



Research Article

STUDY THE RISK FACTORS, BACTERIAL PROFILE AND ANTIBIOTIC RESISTANCE PATTERN IN URINARY TRACT INFECTIONS PEDIATRIC IRAQI PATIENTS

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ABSTRACT

Urinary tract infection (UTI) in pediatric patients is a significant source of morbidity and considerable mortality. There are multiple risk factors as well as wide range of pathogen that associated with recurrent UTI. So, this study aims to determine the risk factor in UTI patients then isolation and identification causative pathogens with determination antibiotic resistance profile. A total of one hundred clinical samples from UTI – pediatric patients attending to Baghdad teaching hospitals during 1-5 till 1-12 /2017 were included. The study shows that Female sex, lack of circumcision, vesico-ureteral reflux (VUR), neurogenic bladder (NB), urinary tract abnormalities and inadequate water intake had significant effect on recurrent UTI. The results revealed that 86 bacterial isolate were gave positive bacterial growth, from which 52 were Gram negative bacteria that represent by 30(57.6%) *E. coli* isolate followed by 11(21.15) *K.pneumonia* isolate and 8(15.38%) isolates *Proteus* sp finally 3(5.76%) *P.aeruginosa* while gram-positive bacteria that comprised 34 (39.5%) divided into 12 (35) isolate were *S.aureus*, *S.saprophyticus* and 10 (30 %) isolate were *Enterococcus* sp. Antibiotic resistance profile confirmed that all uropathogens were multidrug resistance, 100% resistance toward Amoxicillin and show variable resistance toward Cephalosporins, Quinolones and sulfa drugs while the Imipenem and Vancomycin consider drug of choice in treatment. In conclusion, risk factors had a significant role in recurrent UTI. So precise diagnosis to causative agents and susceptibility pattern increase the success of therapy and prevent complication of disease

Key word: UTI, Risk factor, bacterial infection, antibiotics

INTRODUCTION

Urinary tract infection (UTI) is a significant public health problem that consider one of the common sources of infections causing systemic illness in infants and children, if not treated the complication may lead to renal scarring, end stage renal failure and hypertension.¹ UTI is the third most common infection after respiratory and gastrointestinal infections. It causes a significant morbidity and considerable mortality that affects about 150 million people each year worldwide.² The chances of urinary tract infection increase in the presence of risk factors which including familial predisposition, genitourinary abnormalities (posterior urethral valves, ureter- pelvic junction obstruction, constipation), neurologic impairment (myelo meningocele with neurogenic bladder) and function disorder as well as periurethral flora.^{3,4} The determination of risk factors in a child presenting with UTI is important in preventing further recurrences or subsequent complication.⁵ Increase resistance of bacteria toward antibiotics even for newly types lead to emergence the multidrug resistance pathogens and failure therapy in infection anywhere in the world that regard a series problem globally.⁶ However variable antimicrobial resistance rates in different geographical and regional locations⁷. Therefore, the accurate and rapid bacterial identification and antimicrobial susceptibility investigations for patients with bacterial infection was the primary step for both precise therapy and antimicrobial resistance prevention.⁸ Thus, the aim of the current study is to investigate the important risk factors that associated with recurrent UTI, identify the bacterial causative agents and antibiotic susceptibility profile to decrease the reinfection and prevent complication of recurrent UTI.

MATERIAL AND METHODS

Patient characterization

A total of 100 urine samples were obtained by midstream clean catch, bladder catheterization under aseptic conditions during the study period from 1-5 till 1-12/ 2017 from pediatric patients (1-15 years) suffering from recurrent UTI attending to Baghdad Teaching Hospital after received the Ethical Considerations that carried out in according with Declaration of Helsinki guidelines which approved by the Medical Ethics Committee of the Ministry of Health in Iraq with numbering 112115.

The microbiological diagnosis of UTI required $\geq 10^5$ CFU /mL of a single microorganism in midstream, or $\geq 10^4$ CFU/mL of a single microorganism in catheter samples. Urine cultures with growth of more than two microorganisms were considered contaminated.

The following samples were excluded: urine samples obtained by plastic bag applied to the perineum, only valid when the result is negative; samples from previously hospitalized individuals; those from individuals who had received antibiotic therapy during UTI episode; patient not fill all information or correct sample and sample from children above 16 years.

