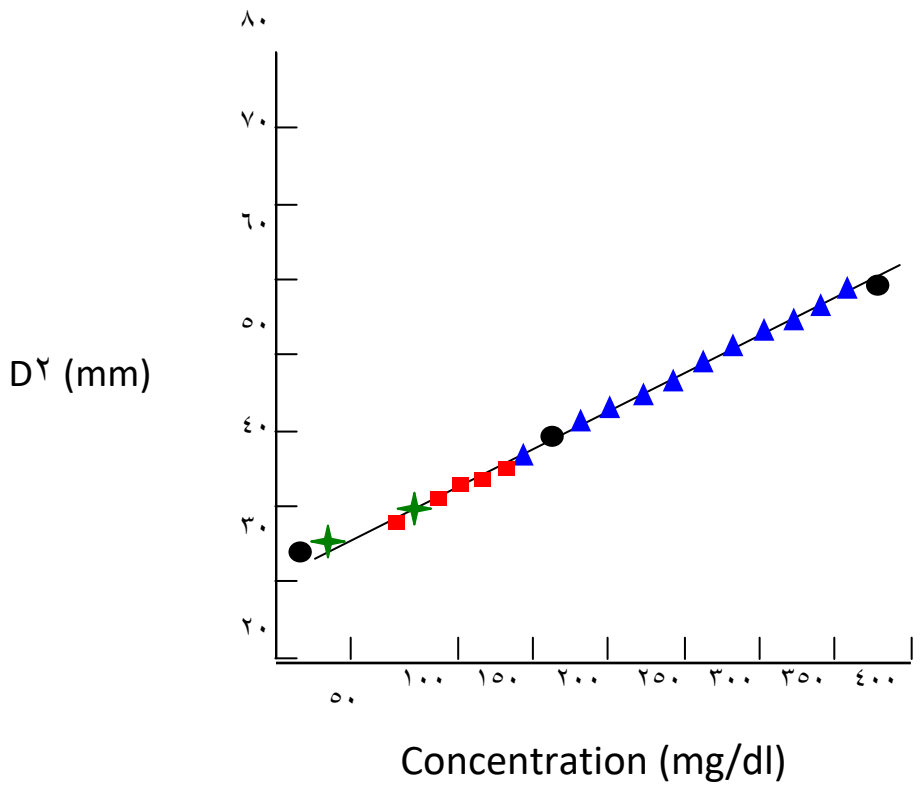


Figure (٤.٣)The relation between concentration of IgM and square of diameter of three standers of radial immunodiffusion kit.

