PREVALENCE AND ANTIMICROBIAL SENSITIVITIES OF UROPATHOGENIC BACTERIA IN A GROUP OF PATIENTS IN KIRKUK CITY [+]

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Abstract:

A total of (405) urine samples were collected from patients with suspected urinary tract infections in the ages between (1-12) years old and from both sexes attending as the out patients of the main Kirkuk city hospital during the period from January to September 2012. Collected urine samples were subjected to different diagnostic tests for the isolation and identification of the infecting bacteria. Results indicated a seasonal variation in the frequency of urinary tract infections incidence among the patients during the research period where the higher frequency of urinary tract infections was recorded during the hotter seasons from June to September. In addition, it was found that, *E.coli* was the most infecting microorganism showing the percentage of (44.44%) followed by *Klebsiella oxytoca* that recorded the percentage of (25.92%) and *Proteus mirabilis* that spotted the third incidence percentage as (23.7%) and *Staphylococcus aureus* that recorded the incidence as (5.9%) from the cultures of the urine samples. Moreover, and according to the age group; infant patients recorded an incidence of urinary tract infections as (57.53%) and children patients recorded an incidence as (31.85%),while, adult patients recorded an incidence as (10.62%). Besides, it was found that, urinary tract infections was more prevalent in female patients in a percentage of (56.54%) than male patients whom recorded the percentage of (43.46%) at different age groups. Furthermore, the results of antibiotic sensitivity test for the urinary tract infections bacterial isolates showed resistance and sensitivity to different types of antibiotics.

انتشار و حساسيات الضد ميكروبية لبكتيريا مسببة لأمراض البول في مجموعة من المرضى في مدينة كركوك

أسل عزيز توفيق

المستخلص :

ما مجموعه (٤٠٥) عينة إدرار تم جمعها من مرضى يشتبه بإصابتهم بالتهاب في المجاري البولية تراوحت إعمارهم بين (١-١٢) سنة ومن كلا الجنسين حيث كانوا من المراجعين الخارجيين للمستشفى الرئيسي في مدينة كركوك للفترة من كانون الثاني الى أيلول ٢٠١٢. خضعت العينات بعد ذلك الى مجموعة من فحوصات التشخيص لغرض تحديد أنواع البكتريا المسببة للإصابة وقد أظهرت النتائج تفاوتا موسميا في وتيرة حدوث التهاب المسالك البولية بين المرضى خلال فترة الدراسة حيث تم تسجيل ارتفاع وتيرة التهاب المسالك البولية خلال المواسم الحارة من حزيران

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الى أيلول وبالإضافة إلى ذلك، فقد وجد أن بكتريا *E.coli* كانت من أكثر المسببات لهذه الاصابة بنسبة (٤٤,٤٤٪) تلتها بكتريا *Klebsiella oxytoca* التي سجلت نسبة (٩٢,٢٥٪) وبكتريا*Proteus mirabilis* التي حصدت نسبة الإصابة الثالثة (٧,٢٣٪) و بكتريا *Staphylococcus aureus* التي سجلت نسبة (٩,٥٪) في عينات البول التي تم جمعها. بالإضافة إلى ذلك ومقارنة مع مجموعة العمر فقد سجل المرضى الرضع نسبة انتشار لالتهابات المجاري البولية (٥٣,٥٧٪) والمرضى الاطفال سجلوا نسبة (٨٥,٣١٪) والمرضى الكبار سجلوا نسبة (٦٢,١٠٪) الى جانب ذلك، فقد تبين أن التهابات المجاري البولية كانت أكثر انتشارا في المرضى من الإناث بنسبة (٥٤,٥٦٪) من المرضى الذكور الذين سجلوا نسبة (٤٦,٤٣٪) في مختلف الفئات العمرية. علاوة على ذلك فقد أظهرت نتائج اختبار الحساسية للمضادات الحيوية مقاومة مختلفة للعزلات البكتيرية و حساسيتها لأنواع أخرى مختلفة من المضادات الحيوية.

