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Bacteriology and Epidemiology Study of Uropathogens Associated with Urinary Tract Infection in Children

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Abstract :-

(375) urine specimens were collected from children suffering from UTI contacting Educational Hospital of Delivery and children in Al Qadisiah city , and (50) urine specimens were collected from healthy children , (211) isolated were recovered from patient children and (4) isolates from healthy children , isolated bacteria from infected children contain :

Escherichia coli with (81) isolates (38.38 %) , then *Klebsiella pneumonia*e (34) (16.11 %) , *Proteus mirabilis*(27) (12.79 %) , *Enterobacter cloaca* (18) (8.53 %) , *Staphylococcus aureus* (13) (6.16 %) , *Proteus vulgaris* (12) (5.68 %) , *Enterobacter aerogenes* (10) (4.73 %) , *Klebsiella oxytoca* (4) (1.89 %) , *Pseudomonas aeroginosa* (4) (1.89 %) , *Streptococcus agalactiae* (3) (1.42 %) , *Enterococcus faecalis* (3) (1.42 %) , *Morganella morganii* (2) (0.94 %) , while isolated bacteria from healthy children contain : *E. coli* with (50 %) , *Proteus mirabilis* (% 25) , *Enterobacter cloaca* (% 25) .

Results showed there were (47) samples with percentage (22.27 %) given bacterial urine without pus and (164) (77.72 %) with pus , and the highest percentage of bacteria causative of infection were recovered to *E. coli* (46.34 %) and the highest percentage of bacteria without pus cell to *Proteus spp* (51.06 %) . Study of epidemiological factors recovered percentage (53.98 %) infection in female children and (45.86 %) in male , specimens with significant bacterial growth were (64.03 %) and without significant growth (9.35 %) in female , and (47.09) specimens with significant bacterial growth and (6.39 %) without significant growth in males, *E. coli* was predominance in female (43.84 %) and *Proteus spp* (30.86 %) in males . The highest percentage of UTI infection in the age group (2-4) (40.28 %) with predominance of *E. coli* (40.74 %) and the highest percentage of UTI infection (61.13%)in children of countryside ,while (38.86 %) to the town children with predominance of *E. coli* (38.75 %) (37.88 %) respectively , and highest percentage of UTI infection in children (12.59 %) were recovered in July , while the lowest percentage (1.01 %) recovered in December .

Results showed that Ciprofloxacin is reactively expensive when compared to most antibiotics used with percentage to *E.coli* (92.69%) , *proteus spp*. (85.17%) , *klebsiella spp* .(92.53%) , *Enterobacter spp.* (93.50%) , *S.aureus* (95.06%) , *pseudomanas aeruginosa* (94.64%) , *S.agalactiae* (100%) ,*Enterococcus spp* (100%), *M.morganii* (100%).There were no significant differences in Lactoferrin Concentration was observed between the healthy children and children negative UTI

, whereas , the differences between UTI negative and UTI positive children was significant ($p<0.001$) .
Results showed too that pathogen –specific antibody secreting cells (ASC) were found in the blood of (77 / 81) children with UTI caused by *E. coli* , and predominance of IgM (67.53 %) ,while IgA dominate (32.46%) in this patients.

Introduction

Urinary Tract Infection (UTI) is defined by the presence of organisms in urinary tract , which is usually sterile [1] however , since asymptomatic colonization of the urinary tract can occur , other features such as the presence of inflammatory markers or follow – up cultures may be need to difinitively diagnose as (UTI) – Clinically important infections usually occur due to bacteria although viruses , fungi , and parasites can also cause infection . Common non bacterial causes of (UTI) include hemorrhagic cystitis from adenovirus and Candida infection in immunocompromised individuals - (UTI) the most commonly diagnosed bacterial infection in childhood [2] , common bacterial pathogens include – gram negative – species such as *Escherichia coli* , *Klebsiella spp* , *Proteus spp* , *Enterobacter spp* , *Pseudomonas aeruginosa* and *Serratia spp* , and gram positive organisms , including group β Streptococci , *Enterococcus spp*, and *Staphylococcus aureus* [3] [4] [2] . In general bacteria infect the urinary tract by ascending from the urethra , although hematogenous infection may occure in rare instances among young infants . (UTI) can be further sub divided in to infection localized to the bladder and urethra (cystitis and urethritis) versus upper parenchyma (pyelonephritis) . Ascending infection of the urinary tract is a complex process that has been associated with bacterial adhesion , virulence , and motility properties as weel as host anatomic , humoral , and genetics factors [3] [5] , the presence of fever , chillis , and flank pain has usually been identify the presence of upper tract infection [6 , 7] . The epidemiology of (UTI) during childhood varies by age , gender , populations and circumcision in male [8] , [9] , [10] . The urinary tract is related common site of infection in young children , (UTI) are important because they cause morbidity and may result long – term medical problems , including hypertension and reduced renal infection [11] . Antibiotic treatment should be limited to symptomatic (UTI) and iniated after sensitivity testing only Empiric use of antibiotics must be limited to highly symptomatic until the results sensitivity testing [12] , antibiotic therapy could be effective in the treatment of lower (UTI) and can be recommended as a safe treatment modality in patients [13] , the measurement of urinary lactoferrin (LF) released from polymorphonuclear leukocytes consider simple and rapid diagnosis of (UTI) [14] ,knowledge on the human immune defense against(UTI) in children is sparse specific antibody responses to infecting pathogen have been observed both in sera and in urines from both adults and children with(UTI) [15] , so , the aim of this study are:

1. Isolation and Identification of bacteria from urine specimens of infected and healthy children .
2. Study of epidemiological factors of (UTI) in children

3. Study the susceptibility of isolated types of bacteria to some type of antibiotics
4. Measuring urinary lactoferrin for the diagnosis of (UTI)
5. Assay of specific antibody secreting cells (ASC) to each children own *E.coli* (the first cause of (UTI) and ASC specific to *E.coli* fimbria

Methods and Materials :-

1 – Collection of specimens : the (375) urine specimens were collected from infected (patients) children contacting the Educational Hospital for children and Delivery in Al- Diwanya city and (50) urine specimens were collected from healthy children , the age of patients and healthy children ranged from (2 - 14) year , specimens were collected between January 2008 to January 2009 according to [16 , 17]

2 – Microscopical examination : urine specimens put in centrifuge at (3000) cycle / minute for (5) minute to show the presence of puss cells [18]

3 – Isolation of bacteria : each urine specimens was immediately inoculated (in triplicates) into MacConkey agar plates , Mannitol salt agar plates , Cetromide agar plates , Blood agar plates on arrival at the laboratory , these were incubated aerobically at 37 ċ for 18 hrs [17 , 18] .

4 – Identification of bacteria

A – Characteristics manifestation : according to [17 , 19]

B – Micriscopic characteristics : according to [17 , 19]

C – Biochemical tests

Tests conducted of production of the enzymes catalase , oxidase , coagulase , voges proskauer [20] , urease , indole , H2S production , sugar fermentation , motility [17] , haemolysis , bacitracin , phenylalanine deaminase , nitrite reduction , gelatin digestion according to [19] , citrate utilization , hydrolysis of asculin , growth in 6.5% Nacl , and growth in 10 -45 ċaccording to [17] [19]

5 – Antibiotic susceptibility

Antibiotic susceptibility of the isolated test organisms namely :

E.coli (n = 81) , *Proteus spp* (n = 39) , *Klebsiella spp* (n = 38) , *Enterobacter* (n = 28) , *s. aureus* (n = 13) , *Pseudomonas aeruginosa* (n = 4) , groub β Streptococci (n = 3) , *Enterococcus faecalis* (n = 3) , *Morganella morganii* (n = 2) , against antibiotics were determined using standard microbiological protocol [21] . The standard antibiotics discs used were those of

Ciprofloxacin (IP) , Gentamycin (GN) , Erythromycin (E) , Amoxicillin (Amo) , Ampicillin (A) , Amikacin (AN) , Penicillin(PG) , Streptomycin (S) , Tetracycline (TE) , Spectinomycin (SPT) . The diameter of the zone of inhibition proceed by each antibiotic disc was measured using earlier described as susceptible (s) or resistance (r) to the antibiotic agent used , depending on the length of zone of inhibition produced compared to reported standard length [22] .

6 – Lactoferrin concentration : liquid of LF solution was added to (1) ml of each urine specimens and the mixture was diluted to a final volume of (5) ml with the sample dilution buffer , each value is the mean ± SD (cutoff value) , LF concentration in urine specimen was determined by a sandwich ELISA with rabbit

poly clonal antibody [14] , the quantitative detection range of LF by a sandwich ELISA were analyzed by using students test , where the standard error of the mean (SEM) was used as a measure of variance among the means .

7 – Collection of blood samples : according to [23]

8 – Assay of specific antibody secreting cells (ASC) : specific ASC were enumerated by [24]

9 – Statistical analysis : the data were analyzed statistically using X^2 and t test [25]

Results and Discussion :-

1 – Isolation and Identification of Bacteria

Two hundred and eleven isolates were recovered from (375) urine specimens contain :

Escherichia coli , Klebsiella pneumoniae , K.oxytoca , Proteus mirabilis , Proteus vulgaris , Enterobacter aerogenes , Enterobacter cloaca , Pseudomonas aeroginosa , Morganella morganii as gram negative , *S.aureus , Enterococcus faecalis* and group Streptococci(*S.agalactiae*) as gram positive , the results of most important characteristics and required for isolation and identification of this type of bacteria with their important relatives species are shown in table (1)

Table (1) Biochemical test of gram negative uropathogens isolated from children

Test / Teype Of bacteria	IND	VP	CIT	PDA	URE	H2S	MOT	GAS	LAC	INOS	GEL	NIT	OXID	CAT
Escherichia coli	+	-	-	-	-	-	+	+	+	-	-	+	-	+
Klebsiella pneumoniae	-	+	+	-	+	-	-	+	+	+	-	+	-	+
Klebsiella oxytoca	+	+	+	-	+	-	-	+	+	+	-	+	-	+
Proteus mirabilis	-	±	±	+	+	+	+	+	-	-	+	+	-	+
Proteus vulgaris	+	-	-	+	+	+	+	+	-	-	+	+	-	+
Enterobacter aerogenes	-	+	+	-	-	-	+	+	+	+	-	+	-	+
Enterobacter cloaca	-	+	+	-	±	-	+	+	+	-	-	+	-	+
Pseudomonas aeruginosa	-	-	+	-			-	-	-	+		+	+	+
Morganella morganii	+	-	-	+	+	±	+	+	-	-	+			

IND: indol , VP: voges proskauer , CIT: cirate utilization, PDA: phenylalanine deaminase , URE: urease , H2S : H2S production , MOT: motility , GAS : gas from glucose , LAC , INOS : fermentation of lactose and inositol , GEL:gelatin digestion, NIT: nitrate reduction , OXID:oxidase , CAT: catalase , + : positive , - : negative , ± : some positive and some negative