Bacterial Isolation and Identification

All inclusion samples were cultured on the MacConkey agar plates, Blood agar plates and incubated at 37 °C under aerobic condition for 18-24-hour, Gram's stain was used to examine the isolated bacteria for studying the microscopic properties such as gram reaction and shape. While Biochemical tests used according

to MacFaddin JE⁹ in addition to use VITEK-2 system to confirmed identification.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was accomplished by the "Kirby-Bauer disc diffusion method using Mueller-Hinton agar" as recommended by Clinical Laboratory Standard Institute¹⁰. The antimicrobial agents tested, and their corresponding concentrations were as follows: Amoxicillin (25µg), Ampicillin/sulbactam, Amikacin (10µg), Norfloxacin (10µg), Ciprofloxacin (5µg) Cotrimoxazole, Nitrofurantion (300) Ceftriaxone (10µg), Cefotaxime (10µg), Vancomycin (10) Imipenem (10µg).

A small inoculum of bacterial suspension (which prepared by inoculate 5 isolated grown on BHI agar to 5 ml of tryptic soy broth then incubated for 2hour to produce a bacterial suspension of moderate turbidity that compared with turbidity of ready-made 0.5 McFarland tube standard) was inoculated on Mueller-Hinton plates and antibiotic discs were placed on the plates, spacing them well to prevent the overlapping of inhibition zones. After incubating the inoculated plates aerobically at 37 C° for 18 to 24

hours, the susceptibility and resistance of the isolates to each antimicrobial agent was measured and the results were interpreted in accordance with criteria provided by Clinical and Laboratory Standards Institute.¹⁰

Statistical Analysis

The analysis of variance (ANOVA) was applied by using Statistical Package for Social Science (SPSS) system/ version 17 and Microsoft Office Excel 2007. Results were expressed as mean ± S.D. P value of <0.05 was considered significant. L.S.D. values were compared with values of means difference.

RESULTS

Patients characterization

Out of 100 clinical specimens (urine), only 86 (86%) samples were giving positive results (bacterial growth) on general and selective media and those patients were searched for risk factors. While the rest 14 samples gave negative results for culturing as shown in the Figure (1 -A).

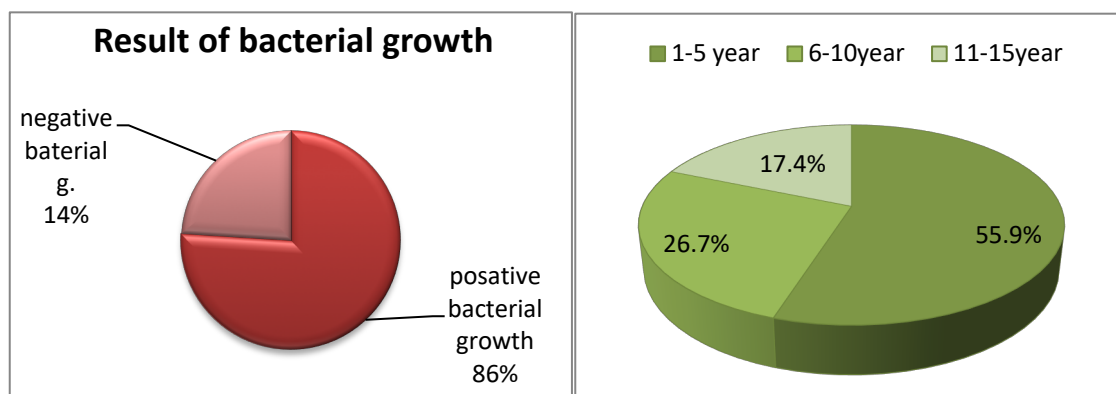


Figure 1: Distribution according to A- bacterial growth B- age of patients

The result clarified that 86 patients were 54(63%) female 32(37%) male. Figure (1-B) shows that UTIs is related to age of the pediatric patients. The highest percentage of infected patients 48 (55.9%) and 23(26.7%) were found within the age brackets of 1-5 years and 6-10 years respectively, followed by 15(17.4%) patients with UTIs were obtained from age groups 11-15 years.

A result was illustrated that majority of patients 26 (30.2%) suffering from vesico-ureteral reflux, urinary tract abnormality 22

(25.6 %) the second leading risk factor causing urinary tract infection, 18 (20.9 %) patients had neurogenic bladder. As show in Table 1 minor contribution was made by risk factors like low water intake 6 (6.9) while in almost 14(16.4) percent cases the risk factor could not be identified, and no cause was detected from which the female recorded 10 and 4 males. In male the out of 32 patients, 22(68.7%) uncircumcised boys while 10(31.3%) circumcised boys.