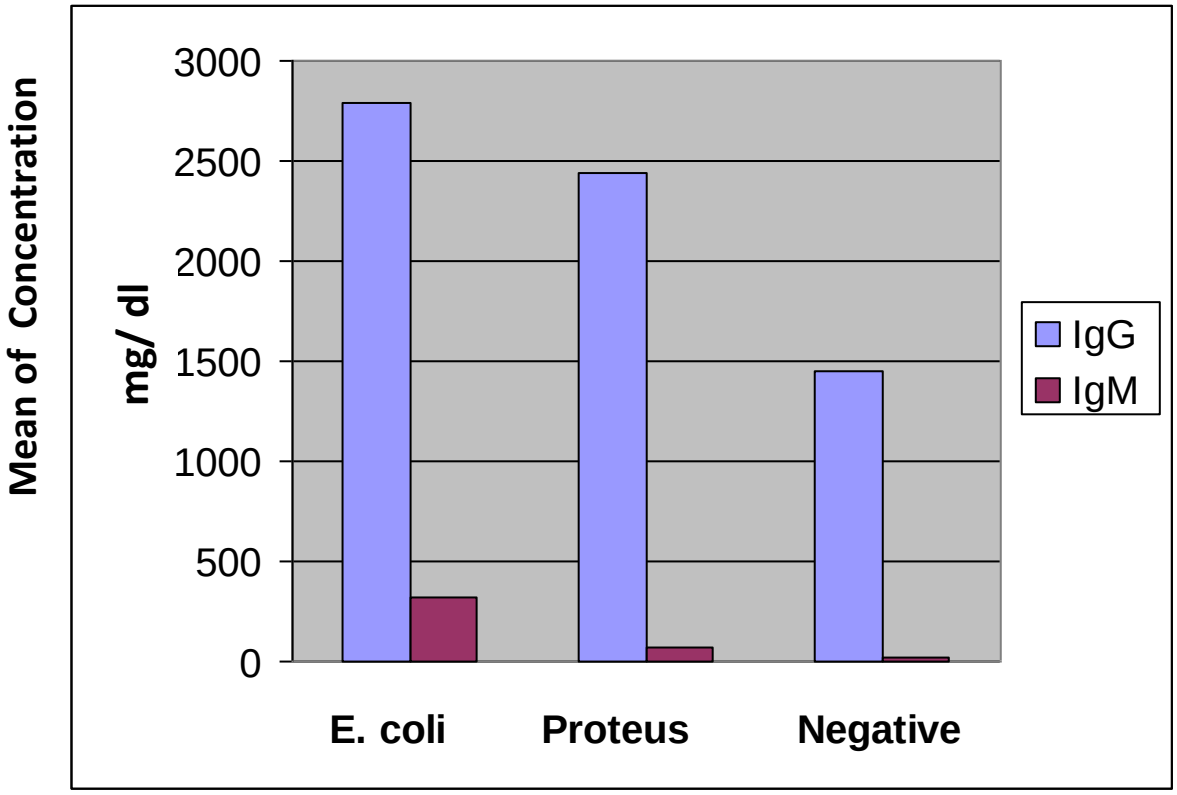


Figure (٤.٤)

The means of concentrations of IgG,IgM antibodies according to type of bacteria.

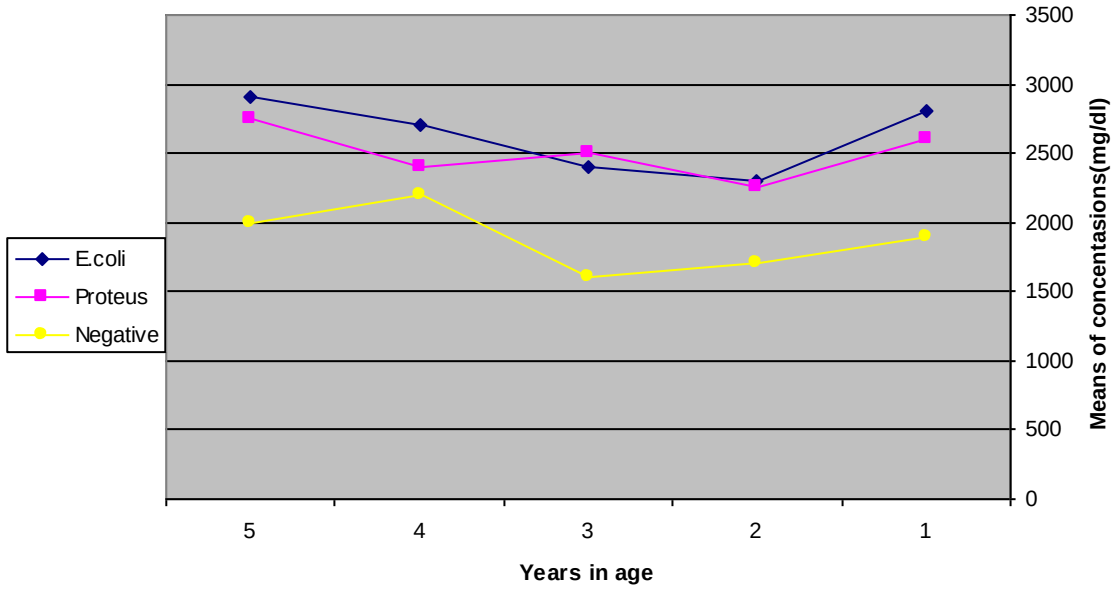


figure (٤.٥) The relation between the mean of concentrations of IgG antibodies and the age groups.