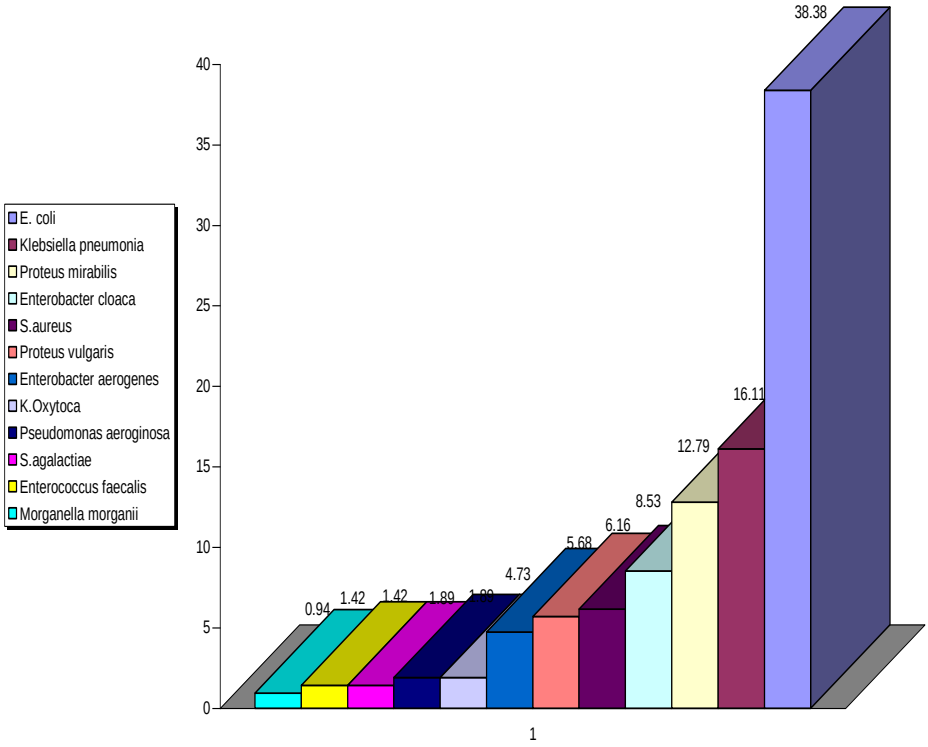
Table (2) Biochemical test of gram positive uropathogens isolated from children

Type of bacteria / Test	*Staphylococcus aureus*	*Enterococcus faecalis*	*Streptococcus agalactiae*
Oxidase	-	-	
Catalase	+	-	
Bacitracin	R		R
Pigment	+		
Coagulase	+		
Mannitol	+	+	-
Haemolysis	β	γ	β
Nitrate reduction	+		-
Arabinose		-	
Lactose			±
Raffinose		-	-
Ribose		+	+
Sorbitol			-
Hydrolysis of asculin		+	-
Growth in 6.5% Nacl		+	
Growth in 10 -45 ċ		+	
Gelatinase	±	+	

+ : positive , - : negative , ± : some positive and some negative , β and ά : beta and alpha hemolysis

Results showed predominance of *E. coli* than other type of isolated bacteria from urine specimens of infected children (fig 1) , *E. coli* isolated with (81) isolates (38.38 %) , then *Klebsiella pneumonia*e (34) (16.11 %) , *Proteus mirabilis*(27) (12.79 %) , *Enterobacter cloaca* (18) (8.53 %) , *S.aureus* (13) (6.16 %) , *Proteus vulgaris* (12) (5.68 %) , *Enterobacter aerogenes* (10) (4.73 %) , *K.oxytoca* (4) (1.89 %) , *Pseudomonas aeroginosa* (4) (1.89 %) , *S.agalactiae* (3) (1.42 %) , *Enterococcus faecalis* (3) (1.42 %) , *Morganella morganii* (2) (0.94 %) .

All previous reports as well as the present study have documented that *E. coli* is the principle pathogen responsible for urinary tract infection [26] [27] ,Stamm. W.E [28] have compared *E. coli* (73 isolates) , *Klebsiella spp* (22) , *Pseudomonas aeruginosa* (17) , *Enterococcus spp.* (14) , *Proteus spp.* (8) , *Enterobacter spp* (5) isolates from (110) children with (UTI) ,while [3] isolated *E. coli* , *Proteus spp* and *Klebsiella spp* in (90 %) of (UTI) reposted case , and [29 , 30] were isolated *E. coli* with percentage (65.1%) , *Proteus spp.* (4.60%) , , *Morganella morganii* (4.6 %), *Pseudomonas spp* (1.80%) , *Enterococcus spp.* (2.4%) and *S.agalactiae* (0.70%) .