Table 1: Risk factors in UTI patients

Type of risk factor	Female	Male	Total
Vesico-ureteral reflux	19	7	26
UT abnormalities	10	12	22
Neurogenic bladder	11	7	18
Low water intake	4	2	6
Non -observed risk	10	4	14
Total	54	32	86

Bacterial isolation and Identification

Out of these 86 culture positive samples, 52 (60.4% %) were Gram negative bacteria that represent by 30(57.6%) *E.coli* isolate followed by 11(21.15) *K.pneumonia* isolate and 8(15.38%)

isolates *Proteus* sp finally 3(5.76%) *P.aeruginosa* as shown in Figure (2-B), while gram-positive bacteria that comprised 34 (39.5%) divided into 12 (35.29) isolate were *S.aureus* , *S.saprophyticus* and 10 (29.41%) isolate were *Enterococcus* sp Figure (2-A).

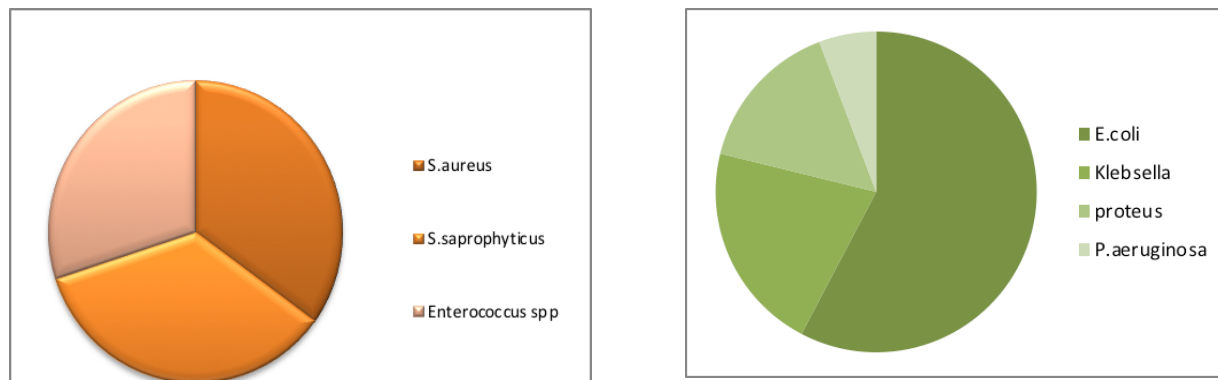


Figure 2: Distribution of gram-positive bacteria (A) and gram-negative bacteria (B)

Antibiotic susceptibility test

All gram-positive bacteria showed 100% susceptibility to Vancomycin while recorded 100% resistance to Amoxicillin, followed by Ampicillin/sulbactam, Ciprofloxacin and revealed moderate resistance to Cephalosporins and macrolides as shown

in Figure 3. In case of gram-negative bacilli, Imipenem showed no resistance and the Amikacin (AK) appeared least resistance to the gram-negative organism while Amoxicillin revealed 100% resistance followed by Ampicillin/sulbactam, Cotrimoxazole, finally moderate resistance was recorded to other tested antibiotic Table 2.