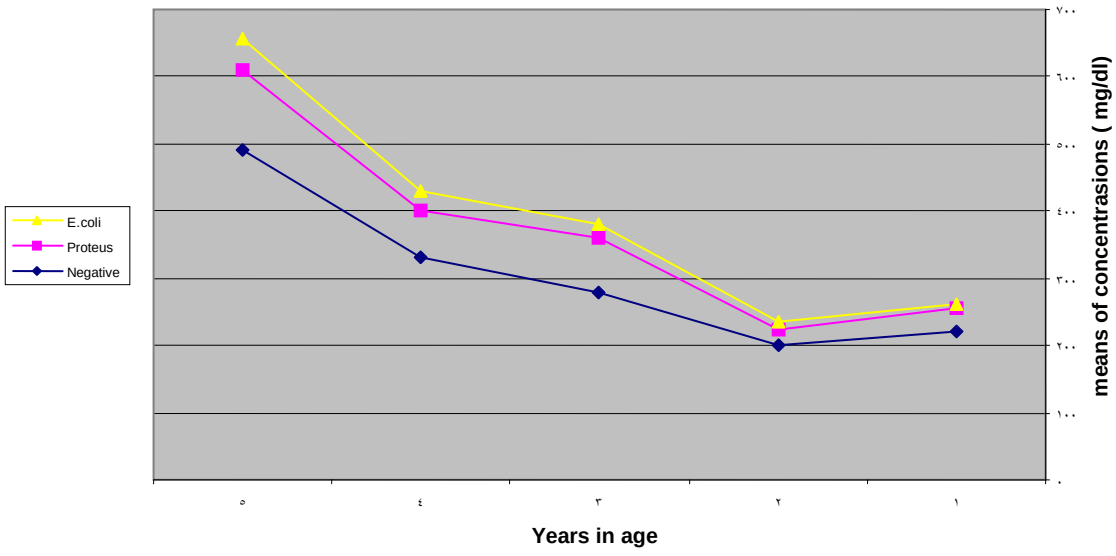


figure (4-7) The relation between the average of concentration of IgM antibodies with the age groups.

Discussion

Chapter Five

Disc on

٥- Discussion

٥.١. Sex related disease

This study has covered ٣٨٥ children (١٥٦ male and ٢٢٩ female) ending during the period of the study in the Pediatric Hospital of Karballa who had UTI . Pyuria was diagnosed in ١٠٠ of them. They were ٦٣ female and ٣٧ for male. The presence of positive urine culture was ٥٦ . They were ٣٥ girls and ٢١ boys. The percentage of urinary tract infection under ٥ years was higher in girls (١٥.٣٪) than in boys (١٣.٥٪) as listed in table (٤-٢).

These results agree with the results obtained by (Shahab and Navideh ,١٩٩٧), who reported the rate of urinary tract infection in female (٣٥.٣٪) higher than male (١٨.٣٪) in children under ١٢ years of age .

. The present data agrees with those obtained by Zainal and Baba, ١٩٩٦. study in Malaysia , in which the prevalence was ٠.١٩٪ in boys

and ٠.٣٣٪ in girls.

In general girls are more susceptible to urinary tract infection than boys because the urethra is shorter and nearer to the anus more in female leading to more possibility of infected with enteric bacteria, in addition, the vaginal pathogen may invade the urinary tract causing urinary tract infection (Mims *et.al.,* ٢٠٠٤).

٥.٢. Urinary tract infection according to age groups

with urinary tract infection were taken in this study under ٥ years and The children also were divided into ٥ age groups .The first age group covered patient from birth to ١ year, the second from ١ to ٢ years ,the third from ٢ to٣ years ,the fourth from ٣ to٤ years and the fifth from ٤ to ٥ years .

This data indicate that the age group more affected was the first age group who were ٢٠ cases from ٥٦ (٣٥.٧٪)and the second were ١٨ from ٥٦ (٣٢.١٪)and the third were ٧ from ٥٦ (١٢.٥٪)and the fourth were ٤ from ٥٦ (٧.١٪)and the fifth were ٧ from ٥٦ (١٢.٥٪) .