Key words: Urinary tract infection, UTI, uropathogenic bacteria, Kirkuk city, *E.coli*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Staphylococcus aureus*

Introduction :

Urinary tract infections (UTIs) are the most common bacterial infections ranging from asymptomatic to sever sepsis [1&2]. It also contributes to the most nosocomial infection in many hospitals and accounts for approximately 35% of all hospital-acquired infections [3&4]. Bacteria are the primary organisms that cause UTI and gram negatives causes 80-85% of the infections and among them exist the *Escherichia coli* that is the most frequent pathogen [4&5]. But, in complicated UTIs the prevalence is for other antibiotic resistant bacteria such as *Klebsiella* and *Proteus* [6]. Yet, *Escherichia coli* as well as other members of the Enterobacteriaceae family are a leading cause of infectious diseases in the community due to multi-dug resistance attribute that increased in the last few years leading affecting the prognosis and survival of most UTI patients [7, 8 &9].

Objective: This study was designed to investigate the prevalence of a number of uropathogenic bacteria isolated from a group of patients with suspected urinary tract infections in Kirkuk City and to evaluate the relationship between patient's age and gender on the frequency of urinary tract infections beside the determination of the antimicrobial sensitivity of the bacterial isolates against different antibiotics.

Materials and Methods :

1. Collection of urine samples:

Fresh urine samples were collected from (405) patients ranging in age between (1-12) years old from both sexes suffering from signs and symptoms of urinary tract infections that included (fever, back and abdominal pain, strong-smelling urine, hematuria, vomiting and frequency of urination with burning) that were diagnosed by urologist as having urinary tract infection. Those patients were attending as the out patients of Azadi Teaching Hospital (the main hospital in Kirkuk city) during the period from January to September 2012. Besides, urine samples were also collected from (20) healthy volunteers from each age group as the age groups of the urinary tract patients (infants, children and adults) to be considered as control through data comparison. Those samples were all collected in sterile glass containers

of 50ml volume using clean –catch midstream technique according to standard procedures [10 &11].

2. Laboratory testing for suspected Urinary tract infections:

All patients with suspected urinary tract infection were subjected to urinalysis procedures which comprised physical examinations that involved the examination of urine (color, turbidity and odor), beside examining the chemical properties of the urine using dipstick methodology which involved examining (the pH, specific gravity, protein, blood, glucose, ketones, leukocytes and nitrites), in addition to the microscopic examinations of the collected urine samples that included searching the urine sample microscopically for the presence of (cells, casts, bacteria, yeast, parasites and crystals) according to standard procedures [12, 13 & 14]. Accordingly, any urine sample showed cloudy color, and turbid with acidic pH and ammonia smell containing red blood cells, protein, nitrite and bacterial cells supported the diagnosis that, the patient had urinary tract infection.

3. Identification of bacteria:

About 10 ml of each urine sample was centrifuged at 3000 rpm for 5 min, and the drops of the retained sediment were examined under 40 x power of a light microscope [10]. Consequently, for the quantification of the organisms in the urine, a calibrated (10 µl) inoculation loop (COPAN/ USA), taken from each urine sample was spread on enriched media (blood agar) and selective media (MacConky agar), the samples were incubated at 37C° for 18-24 hr, then, the number of bacterial colonies on the agar was calculated and the number of organism in 1 ml of urine was measured [12&13] . Subsequently, isolated bacteria were further identified according to using Gram stain and biochemical tests including: oxidase, indole, catalase, urea hydrolysis, geletinase, kiliglar iron agar, coagulase, phenylalnine deaminase and motility test [13&14].

4. Antibiotic sensitivity test:

This test was carried out according to standard techniques using disk diffusion test [15] by mixing colonies in peptone water and then streaking it on Mueller-Hinton agar plate using sterile cotton swab. Then, antibiotic disks (Bioanalyse/ Turkey) including (Ciprofloxacin 5µg, Amikacin 10 µg, Gentamycin 10µg, Cefotaxime 10µg, Ampicillin10µg, Trimethoprim 5µg, Amoxycillin 10µg, Pencillin 10µg, Erythromycin 15µg, Tetracycline 30µg and Streptomycin 10µg) and plates were incubated at 37˚C for 24 hr. The susceptibility of different bacterial isolates to antibiotics was determined according to the size of zone of inhibition according to [15&16].

5. Statistical Analysis:

Data from the study were analyzed using T- test by using SPSS program Ver.10 for Windows. A *P* value of <0.05 was considered indicative of a statistically significant difference .