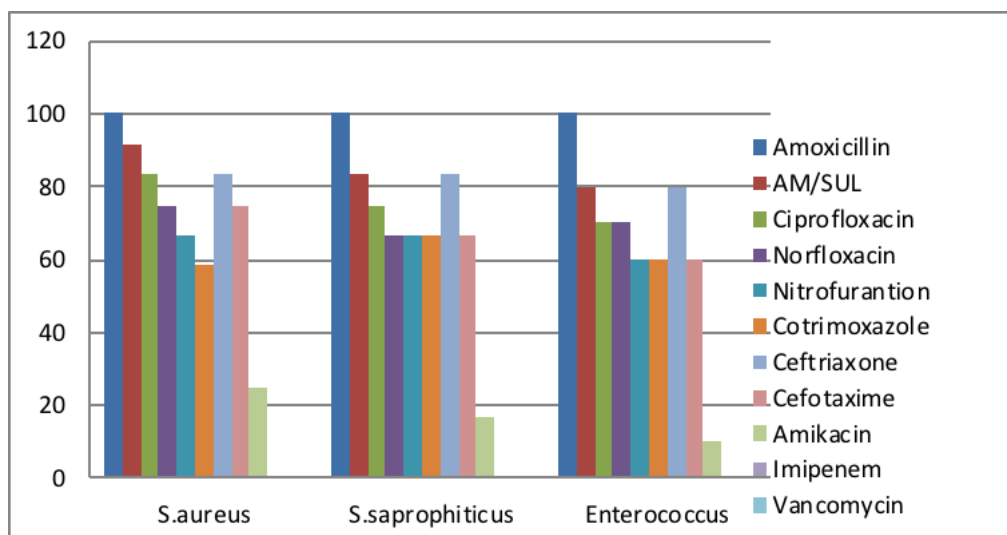


Figure 3: Antibiotic resistance profile in gram-positive bacteria

Table 2: Antibiotics resistance profile in gram-negative bacteria

Antibiotics	<i>E. coli</i> N=30	<i>K. pneumonia</i> N=11	<i>Proteus sp</i> N=8	<i>P. aeruginosa</i> N=3
Amoxicillin	100%	100%	100%	100%
Ampicillin /sulbactam	100%	10(90.0%)	100%	100%
Ciprofloxacin	26(86.6%)	9(81.8%)	6(75%)	2(66.6%)
Norfloxacin	21(70%)	8(72%)	5(62.5%)	2(66.6%)
Nitrofurantion	13(45.6%)	4(36.3%)	4(50%)	1(33.3%)
Cotrimoxazole	28(93.3%)	10(90%)	7(87.5%)	100%
Ceftriaxone	17(56.6%)	6(54.4%)	5(62.5%)	2(66.6%)
Cefotaxime	18(60%)	5(45.4%)	4(50%)	2(66.6%)
Amikacin	2(6.66)	3(27.2)	1(12.5)	1(33.3)
Imipenem	0%	0%	0%	0%

DISCUSSION

UTI including cystitis and pyelonephritis is a serious infection disease in childhood that represents one of the most recurrent UTI. The current study appear that female baby is more prone to developed UTI than male baby (p<0.05) and this observation seems to agree with the findings of Foxman B¹² who reported a prevalence of UTI is more frequency in female. Also, other report

observed that the estimated incidence of UTI is 1% in boys and 3% in girls during the first ten years of life.¹³

The predominant UTI risk factors in young girls are anatomic and physiologic factors since the short and wider urethra of female permits easy entry of bacteria into the bladder from fecal flora and causes UTI. Hossain MA *et al*¹⁴ supported our results and confirmed that female regard as risk factor in UTI.

The results of age distribution in line with Jitendranath A *et al*¹⁶ they illustrated that the maximum numbers of cases were in the 0-6 age group with 80% of all cases followed by age 7-12. The high percentage of UTI in this age may be regard to not toilet trained. Montini G *et al*¹⁷ in another investigation they demonstrated that out of 62 isolates recovered from UTI pediatric patients about 67.8 % were from children under 5 years of age.¹⁸

This study observed increase incidence of uncircumcised boy and this finding agree with Riccabona M¹⁹ who explain that about 10-12 folds increased risk of UTI in uncircumcised boys compared with circumcised. The periurethral area was found to be more frequently and more heavily colonized with uropathogens, especially *E. coli*, in uncircumcised infants than in circumcised infants.²⁰ During the first seven years, boys record less than girls in incidence of UTI also, uncircumcised boys about 10-folds increased incidence.²¹ However, several factors associated with increased risk of recurrent UTI include grade 4 to 5 vesicoureteral reflux and age 3 to 4 years.²² In same line researchers revealed that vesico-ureteral reflux its congenital disorder affects girls more than boys and the main presentation for these children is UTI which associated with renal abnormalities or obstruction, or high-grade vesico-ureteral reflux.²³

A prospective study of 128 Spinal Cord Injury patients demonstrated that patients with vesico-ureteral reflux had a 23-fold risk for the development of repeat infection.²⁴ UTI patients diagnosed with vesicoureteral reflux (VUR) is more likely to encounter recurrent UTIs and subsequently develop renal scar formation.²⁵ The unstable storage function and uncoordinated emptying are a high risk for infection in neurogenic bladder patients as a result of their physiological cleansing of the urethra by bladder emptying does not take place, also confirmed that chronic or recurrent urinary tract infections (UTI) pose a significant risk to all patients with neurogenic bladder.²⁶ UTI consider as one of the most difficult complications to treat and prevent in patients with a neurogenic bladder.⁵ The incidence of UTI in patients with a neurogenic bladder is high and estimated that the overall rate of UTI in patients with a neurogenic bladder is 2.5 episodes per patient per year.²⁷

UTI in patients with a neurogenic bladder reach to one third (36.4%) of patients diagnosed with a lower UTI at least one-year post neurogenic bladder diagnosis.²⁸ Urinary tract obstruction is one of the main causes of urinary tract infection, which produces the favorable environment for the growth of urinary tract pathogens.²⁹ Infections occurring due to anatomical abnormalities urinary tract can also be lead to treatment failure, repeat infections, or significant morbidity and mortality with a poor outcome. In same regard Hang J & Kuo H³⁰ explain that urogenital tract anatomical abnormalities have been represent the essential risk factors for recurrent UTI that help bacterial survival as a result of defect in pathogen recognition and urethral barrier function impairment.