These results agree with those obtained by (chow *et.al.,*١٩٨٨) that

show age below ١ year is more affected then decreases above one year , to fourth year , it became less above fourth year. Also the results agree with those obtained by (Shahab and Navideh,١٩٩٧)who reported that more age group affected is that under ٢ years then the incidence decreases till ٤years and even decreases more above ٤ years of age .

٥.٣. Effect of feeding on UTI .

The breast feeding is an important protective factor against most diseases of childhood. This study investigated the impact of breast feeding on the occurrence of urinary tract infection in children under two years age. The children number with urinary tract infection and on breast feeding exclusively were ٢٤ (٣٥.٨ %). while on mixed feeding (breast and bottle feeding) ١٢ (١٧.٩ %) and on bottle feeding were ٣١ (٤٦.٣ %) as show in table (٤-٤) .

These results show increase of urinary tract infection in children on bottle feeding if we compared with other study on types of feeding that done in Basrah that show ٥٠.٧ % were still receiving breast milk as

their only source of milk and ١٤.٤ % were receiving infant formula in addition to breast feeding (Benyamen. ١٩٩٨). Also the results agree with those obtained by (outerbridge,١٩٩٨) who reported that breast feeding is very effective in reducing incidence of UTI in children .

٥.٤. Urinary tract infection in boys under five years according to circumcision affect.

The effect of circumcision was investigated in boys as its predisposing factor of UTI . Male patients were divided into circumcised and un circumcised boys. The circumcised boys in the first age group were ٢ (١٥.٤٪)from ١٣ boys and in the second age group were ٣(٣٣.٣٪) from ٩ boys and in the third age group were ٢(٥٠٪) from ٤ boys and in the fourth age group were ٤(٦٦.٧٪) from ٦ boys and in the fifth age group were ٣(٦٠٪) from ٥ boys. The total circumcised boys under ٥ years were ١٤ (٣٧.٨٪) from ٣٧ boys with UTI .

These results agree with the results obtained(Craig *et.al.*,١٩٩٦) in Australia in boys under ٥ years of age (mean ٦ months)that ٦٪ of uncircumcised got UTI but only ١٪ of circumcised got it. Also the results agree with those of (Charles *et.al.*,١٩٨٤)who reported that ٩٥٪ of UTI in the uncircumcised boys and ٥٪ of UTI in circumcised boys in the age from ٥ days to ٨ months .These results were confirmed by (Wiswell,١٩٨٩) who established that uncircumcised boys had ١١-fold higher incidence of getting UTI than circumcised boys.

٥.٥. Recurrent urinary tract infection in children .

This study proved that many of the urinary tract infection were recurrent in children that ٣٩ (٧٠٪) out of ٥٦ with recurrent urinary tract infection and ١٧ (٣٠ %)with out recurrence (acute urinary tract infection)as listed in table(٤-٦).These results agree with those obtained by (Zymslowska and Kozlowski,٢٠٠٣) who reported that acute UTI was observed in ٢٩٪ of children , while recurrent UTI was diagnosed in ٧١٪of patients.

Recurrent UTI in girls was higher than boys that about ٥٧٪ of UTI in boys were recurrent while ٧٧٪of UTI in girls were recurrent. These results agree with those obtained by (Hellerstein,١٩٩٨)who reported that UTI in girls are more recurrent than in boys.

٥.٦. Bacterial causes of UTI in children.

The type of bacteria responsible for urinary tract infection were

presented in table (٤-٧). *E. coli* was the major causative bacteria of UTI which was isolated from ٣٧ (٦٦.١ %) children so *E. coli* was the commenst organism in children's under ٥ years. These results agree with what found by (Bouallegue *et.al.,* ٢٠٠٤) in Tunisia (٧١ %) and those of a study by (Shahab and Navideh,١٩٩٧) in Iran (٧١ %). *Proteus mirabilis* isolated from ١٠ children (١٧.٩٪) .