Results and Discussion :

A total of (405) patients showing the signs and symptoms of urinary tract infections were enrolled in this study and results of figure (1) shows the seasonal distribution of UTI cases during the research period from January to September 2012.

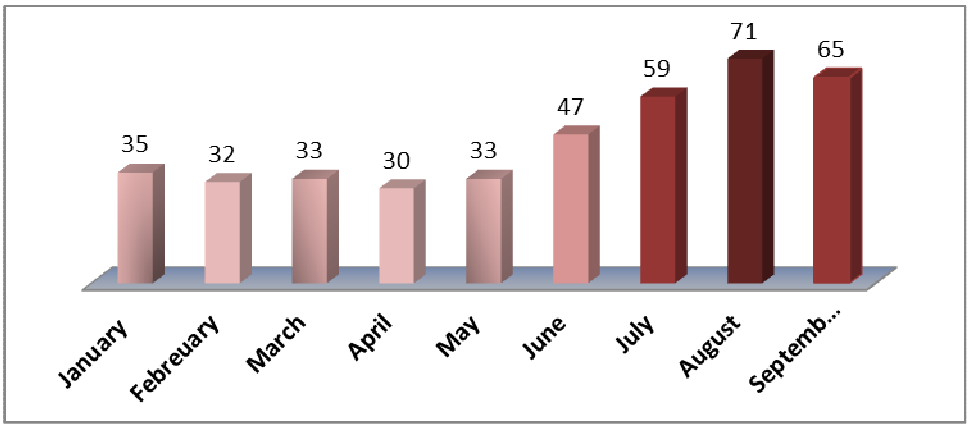

Figure (1): Seasonal Variation of Urinary Tract Infection (UTI) prevalence during the period from January to September 2012 in Kirkuk city.

The results of the figure above indicated a seasonal variation in the frequency of UTI incidence among the children during the period from January to September 2012 where the higher frequency of UTI was recorded during the hotter seasons (from June to September) with a significant increase (P <0.05) during August. There was no evidence that urine specimens were processed differently at different times of the year. Still, different reasons can be postulated for this seasonality in UTI in children; the hotter weather may induce relative dehydration which, in turn, may lead to more concentrated urine and less frequent voiding leading to increased UTIs, in addition to the increased rate of swimming in natural (with potentially contaminated water) could increase the risk of developing UTI concentrated urine and less frequent voiding; adding to that, the hot weather may lead to a moisture perineum which could facilitate bacterial transfer from the rectum to the urethra. However, the same results were recorded by other studies [3, 17 &18] whom suggested the same reasons for UTI seasonality in different world parts.

More to the point, it was declared that, Gram negatives causes 80-85% of the infections and among them exist the *Escherichia coli* that is the most frequent pathogen as recorded by other studies [3&19]. Results of figure (2) showed the same results.

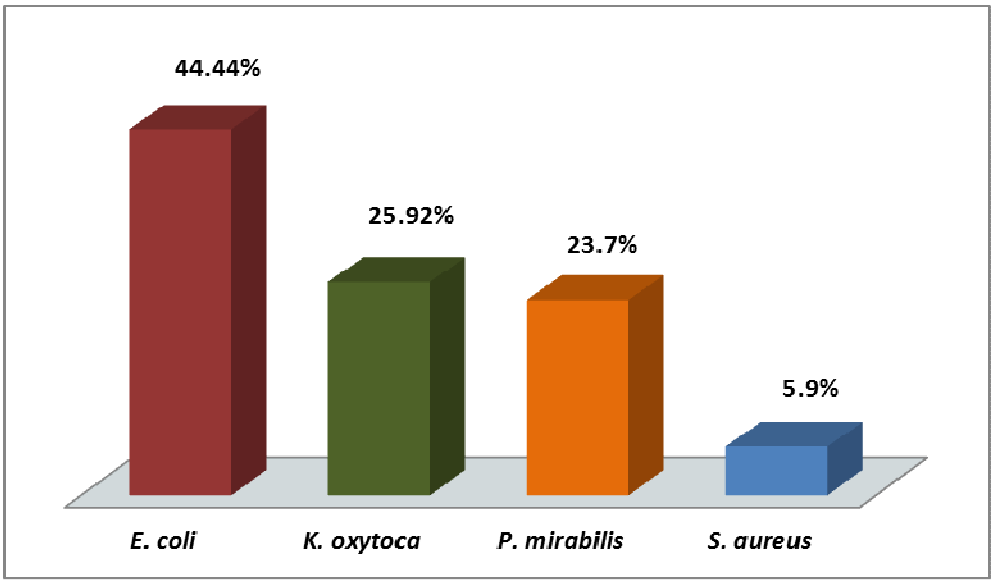

Figure (2): Frequency and distribution of bacterial isolates from cultivated urine samples.