Type of bacteria

Fig (1) Aerobic bacteria recovered from urine specimens of children

The predominance of *E. coli* on other type of bacteria may belonged to precence of *E. coli* in stool with big number , so , it may be source to auto – infection , mean it may leave the normal part (intestine) and transport to urinary tract causing (UTI) [31] [27] , in addition *E. coli* have many virulence factors , the most important of this factors is cilia that enable *E. coli* to adhesion on the surfaces of epithelial cells in urinary tract [32] . *Klebsiella pneumoniae* were recovered with percentage (16.11 %) , it is a common gram negative bacteria that exhibit multiple antibiotics resistance , *Klebsiella pneumoniae* have many virulence factors that helps it to resistance of body defence such as phagocytosis [33], [34] refere to that *Klebsiella*

pneumonia appears programmed for minimal expression type 1 pilli , and *Klebsiella pneumonia* carries an extra gene on function at the 3 end list type operon , that may be important in their pathogensis , while other type of gram negative bacteria (except *Pseudomonas aeruginosa*) consider as normal flora or opportonustic pathogens of intestine , so , they made important role in UTI , *S.aureus* and *Enterococcus spp* have important role too in UTI [35][17] .The results showed percentage of *E. coli* (50 %) , *Proteus mirabilis* (25 %) , *Enterobacter cloacae* (25 %) in the urine specimens of healthy children (table 3) and there were no growth recovered in (134) specimens with percentage (35.73 %) , that may be belonged to the precence of one type of bacteria needs special condition for growth or may the cause was one type of anaerobic bacteria or viruses or parasites .

Table (3)Aerobic bacteria recovered from healthy children

Type of bacteria	No.of isolates	%
E. coli	2	50
Proteus mirabilis	1	25
Enterobacter cloacae	1	25
Total	4	100

Table (4) showed relationship between isolated bacteria and pus cell , there were (47) samples with percentage (22.27 %) given bacterial urine without pus , while (164) (77.72 %) given bacterial urine with puss cell (more than 10 cells) , results showed the highest percentage of bacteria causative of infection were recovered to *E. coli* (46.34 %), and the highest percentage of bacteria without pus cells were to *Proteus spp* (51.06 %) , [36] refers that *Proteus spp* have ability to destroyed urea in urine and realize ammonia that help for rising of urine alkalinity then lyses of pus cells .

Results showed presence of *E. coli* , *Proteus mirabilis* , *Enterobacter cloacae* in (33.33 %) (less than 10 cells) and only *E. coli* more than (10 cells) in urine of healthy children (Table 5)

Table (4) Relationship between isolated bacteria and pus cells in children with UTI

Pus cell / Bacteria	Lessthan 10cells		More than 10 cells		Total
	No	%	No	%	
E. coli	5	10.63	76	46.34	81
Klebsiella spp	4	8.51	34	20.73	38
Proteus spp	24	51.06	15	9.14	39
Enterobacter spp	7	14.89	21	12.80	28
S.aureus	3	6.38	10	6.09	13
S.agalactiae	1	2.12	2	1.21	3
Ps. aeruginosa	1	2.12	3	1.82	4
En. faecalis	1	2.12	2	2.43	3
M.morganii	1	2.12	1	6.09	2
Total	47	22.27	164	77.72	211

$X^2 = 214.61$, df = 8 , P < 0.05 *there were significant differences

Table (5) Relationship between isolated bacteria and pus cells in healthy children

Pus cell / Bacteria	Less than 10 cells		More than 10 cells	
	no	%	no	%
E. coli	1	33.33	1	100
P. mirabilis	1	33.33	0	0.0
En. cloacae	1	33.33	0	0.0
Total	3	100	1	100

$X^2 = 1.07$, df = 2 , P < 0.05
***there were significant differences**

2- Epidemiological factors

A-sex

There were (203) samples with percentage (53.98%)had taken from females and (172) (45.86%) had taken from males children, specimens with significant bacterial growth were (64.03%) and (9.35%) without significant growth in female, and (47.09%) specimens with significant bacteria growth and (6.39%) without significant growth in males (table 6), while healthy children given (8%) significant growth and (4%) non significant in females and (4%) significant males (table 7) .

Results recovered predominant of *E.coli*(43.84%) and *klebsiella spp*(20.76%) in female and *proteus spp*. (30.86%),*E.coli* (29.62%) in males (table 8), the reason of predominant of *E.coli* in female may caused by carbohydrates and glycoproteins in vaginal liquids that help *E.coli* to adhesion vaginal epithelial cells in addition to ability of serotype of *E.coli* to resistant lower PH in vaginal liquids [37] while *Proteus spp* predominance in males with percentage (30.86%) belonged to harmony

high injury of cells lining the urethra in male and channels of prostate[37][3], bacteria periodically enter the female urinary bladder from urethra in small numbers , they are able to bind, multiply colonize, and invade the urinary tract in sequential [38]. and the higher incidence of UTIS in girls has been attributed to the short female urethra [38].

Table(6) distribution of infection according to bacterial growth in patients children and it's relationship with sex

Type of growth / sex	No. of specimens'	specimens' with significant bacterial growth		specimens without significant bacterial growth		Samples with no growth	
		N	%	N	%	N	%
Female	203	130	64.03	19	9.35	54	26.60
Male	172	81	47.09	11	6.39	80	46.51*
Total	375	211	56.26	30	8.00	134	35073**

*** percentage to the total number of genus isolates X^2=16.07 df=3**
**** percentage to the total number of samples there were significant differences**

Table (7) Distribution of infection according to bacterial growth in healthy children and its relationship with sex

Type of growth / sex	No. of specimens'	specimens' with significant bacterial growth		specimens without significant bacterial growth		Samples with no growth	
		N	%	N	%	N	%
Female	25	2	8%	1	4	22	88
Male	25	1	4%	0	0	24	96*
Total	50	3	6	1	2	46	92**

*** X^2= 1.4 , df = 3 , there were significant differences**

Table (8) Type of bacteria isolated from urine specimens of patients children according to sex.