These results were higher than what was found by (Shahab and Naviden , ١٩٩٧) (٦.٣ %) and even higher than those obtained by (Bouallegue *et.al.,*٢٠٠٤)(٨%) and also higher than those of (Azhar,٢٠٠٠) (١١٪).while *Klebsiella* isolated from ٣ children only (٥.٤٪) .

This result agrees with those found by (Olli *et.al.*, ١٩٩٩) study in Turkey (٤.٤٪) and less than of (Bouallegue *et.al.*, ٢٠٠٤) (١٠٪).

Staphylococcus saprophyticus was isolated from ٤ children (٧.١٪) These results agrees with the results found by (Azhar , ٢٠٠٠) ٤٪ and with (Olli *et.al.,* ١٩٩٩) (٣.٨٪)

Pseudomonas organism was isolated from ١ child (١.٨٪) This result agrees with (Bouallegue *et.al.,* ٢٠٠٤) (١٪) and less than those of (Shahab and Navideh ,) (٣.١٪) and agrees with the results obtained by (Azhar , ٢٠٠٠) (١٪) .

Enterobacter organism was isolated also from ١ child (١.٨٪) This result is less than that of (Shahab and Navideh , ١٩٩٧) (٣.٧٪)

٥.٧. Antibiotic sensitivity results

The antibiotic resistance percent of specific type of bacteria is shown in table (٤-٩) and the resistance percentage in general is present in figure (٤.١) .

Regarding Amoxicillin, ١٠٠٪ of *P. aeruginosa* , *Enterobacter* , *P. mirabilis* were resistant . These results correlate well with the results obtained by(Saurez *et. al.,*٢٠٠٢) , (Orett ,١٩٩٩) and (Yamaguchi *et.al.,*١٩٩٩) who observed that these bacteria had high resistance to Amoxicillin. In addition ٨٣.٨٪ of *E .coli*, ٦٦.٧٪ of *Klebsiella* were resistant to Amoxicillin. These results agrees with the results obtained by (Bouallegue *et.al.,*٢٠٠٤) who reported that high resistance of *E. coli* to Amoxicillin and *Klebsiella* in addition to ٥٠٪ of *Staph. Saprophyticus* were resistant to Amoxicillin. These results agrees with the results obtained by (Jureen *et.al.,*٢٠٠٣) who showed the coagulase negative Staph. had high resistance to Amoxicillin . Generally ٨٤.٩٪ of bacteria isolated from children in this study were resistant to Amoxicillin .The result agrees with the result obtained by (Shahab and Navideh, ١٩٩٧) who reported that about ١٠٠٪ of the isolated bacteria were resistant to amoxicillin.

Regarding Nitrofurantoin , ١٠٠٪ of *P. aeruginosa,* ٦٠٪ of *P. mirabilis* ,٢٢.٥ % of *E .coli* , ٣٣.٣٪ of *Klebsiella* were resist. However no *Staph. Sp*. Or *Enterobacter* were resistant . These results are in agreement with the results obtained by (Boullegue *et.al.,*٢٠٠٤) who reported that low resistance of *E .coli* and *Klebsiella* toward nitrofurantoin and these results agrees with the results obtained by (chow *et.al.,*١٩٨٨) that found that ٥٠٪ of *proteus* were resistant to nitrofurantoin . In addition these results agrees with those of (Sedlock *et.al.,*١٩٩٠) who reported no resistance of *Staph. saprophyticus* toward Nitrofurantoin. Also these results agrees with (Zhou *et.al.,*٢٠٠٢) who reported that most of *Enterobacter* were sensitive to nitrofurantoin and also the result agrees with (chiner *et.al.,* ١٩٩٩) , (Barrett *et.al.,*١٩٩٩) who reported high resistance of *P. aeruginosa* to Nitrofurantoin .

Generally ٢٦.٨٪ of the isolated bacteria from children in this study were resistant to Nitrofurantoin. These results are in agreement with the results obtained by (Radi,١٩٩٤) who reported that about ٢٠.٩٪ of isolated bacteria were resistant to nitrofurantoin.