The results shown in figure (2) revealed a significant increase (P <0.05) in the numbers of the bacterium *E.coli* isolates recording the percentage of (44.44%) asserting the previously mentioned results of other studies [17 &18] whom stated that, *E.coli* was the commonest pathogen in the urinary tract infection. In addition, *Klebsiella oxytoca* had recorded the percentage of (25.92%), *Proteus mirabilis* recorded the percentage of (23.7%), and *Staphylococcus aureus* came the last in the recorded percentages as (5.9%). However, these results came in agreement with many local studies like [20, 12 and 22] who found that *Escherichia coli* was the main pathogen of urinary tract infection.

Moreover, it could be also noticed from figure (2) that, *Proteus mirabilis* was recorded as the third percentage (23.7%) after the percentages of *Escherichia coli* (44.44%) and *Klebsiella oxytoca* (25.92%) which agree with the results established by previous studies [23&24] whom stated that, the prevalence of the multi-drug resistant *Proteus mirabilis* strains has increased in the last few years due to the presence of extended spectrum beta- lactamase genes that help this bacteria to resist many kinds of antibiotics leading to their spread.

Yet, this study included (405) UTI ranging in age between (1-12) years old and the relationship between the prevalence of urinary tract infection pathogens and UTI patient's age was also investigated and results were shown in figure (3).

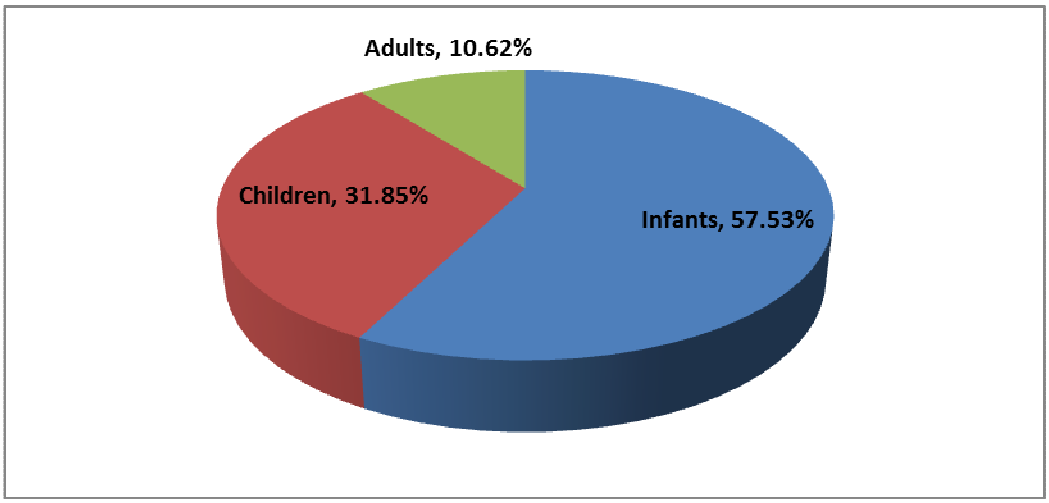

Figure (3): The frequency of urinary tract infection incidence between patient's age in three different cohorts including infants, children and adults.

Results of figure (3) showed that, UTI was significantly prevalent (P <0.05) in infants (> 1-2) years recording the percentage of (57.53%) more than UTI incidence in the children (3-10) years and adults (10-12) years ; where the recorded percentages of UTI incidence were (31.85%) and (10.62%) respectively. Yet, these results were obtained probably due to the following reasons; it is usually very easy to miss UTI symptoms in children especially for the young ones (>1-4) years old where UTI symptoms which includes fever, recurrent urination and sometimes vomiting might be confused with the symptoms of other disease like cold and tonsillitis. In addition, the reduction of child cleaning up and frequent dipper change during the cold seasons might increase the risk of developing UTI among these age groups. The same results were obtained by other studies [25, 26 &27]. Moreover, table (1) shows the frequency of the UTI causative bacteria compared to the age of the UTI patients.