Sex / Typeof bacteria	Male		Female	
	no	%	no	%
E.coli	24	29.62	57	43.84*
Klebsiella spp	11	13.58	27	20.76
Proteus spp	25	80.86	14	10.75
Enterobacter spp	11	13.58	17	13.07
S.aureus	4	4.9	9	6.92
S.agalactiae	1	1.23	2	1.53
Ps.aeruginosa	3	3.70	1	0.76
Ent. facales	1	1.23	2	1.53
M.morganii	1	1.23	1	0.76
Total	81	100	130	100

***percentage to total no of isolates in the same sex**

*** X^2=27.57 df=8 , there were significant differences**

Table (9) Type of bacteria isolated from urine specimens of healthy children according to sex

Sex / Type of bacteria	Male		female	
	No.	%	No.	%
F.coli	0	0	2	66.66
Pr. mirabilis	1	100	0	0
En.clouae	0	0	1	33.33
Total	1	100	3	100

X^2=3.24 df=2 , there were significant differences

B-Age group

Results showed the highest percentage of infection in the age (2 - 4) years (40.88%), then (5-7) years (33.17%), while the lowest percentage (8.53%) to (12-14) years, (table 10), the results showed too the highest infection with *E.coli* recovered to (2-4) years (40.74%), and *klebsiella spp* (44.73%), *Enterobacter spp* (39.28%), *S.aureus* (15.38%), *Ps.aeruginosa* (50%) to the same age (2-4 years) .A high incidence of infection in age group (2-4) may be due to lack of a awareness of health and cultural and the absence of mother role in attention and care of child health and the lack of attention to the child himself in this age group, or the pathogenic bacteria after child birth begins to endemism area around urethra and then gravelly less prepared during the first year of life to become presence a too little after 5 years of

age, which reduces the risk of exposure to UTI caused by this bacteria, while the low incidence of infection in progress group of age was belonged to maturation of defense mechanisms in host body then development of resistance to infection, in addition to increasing of a wariness of helth [36], Table(11) showed isolated bacteria from urine specimens of healthy children, there were two infection with *E.coi*, in age group (2-4) , (9 – 11) and one infection with *En.cloacae* in age group (5 – 7) and one isolates of *Pr.mirabilis* in the age group (2-4).

Table (10) Isolated bacteria from urine specimens of children according to age

Age(year) / Type of bacteria	No.of isolates	2-4		5-7		9-11		12-14	
		No	%	No	%	No	%	No	%
E.coli	81	33	40.74	27	33.33	14	17.28	7	8.64*
Klebsiella spp	38	17	44.73	14	16.04	5	13.15	2	5.26
Proteus spp	39	18	46.51	13	33.33	5	12.82	3	7.69
Enterobacter spp	28	11	39.28	9	32.14	6	21.42	2	7.14
S.aureus	13	2	15.38	3	23.07	5	38.46	3	23.07
S.agalactiae	3	0	0.00	1	33.33	1	33.33	1	33.33
Ps.aeruginosa	4	2	50	1	25	1	25	0	0
Ent. facales	3	1	33.33	1	33.33	1	33.33	0	0
M.morganii	2	1	50	1	50	0	0	0	0
Total	211	85	40.28	70	33.17	38	18.00	18	8.53**

*** parentage to total no of isolates same type of bacteria**

**** parentage to total no of isolates to all type of bacteria**

***** X^2= 19.65 , df = 24 , there were significant differences**

Table (11) Isolated bacteria from urine specimens of healthy children according to age

Age(year) / Type of bacteria	No.of isolates	2-4		5-7		9-11		12-14	
		No	%	No	%	No	%	No	%
E.coli	2	1	50	0	0.0	1	50	0	0
Pr.mirabilis	1	1	10.03	0	0.0.	0	0	0	0
En.cloacae	1	0	0	1	100	0	0	0	0
total	4	2	50	1	25	1	25	0	0

X^2=3 df=6 , there were significant differences

C – Residential area

Table (12) showed the highest percentage of infection (61.13 %) were recovered to the children of countryside , while (38.86 %) to the town children , the results showed predominance of *E.coli* in urine samples of countryside and town children with percentage (38.75 %) and (37.88 %) respectively , and *Proteus spp* (20.15 %) (15.85 %) , and *Klebsiella spp*. (18.10%) (17.07 %) , the high percentage of infection in village children may belonged to absence of healthy condition and lack awareness of health and using of untreated water (polluted water) [36] , the results showed three infections in village and one in the town (table 14) .

Table (12) type of bacteria isolated from urine specimens of patients children according to region of residential area

Region / Type of bacteria	Village		Town		total
	No of isolates	%	No of isolates	%	
E.coli	50	38.75	31	37.88*	81
Klebsiella spp.	24	18.10	14	17.07	38
Proteus spp.	26	20.15	13	15.85	39
Enterobacter spp.	16	12.40	12	14.63	28
S.aureus	8	6.20	5	6.09	13
S.agalactiae	1	0.77	2	2.43	3
Ps.aeruginosa	2	1.55	2	2.43	4
Ent. facales	1	0.77	2	2.43	3
M.morganii	1	0.77	1	1.21	2
Total	129	61.13	82	38.86**	211

*** percentage to isolated no of the same region of residential area**

**** percentage to total no of isolates bacteria.**

***** X^2= 2.21 , df = 8 , there were no significant differences**

Table (13) type of bacteria isolated from urine specimens of healthy children according to region of residential area

Region / Type of bacteria	village		town		total
	no	%	no	%	
E.coli	1	33.33	1	100*	2
Pr.mirabilis	1	33.33	0	0	1
En.cloacae	1	33.33	0	0	1
total	3	75	1	25**	4

*** percentage to isolated no of the same region of residential area**
**** percentage to total no of isolates bacteria.**

D- Seasonal variation

The highest percentage of UTI infection in children (12.59%) were recovered in July , then August (12.12%) , Jun (11.45%) ,May (11.11%) , while the lowest percentage (1.01%) were recovered in December (fig 2) , this results of rising of infection with UTI in summer season may belonged to the rising of temperature in this months , so, sweat will be increasing in human body and urine become with a little amount ,that help the bacteria to have a long incubation time , then help the bacteria to reproduction, then occur of inflammation , in addition using of drinking water is rise in this season (summer) , that may be source of infection [36] , [39].