Regarding Cefotaxime , ١٦.١ % of *E .coli*, ٤٠ % of *proteus* , ٦٦.٧٪ of *Klebsiella* and ١٠٠ % of *P. aeruginosa* ٢٥٪ of *Staph. Saprophyticus* were resistant but no *Enterobacter* resistance was found.

These results are in agreement with results obtained by (Boullegue *et.al.,*٢٠٠٤) who reported very low resistance of *E .coli* and high resistance of *Klebsiella* toward cefotaxime . These results agrees with those of (Blandino *et.al.,*١٩٩٠) who reported that most of *P. mirabilis* were sensitive to cefotaxime while he reported low resistance toward to coagulase negative bacteria and these results agree with those obtained by (Irgbu *et.al.,*٢٠٠٣).

Deguchi *et.al.,*(١٩٩٠) and Sedlock *et.al.,*(١٩٩٠) reported that most of *Staph. saprophyticus* were sensitive toward cefotaxime , an observation that agrees with the results obtained by (Szaba *et.al.,*١٩٩٠) who reported *P aeruginosa* with high resistance to cefotaxime and a similar observation by (Thomson *et.al.,*١٩٩١) who reported that most of *Enterobacter* were sensitive to cefotaxime .

Generally ٣٠.٤٪ of the isolated bacteria from children in this study were resistant to cefotaxime. This result is in agreement with those obtained by (Radi,١٩٩٤)who found that about ٢٤.٧٪ of bacterial causes of UTI were resistant to cefotaxime.

Regarding Trimethoprim sulfamethoxazole , ٤١٪ of *E. coli* , ٤٠٪ of *proteus* , ٦٦.٧ % of *Klebsiella* , ٢٥٪ of *Staph saprophyticus* and ١٠٠٪

of *P. aeruginosa* and *Enterobacter* were resistant . These results are in agreement with results obtained by (Bouallegue *et.al* .,٢٠٠٤) who reported that ٤٠٪ of *E. coli* , ٦٥٪ of *Klebsiella* and ٣١٪ of *proteus* were resistance toward Trimethoprim .And these results agree with (Gladin *et.al.,*١٩٩٩). (Barsic *et.al.,*١٩٩٧) and (Guirguitzova *et.al.,*٢٠٠٢) who reported that *P. aeruginosa* and *Enterobacter* had high resistance toward Trimethoprim and also the results agreed with (Chow *et.al.,*١٩٨٨) Who reported that ٧٥٪ of coagulase negative bacteria were sensitive to Trimethoprim.

Generally ٦٦.١٪ of bacteria isolated from children in this study were resistant to Trimethoprim sulfamethoxazole . These results agree with results obtained by (Radi,١٩٩٤) who reported that about ٧٠.٩٪ of bacteria were resistant to Trimethoprim sulfamethoxazole.

Regarding Gentamicin ٣٧.٨ of *E. coli* , ٤٠٪ of *proteus mirabilis* , ٣٣.٣٪ of *Klebsiella pneumonia* ٥٠٪ of *S. Saprophyticus* were resistant and no resistance of *P. aeruginosa* and *Enterobacter* bacteria.

These results agree with the results obtained by (Boullegue *et.al* .,٢٠٠٤) and(Kevin *et.al.,*٢٠٠٣) who reported that low resistance of *E. coli* and very low resistance of *P. aeruginosa* toward gentamicin . it also agrees with (Blandino *et.al.,*١٩٩٠) who indicated that most of *Enterobacter* and *Proteus mirabilis* were sensitive to gentamicin and one of two of *S. Saprophyticus* were resistance to gentamicin .

Generally ٣٧.٥٪ of isolated bacteria from children in this study were resistance to gentamicin. These results are in agreement with results obtained by (Radi,١٩٩٤) who reported that about ٢٦.٤٪ of bacteria were resistant to Gentamicin.