Table (1): The frequency of the uropathogenic bacteria isolated from both the urinary tract infection patients and from the control group from different ages; where, Infants age range=(1-2) years , children age range = (3-10) years and adults age range =(10-12) years.

Uropathogenic bacteria isolated from urine samples from patients and control groups	Isolated bacterial frequency in UTI patients and controls in different ages					
	Infants With UTI (%)	Infants Of Control group (%)	Children With UTI (%)	Children Of Control group (%)	Adults With UTI (%)	Adults Of Control group (%)
E. coli	64.44%	25%	26.67%	15%	9.44%	1%
K. oxytoca	55.24%	15%	43.8%	5%	10.48%	3%
P. mirabilis	56.25%	5%	28.13%	5%	15.62%	1%
S. aureus	41.67%	3%	33.33%	3%	25%	1%

Results of table (1) clearly showed that, the frequency of the uropathogenic *E.coli* was significantly high (P <0.05) in infants in both groups of infant UTI patients (64.44%) and in the control group of infants (25%) more than that of the children UTI patients and control groups that recorded the percentages of (26.67%) and (15%) respectively. Besides, the results of *E. coli* incidence recorded by the infants group were also higher than the results that were recorded by the UTI adult group (9.44%) and adults control groups. The same results were obtained with the other uropathogenic bacteria that included *K. oxytoca*, *P. mirabilis* and *S.aureus* where the infants showed significant increase in the incidence of three other types of bacteria revealed by the recorded percentages as (55.24%) for *K. oxytoca* ,(56.25%) for *P. mirabilis* and (41.67%) respectively. More to the point the same results were obtained by other studies [25 &26] whom documented that, the percentage of infection with *E.coli*, *Klebsiella* spp. and *Proteus mirabilis* was high among the infantile age group (>1-2 years old), than the children group and the adults group where they acknowledged that, UTI has accounted for the commonest infections in infants and children less than five years old. In addition, several studies have shown a 10-12 fold increased risk of UTI in the uncircumcised boys and in juniors children warring diapers due to the accumulation of bacteria for a long time in the diapers [27 &28]. Furthermore, it was found that, UTI was more prevalent in females than males as figure (4) where results had clearly showed that, UTI was higher in females (56.54%) in comparison to the ratio in their male partner that recorded (43.46%). These results however, agree with the results obtained by other studies [1,3& 24] where they confirmed that, urinary tract infections are one of the most common bacterial infections seen in children and it has been estimated that UTI are diagnosed in 1% of boys and 3-8% of girls.

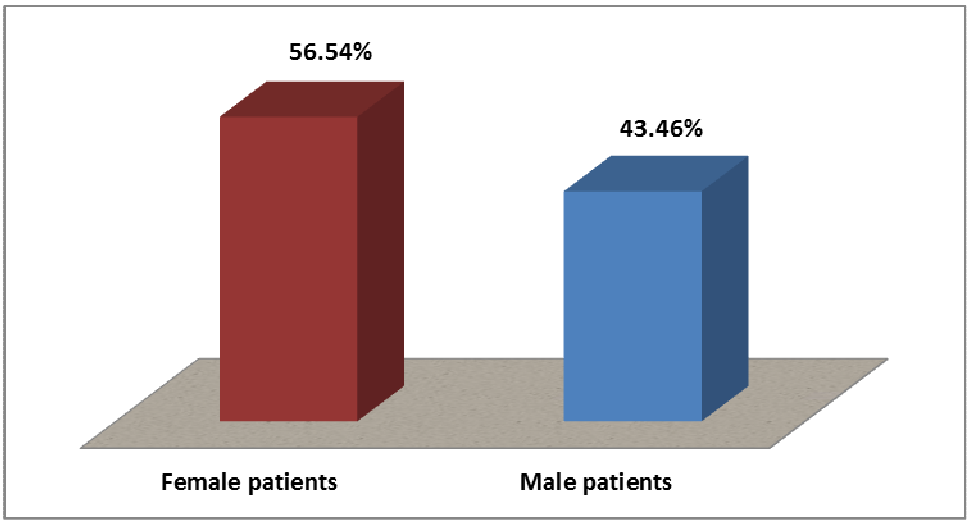

Figure (4): Prevalence of urinary tract infection according to the patient's gender.