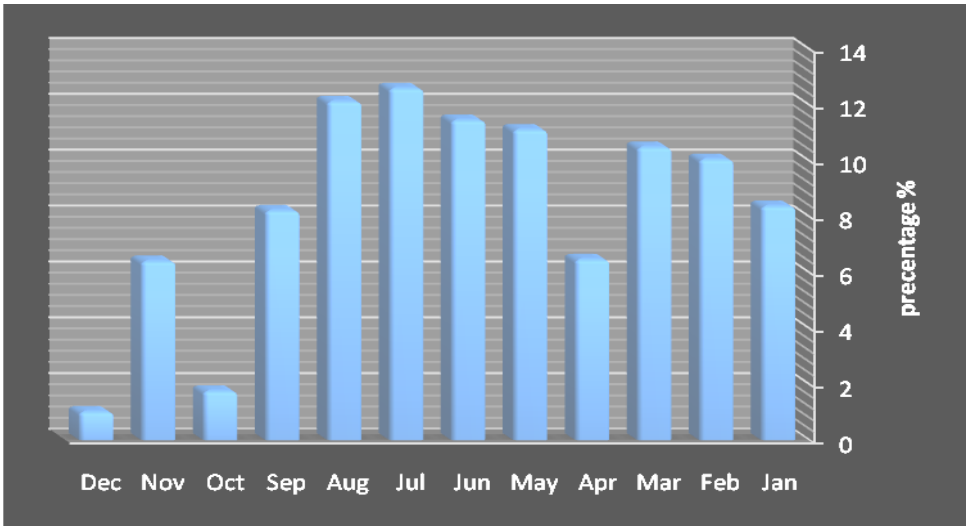


Figure (2) Distribution of infection with UTI in children according to the month.

3- Antibiotic susceptibility

The results showed that ciprofloxacin is reactively expensive when compared to most antibiotics used , with percentage to *E.coli* (92.69%) , *proteus spp* (85.17%) , *klebsiella spp* (92.53%) , *Enterobacter spp* (93.50%) , *S.aureus*

(95.06%) , *pseudomanus. aeruginosa* (94.64%) , *S.agalactiae* (100%) ,*Enterococcus spp*. (100%), *M.morganii* (100%) , table (14) , then Amikacin with percentage to *E.coli* (85.21%) ,*probeus ssp* (83.73%) , *Klebsiella spp* .(90.63%) , *Enterobacter spp.* (91.12%) , *S.aureus* (50.63%) , *pseudomonas aeruginosa* (60.37%) , group β Streptococci (66.66%) , *Enterococcus faecalis* (100%) , *M.morganii* (100%) , then Gentamycin with percentage to *E.coli* (80.12%) , *proteus ssp* (70.62%) , *Klebsiella spp* (75.82%) , *Enterobacter spp* (65.12%) , *S.aureus* (93.69%) , *pseudomonas aeruginosa* (89.15%) , group B Sterptococci (66.66%) , *M.morganii* (50%) , while most type of isolated bacteria (*E.coli* , *proteus ssp* , *Enterobacter spp.* , S.*aureus* , *pseudomonas aeruginasa* , *Morganella ssp*. showed perfect resistance to pencillin G.

The observed high level of isolated organisms susceptibility to ciprofloxacin might not be unconnected with the high cost of ciprofloxacin , this high cost has probably restricted is procurement and misuse by the residents investigated , thereby reducing emergence of resistant bacteria strain similarly , the route of administration of Gentamycin may have reduced its misuse hence the reduction in the emergence of resistant bacterial strain [40].

The resistance of *E.coli* to β- lactam antibiotics may be belonged to production of β - lactamase enzyme , the reason of this resistance is presence of coded genes on conjucuted plasmid [41] , in another mean , there are increasing resistance to ampicillin and other β -lactam antibiotics in *E.coli* , the main causative pathogen of urinary tractivfection [42] , [43] .

The resistance of *Klebsiella spp* to many types of antibiotics belong to presence of resistant strain because wide using of antibiotics in treatment of patients and because of being this bacteria in the normal flora group of intestine , the continuous using of this antibiotics lead to selection of resistant strain for it , in addition of the chance of getting multi resistant plasmid from other type of enterobacteria by transductions , transformation , and conjugation [44] , antimicrobial resistance genes are often associated with conjugative plasmids or transposons , which encoded the proteins necessary to initiate and complete their transfer to new hosts , the human large intestine has been proposed as a suitable environment for gene exchange , [45] , refer to transfer of plasmid – mediated B-lactamase from a *Klebsiella pneumoniae* strain to an *E.coli* strain during antibiotic treatment of children , and he demonstrate that aplasmid carrying B -lactamase gene appears to have been transferred from an ampicillin resistant *E.coli* strain to an initially susceptible strain during their co-resistance in the infant gut.