Regarding nalidixic acid .٢٣.٢٪ of *E .coli* and ٢٠٪ of *Proteus mirabilis* , ٢٥٪ of *staphylococcus saprophyticus* , ٣٣.٣ % of *Klebsiella pneumonia* and ١٠٠ % of *Pseudomonas aeruginosa* and *Enterobacte*r were resistant .These results are in agreement with results obtained by(Chow *et.al.,*١٩٨٨) who indicated that very low resistance of *E .coli* ,*proteus* and *Klebsiella* toward nalidixic acid and also agrees with results obtained by (Lazar *et.al.,*٢٠٠٢) who reported that high resistance of *Enterobacter*, *Pseudomonas* aeruginosa toward nalidixic acid . Also agrees with the results obtained by (Mabrook and Yahia,٢٠٠١) who reported only ٣٣.٣٪ of *Staphylococcus saprophyticus* were resistant to nalidixic acid .

Generally ٣٣.٧٪ of isolated bacteria in this study were resistant to nalidixic acid. This result is similar to the result obtained by (Radi,١٩٩٤) who reported ٢٠.٩٪ of isolated bacteria were resistant to nalidixic acid.

٥.٨. Immune response of urinary tract infection

The results of immune response in the children were that the mean of concentrations of serum IgG, IgM antibodies in cases of *E .coli* in urine culture was higher than that of *Proteus mirabilis* in urine culture and was higher in *Proteus mirabilis* in urine culture than negative urine culture as seen in figures (٤.٢-٤.٣-٤.٤).These results are in agreement with the results obtained by (Agata *et.al.,*١٩٨٩) who found a high concentration of antibodies to P .fimbiria of *E .coli* in cases of urinary tract infection and also agrees with(Bergman *et.al.,*١٩٧٩)who reported that ٢٤٪ of patients without *E .coli* in urine culture had raised *E .coli* antibody titer but only ٤٪ of patient without growth of *proteus mirabilis* had raised *P .mirabilis* antibody titer .

The distribution of means of concentrations of IgG antibodies according to age groups show increase in the first age group then decrease in the second and third age group then increase after that

in the fourth and fifth age group as seen in figure (٤.٥).

These results are in agreement with results obtained by (Vlug *et.al.,*١٩٩٤) who reported a high concentration of IgG antibody in the first months of life because the placenta transfer from mother during infancy then decrease later in the second and third year then increase later in the fourth and fifth year.

The distribution of the means of concentrations of IgM antibody show increase gradually during the first five years of age as seen in figure (٤.٦).

These results are in agreement with the results obtained by (Brooks *et.al.,* ٢٠٠١).

who found that IgM antibody formed by the fetus during the first months of life with an infection and increase gradually till reach adult concentration .

Conclusions

&

Recommendations

Conclusions

١. A urine culture is a best method for diagnosis of UTI in children .

٢. The majority of the infected children were females while males were very low and the highest frequency of the disease was in the first year of age.

٣. The most common type of bacterial causes of UTI was *E .coli* followed by *Proteus mirabilis* then *Staphylococcus .saprophyticus* then *Klebsiella pneumonia* then in the last *Enterobacter* and *Pseudomonas aeruginosa* .

٤. The best effective antibiotics for bacterial causes of UTI were Nitrofurantoin then Cefotaxime , Nalidixic acid , Gentamicin , Trimethoprim – Sulfamethoxazole and finally amoxicillin.

٥. Breast feeding is a prophylactic factor against different disease occurring during childhood especially diarrhea and UTI.

٦. The circumcision reduces incidence of UTI in boys during the first year of life.

٧. The immune response for UTI in children differ according to bacterial causes that the immune response for *E .coli* was higher than proteus .

Recommendations

١. The antibiotic choice for the treatment of UTI in children must be correctly and depend on urine culture and antibiotics sensitivity results and also follow up of cases of recurrent and complicated UTI .

٢. Breast feeding is an important protective factor against UTI in children so we must encourage breast feeding and explain the benefits of it for mothers.

٣. The circumcision must be encouraged during the first months of life in boys .

٤. There are specific immune responses for bacterial causes of UTI in children, they differ according to their types and must be studied more that may be useful for us to give some constant ranges of serum immunoglobulins for diagnosis of bacterial causes and for immunization against these bacteria .

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