Bacterial eradication from the urinary tract is partially dependent on urine flow and voiding frequency. Therefore, it seems logical to postulate a connection between fluid intake and the risk of urinary tract infections (UTIs). Hossain MA *et al*¹⁴ in recent study found inadequate water intake predisposes to developed urinary tract infection. Also, this finding observed by Denman SJ & Burton JR³¹ suggested that mild dehydration is a risk factor of urinary tract infection. Contradictory results on the influence of fluid intake on the risk of recurrent UTI by Beetz R³². The study revealed that gram negative bacteria more prevalent than gram positive bacteria and clarify that a clear variation in resistance against different types of antibiotics were appeared by different types of bacteria. Gram- negative bacteria were predominant agents in UTI and *E.coli* was the most frequently occurring pathogen (54.88%) followed by *S.aureus* (9.75% for both), and

P.vulgaris, *P.aeruginosa* (4.88%, for both) and *Enterococcus* species (3.66%).³³ In same regard Sargiary P *et al*³⁴ founded that *E. coli* (42.68%) was most commonly isolated organism in UTI followed by *Klebsiella* sp (16.56%) and *S. aureus* (10.83%) and confirmed that antibiotics that active against gram negative isolates were imipenem, nitrofurantoin. Vancomycin, linezolid, were effective against gram positive isolates.

Hady AA *et al*³⁵ confirmed that *S. aureus* and *E.coli* bacteria were consider the most common gram-positive and gram-negative nosocomial pathogen and the susceptibility profile to routinely used antibiotic in Iraqi hospitals revealed that *S.aureus* were multidrug resist with 100% Amoxicillin, 85% toward, Amikacin and 75% to CTX and 50% CIP and *E.coli* appear as MDR with 100% to Amoxicillin, 85.7% toward Amikacin, CTX and 71% to CIP while all gram-positive and gram-negative isolates revealed 100% sensitive to Vancomycin and IMP with rang 90-100%. Attachment of bacteria to uroepithelial cells is an active process mediated by specific bacterial adhesions and specific receptor sites on the epithelial cells. This process allows bacteria to ascend into the kidney, In the kidney, the bacterial inoculum can produce an infection with an intense inflammatory response that may ultimately lead to renal scarring.³⁶ *S.aureas* a most common gram positive bacteria in incidence of recurrent UTI and *E.coli* a most common gram negative pathogen³⁸.

The present study revealed that isolate was confirmed β -lactam resistant (Amoxicillin, Ampicillin /sulbactam, Ceftriaxone and Cefotaxime). Such resistance may be emerged because of the wide spread use of these drugs in Iraqi hospitals or may be used in pediatric patients for other upper respiratory infections. This result fitted with Kalal BT & Patel RB¹⁸ they founded that all uropathogenic isolate were multidrug resist toward Ampicillin / sulbactam, Ampicillin and Cephalosporins. The resistance of gram negative bacteria to flour quinolones (Ciprofloxacin, Norfloxacin) and nitrofurantion as a result the commonly used of these antibiotics in UTI for long period lead to inhibit the efficiency of antibiotics to eradicated bacteria.³⁸ High percentage of isolates showed resistance to sulfa drugs such as Cotrimoxazole that is line with previous finds.³⁹ Vancomycin appear as the most sensitive drug against Enterobacteraiceae members isolated from UTI infection, also they confirmed that Quinolones and aminoglycosides were found to be highly resistant which is in accordance with our study.⁴⁰

In study done by Yoon JE *et al*⁴¹ they revealed that Enterobacteraiceae had lower resistance rates against Imipenem (100%) and gentamicin (79.0%) which corresponds to results of our study. Also, Sargiary P *et al*³⁴ confirmed that Imipenem was active antibiotics against gram-negative bacteria while Vancomycin and Linezolid were active antibiotics against gram-positive bacteria.

The results of this study revealed that most of the *S.aureus* were resistant to many antibiotics. Antimicrobial resistance among nosocomial pathogens is a significant problem in clinical settings that may be added to the cost of medical care then the morbidity and mortality of patients.³⁵ Mansour mention that emergence of antibiotics unsusceptible staphylococcal strains may become a big trouble in failure therapy of infection in future by *Staphylococcus* sp.⁴²

CONCLUSION

Urinary tract infection increases in the presence of risk factor that lead to sever complication and subsequently leads to high morbidity, poor quality of life and reduced life expectancy. Early diagnosis is important to preserve renal function from severity of complication as well as assessment the pattern of microorganisms

causing UTI and their antimicrobial susceptibility should be done periodically for the empiric treatment.

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