Sensitivity of bacteria to amikacin may belonged rarely using of it in UTI treatment in children , that make the bacteria not able to develop resistance to this type of antibiotics , in addition , amikacin , more constant against modifying enzymes to amino glycosides [46] , the bacteria have many methods to resistant antibiotics such us multi antibiotic resistant (*Klebsiella spp* , *E.coli* , Proteus) , Glycopeptides resistance (*Enterococcus faecalis*) [47] .

Table (14) : antibiotics sensitivity profiles of isolated bacteria from (375) urine samples

Type of bacteria / Type of antibiotics	Bacterial isolates sensitive to antibiotics (%)								
	E.coli n=81	*Proteus spp* N=39	*Klebspp* N=38	*Enteroba ctear ssp* N=28	*S.aureus* n=13	*Ps.aerugi nosa*N=4	*S.agalacti ae* n=3	*Ent.faecal is* N=3	*M.morga nii* N=2
Ciprofloxacin	92.69	85.17	92.53	93.50	95.06	94.64	100	100	100
Gentamycin	80.12	70.62	75.82	65.12	93.69	89.15	66.66	66.66	50
Erythromycin	4.25	60.10	40.65	70.30	45.12	9.74	66.66	33.33	50
Amoxicillin	5.12	20.75	15.79	18.54	8.19	3.25	66.66	33.33	50
Ampicillin	49.02	17.83	12.36	10.90	19.25	41.69	66.66	33.33	0
Amikacin	85.21	83.73	90.63	91.12	50.63	60.37	66.66	100	100
Penicillin G	0.0	0.0	4.02	0.0	0.0	0.0	33.33	33.33	0
Srreptomycin	15.23	65.54	21.18	62.63	35.86	31.65	33.33	66.66	100
Tetracycline	29.67	23.13	2.45	6.32	28.65	34.26	33.33	33.33	0
Spectinomycin	10.37	47.38	20.31	31.14	20.60	22.50	66.66	33.33	0

4 – Lactoferon Concentration

We examined (50) specimens from healthy children , (134) specimens with no growth (children of negative UTI) and (211) specimens from with significant bacterial growth (children of positive UTI) , the results are summarized in table (15) as the mean (±SEM) urinary LF concentration , no significant difference in LF concentration was observed between the healthy children and children with negative (UTI) , we compared the LF concentration specimens from healthy children with those from UTI negative and (UTI) positive populations , there were no significant difference between the healthy and (UTI) negative children , whereas , the difference between UTI negative and UTI positive children was significant ($P<0.001$) . The urinary LF concentration of UTI-positive children was more than 50 fold higher than that the UTI negative amount of LF was released from PMNS into the urine by inflammatory processes and that urinary LF is sensitive marker for diagnosis of UTI, furthermore, LF is stable in urine specimens even after the structure of PMNS are destroyed , for example our preliminary experiments showed that the residual rates of LF from a normal urine specimens which was fortified with 200 ng of LF /ml of urine were 113.5 after storage at 45 c^o for 3days and 92.3% after the cycle of freezing and thawing , therefore, it is not always necessary to specify the time of specimens collection and storage temperature [15].

Table (15): urinary excretion of LF in healthy children and children with and without UTI.

Added (ng/ml) of urine	Healthy children	Children with negative UTI	Children with positive UTI
0	5.2±5.7	6.5±1.4	21.0±3.5
25	36.6±6.7	36.7±4.1	45.8±5.8
50	58.7±13.0	68.9±2.8	71.5±7.5
100	109±9.1	112±14.7	132±19.2
200	191.9±32.5	219±17.4	230.7±33.3
300	302±32.6	276±46.8	310.5±44.4

***each value is the mean ± SD for independent analysis**

5 - Assay of specific ASC to each children own *E.coli*

Pathogen – specific ASC were found in the blood of (77 /81) children with UTI caused by *E.coli* , all four non responders were under (3) years old , the number of ASC was found to increase with increasing age , that may due to the less developed state of the immune system in children , this is supported our notion that the magnitude of the response in children increased with increasing age [14] , in (52/ 77) (67.53 %) cases , the response was dominated by IGM – screting , ASC , while IGA dominated in (25 /77) (32.46 %) cases and none of the patients had an IGG dominated (fig 3) , the high , IGM – ASC response could reflect amore systemic nature of the total ASC response , which is composed of local and systemic responses combined as suggested previously [48] .