Additionally, the development of resistant strains is a common problem in the treatment of urinary tract infections especially in young children [25 &29]. Therefore, all of the isolated strains UTI causative bacteria were tested for antibiotic sensitivity as shown in table (2).

Table (2): Antimicrobial sensitivity profiles of bacterial isolates from urinary tract infection patients ; where *n*, means number of urinary tract infecting bacterial isolates; where, *E. coli* = *Escherichia coli*, *K. oxytoca*= *Klebsiella oxytoca*, *P. mirabilis* = *Proteus mirabilis* and *S. aureus* = *Staphylococcus aureus*; and S = sensitive bacterial isolates, R= resistant bacterial isolates.

Antibiotics	UTI *E .coli* Isolates *n (%)*	UTI *K. oxytoca* Isolates *n (%)*	UTI *P. mirabilis* Isolates *n (%)*	UTI *S. aureus* Isolates *n (%)*
Amikacin	97 (53.9%)-S	100 (95.24%)- S	90 (93.75%)- S	20 (83.3%)-S
Amoxycillin	170 (94.4%) -S	102 (97.14%)-S	53 (55.21%)- S	18 (75%) - S
Ampicillin	35 (19.4%)- R	45 (42.86%) - R	46(47.91%) - S	12 (50%) - S
Cefotaxime	180 (100%) - S	98 (93.33%) - S	61 (63.5%) - S	24 (100%)- S
Ciprofloxacin	77 (42.8%) S	82 (78.09%) - S	96 (100%) - S	22(91.7%)- S
Trimethoprim	164 (91.1%)- S	87 (82.86%) - S	34(35.41%) -S	24(100%) - S
Gentamycin	180 (100%) - S	104(99.05%) -S	14 (14.58%) -S	24(100%) - S
Pencillin	0 (100%) - R	0 (100%) - R	0 (100%) - R	0 (100%) -R
Streptomycin	0 (100%) - R	0 (100%) - R	0 (100%) - R	6 (25%) - S
Tetracycline	0 (100%) - R	0 (100%) - R	0 (100%) - R	0 (100%)- R
Erythromycin	0 (100%) - R	0 (100%) - R	0 (100%) - R	0 (100%)- R

Results of table (2) showed that, different bacterial isolates of urinary tract infection were sensitive to a number of antibiotics and were resistant to the other ones. However, all of the isolates were resistant to the pencillin, streptomycin, tetracycline and erythromycin. While, *E. coli K. oxytoc*a and *S. aureus* were significantly sensitive (P <0.05) to the cefotaxime, gentamycin, trimethoprim and ciprofloxacin antibiotics respectively. Whereas, *P. mirabilis* isolates were more significantly sensitive (P <0.05) to the ciprofloxacin and Amikacin antibiotics than the ones.

Still, the obtained results agree with the results obtained by other studies [16, 30,31 & 32] where they declared that, the incorrect and excessive use of different kinds of antibiotics might add a contribution to the development of antibiotic- resistant strains which can be passed from one bacteria to another through conjugation.

Conclusions:

1. It could be concluded that, UTI in Kirkuk city has a seasonal distribution variation where this infection was more prevalent in hot seasons compared with the cold seasons.
2. The bacterium *E.coli* was more prevalent in causing UTI in infants and children than other types of bacteria.
3. UTI affects the infant's age group and children more than the adults.
4. UTI was more prevalent in females than males in the patients of Kirkuk city.
5. Isolated bacteria showed sensitivity to a number of antibiotics and resistance to other types due to its being multi-drug resistant bacteria.

Recommendations:

1. Extending the examination of urine samples from UTI suspected patients to include more age groups in order to study other bacterial types related to UTI.
2. Extending the study of the uropathogenic bacteria causing UTI to include rural areas to compare between the prevalence of UTI between urban and rural areas surrounding Kirkuk city.
3. Advising families especially mothers who have infants and children less than five years old to take superior care of their children in the matter of regularly changing diapers and frequently inspecting the reason behind fever particularly for their female children.
4. Educating the families about the importance of the treatment of UTI in their children due to the risk of renal scarring.
5. Consulting doctors before using antibiotics to prevent drug abuse.

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