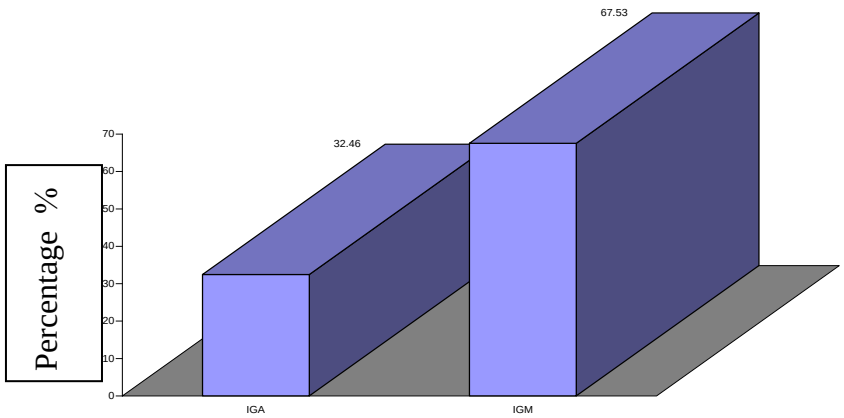


Fig (3) Precentage of IGM and IGA in children with pathogen specific ASC

ASC specific to fimbria were found in the blood of (59 / 65) children with P – fimbrated *E.coli* strain (P +) and in (10 / 12) children with non – P fimbriated *E.coli* strain (P -) , the (geometric means ± SEM)for the P-fimbria specific ASC responses were 32±75 and 8± 4 ASC / 106 PBMC for the P+ and P- groups , respectively , for (51/59) children a response was seen by more than one isotype in most causes , the response was dominated by IGM among the P- children (9/10) had only an IGM response and (1 /10) had only IGA response , these with the highest responses tended to belonged to P+ group , yet most of the responses in both group were low , the observation of a few significant P fimbria – specific ASCresponses in P- children can be explained partly by a possible failure to detect P fimbria with latex test as known that *E.coli* cells are subject to fimbrial phase variation in vivo in the urinary tract [14] [48] [49] .

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دراسة بكتيرية ووبائية لالتهاب المجاري البولية عند الاطفال

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الخلاصة:-

تم جمع (375) عينة ادرار من الاطفال المصابين بالتهاب المجاري البولية والذين راجعو مستشفى الولادة والاطفال التعليمي في مدينة الديوانية ، و (50) عينة ادرار لاطفال اصحاء ، تم عزل (211) عزلة بكتيرية من عينات الاطفال المصابين بالتهاب المجاري البولية و (4) عزلات من الاطفال الاصحاء ، شملت البكتريا المعزولة من الاطفال المصابين بالتهاب المجاري البولية الانواع التالية : (*Escherichia coli* with (81) isolates (38.38 %) , then *Klebsiella pneumoniae* (34) (16.11 %) , *Proteus mirabilis*(27) (12.79 %) , *Enterobacter cloaca* (18) (8.53 %) , *Staphylococcus aureus* (13) (6.16 %) , *Proteus vulgaris* (12) (5.68 %) , *Enterobacter aerogenes* (10) (4.73 %) , *Klebsiella oxytoca* (4) (1.89 %) , *Pseudomonas aeroginosa* (4) (1.89 %) , *Streptococcus agalactiae* (3) (1.42 %) , *Enterococcus faecalis* (3) (1.42 %) , *Morganella morganii* (2) (0.94 %) اما البكتريا المعزولة من الاطفال الاصحاء فشملت على الانواع التالية : *Proteus mirabilis* (% 25) , *Enterobacter cloaca* (% 25) اظهرت النتائج ان (47) عينة وبنسبة (22.27 %) اعطت نمو بكتيري بدون خلايا قيحية و (164) وبنسبة (% 77.72) اعطت نمو بكتيري مع خلايا قيحية ، وان اعلى نسبة للبكتريا بوجود الخلايا القيحية سجلت لبكتريا *E. coli* وكانت (% 46.34) واعلى نسبة للبكتريا بغياب الخلايا القيحية كانت لبكتريا *Proteus spp* (% 51.06) .اظهرت النتائج ان نسبة اصابة الاطفال الاناث (% 53.98) كانت اعلى من نسبة اصابة الاطفال الذكور(% 45.86) وان العينات ذات النمو المعنوي بنسبة (% 64.03) وذات النمو غير المعنوي (% 6.39) عند الاناث ، بينما سجلت العينات ذات النمو المعنوي (47.09) وذات النمو غير المعنوي (% 6.39) مع سيادة بكتريا *E. coli* و بنسبة (% 43.84) عند الاناث و بكتريا *Proteus spp* وبنسبة (% 30.86) لدى الذكور ، كما سجلت اعلى نسبة للاصابة للفئة العمرية 2-4 سنة وبنسبة (% 40.28) مع سيادة بكتريا *E. coli* وبنسبة (% 40.74) ، كما سجلت اعلى نسبة للاصابة لاطفال الريف وكانت (61.13%) بينما كانت نسبة الاصابة لدى اطفال المدينة (% 38.86) مع سيادة بكتريا *E. coli* وبنسبة (% 38.75) و (% 37.88) لاطفال الريف والمدينة على التوالي و قد سجلت اعلى نسبة للاصابة في شهر تموز وبنسبة (% 12.59) واقلها لشهر كانون الاول وبنسبة (% 1.01) ، كما اظهرت النتائج ان اكثر المضادات الحياتية تاثيرا على البكتريا المعزولة كان مضاد الحياة Ciprofloxacin اذ اثر على البكتريا بالنسب التالية : , (92.69%) *E.coli* , (85.17%) *proteus spp*. , (95.06%) *S.aureus* , (93.50%) *Enterobacter spp*. , (92.53%) *klebsiella spp*. , (94.64%) *S.agalactiae* , (100%) *pseudomanus. aeruginosa* ,*Enterococcus spp* (100%), *M.morganii* (100%) كما اشارت النتائج الى عدم وجود فرق معنوي في تركيز اللاكتوفيرين Lactoferrin Concentration بين مجموعة الاطفال الاصحاء والاطفال غير المصابين بالتهاب المجاري البولية بينما يوجد فرق معنوي في تركيز اللاكتوفيرين بين مجموعة الاطفال المصابين وغير المصابين بالتهاب المجاري البولية ، كما اظهرت النتائج وجود(ASC) pathogen –specific antibody secreting cells في دم (81 / 77) الاطفال المصابين بالتهاب المجاري البولية الذي تسببه بكتريا *E.coli* وسيادة الكلوبيولين المناعي IGM وبنسبة (% 67.53) مقارنة بالكلوبيولين المناعي IGA بنسبة (%32.46) .