Causative Microorganisms and Antibiotics Susceptibilities in Children with Urinary Tract Infection

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ABSTRACT

Generally, treatment of urinary tract infection (UTI) in children is a difficult task, due to development of high resistance against antibiotics. The object of this study is to investigate the greatest causative microorganisms, their susceptibility and resistance to antibiotics in children suffering from UTI in Baghdad. A total of 810 urine samples were collected from suspected cases of UTI in children patients of ages (1 day to 12 years) of both sexes. Urine specimens were examined by urinalysis and cultured for isolation of microbial agents. In this study, Bacterial growth was obtained in 202 samples (24.39%) out of 810 urine samples. Various pathogenic organisms were isolated, which represented by A) Gram-Negative bacilli include: Escherichia coli (41.58%), Enterobacter spp. (13.68%), Proteus Mirabilis (12.78%), Acinetobacter spp. (4.45%), Providencia spp. (4.45%), Pseudomonas aeruginosa (3.46), Klebsiella pneumoniae (2.47%), Citrobacter spp. (0.49%), Salmonella spp. (0.49%). B) Gram-Positive cocci: Staphylococcus aureus (11.38%), Enterococcus faecalis (1.98%) and streptococcus agalactiae (1.48%) C) Candida albicans (0.9%). According to antibiotic susceptibility test, mostly the isolates were resistant to Nalidixic acid (67.05%), cefotaxime (59.40%) and cefepime (50.49%). Mostly Gram-Negative bacilli were responsible for UTI, and maximum recurrent isolated bacteria were Escherichia coli. The isolated bacteria were found to be sensitive to Imipenem, Amikacin and ciprofloxacin. Therefore, the selection of the antibiotic therapy in UTI should depend on the native sensitivity form of the infecting organism.

KEYWORDS: Children; Urinary tract infections; causative microorganisms; antibiotic susceptibility; resistance.

خلاصة

يعتبر علاج عدوى المسالك البولية (UTI) لدى الأطفال مهمة صعبة، بسبب زيادة المقاومة للمضادات الحيوية. تهدف هذه الدراسة إلى تحري عن المسببات المرضية ومدى مقاومتها للمضادات الحيوية لدى الأطفال الذين يعانون من التهاب المسالك البولية في بغداد. تم جمع 810 عينة من حالات من الاطفال التمانى من الحالات المرضية بإصاباتها بالالتهابات المسالك البولية لدى الأطفال المرضى بعمر (1 يوم إلى 12 سنة) من كلا الجنسين. تم فحص عينات الادارة وزراعتها لعزل العوامل المرضية. تم الحصول على 202 حالة موجبة للزرع البكتيري (24.39%) من مجموع العينات 810. تم عزل العديد من العوامل المرضية والتي شملت: (أ) العصيات السالبة لصبغة جرام: Escherichia coli (41.58%), Enterobacter spp. (13.68%), Proteus mirabilis (12.78%), Acinetobacter spp. (4.45%), Providencia spp. (4.45%), Pseudomonas aeruginosa (3.46), Klebsiella pneumoniae (2.47%), Citrobacter spp. (0.49%), Salmonella spp. (0.49%). (ب) المكورات الموجبة لصبغة جرام: Staphylococcus aureus (11.38%), Enterococcus faecalis (1.98%).

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INTRODUCTION

Urinary Tract infection (UTI) considers the second most common infection after respiratory infection during childhood in developing countries. Recurrent UTI may cause serious long term complication [1, 2, 3, 4].

The most common sites of UTI are the urinary bladder (cystitis) and the urethra, from these sites; the infection may ascend into the ureter (ureteritis) and subsequently involve the kidney (pyelonephritis). [1, 2, 3, 4]. Females are more prone to infection of UTI than males. Chronic UTI is usually associated with an underlying disease as congenital anomaly of the genito-urinary tract. Risk factors for UTI are urinary tract abnormalities as anatomic anomaly like Vesico Ureteric Reflux (VUR); prematurity, bladder dysfunction, constipation or prolonged retention, uncircumcised boys, and urologic procedures [3,4,6]. Classic UTI symptoms in older children include dysuria, frequency, urgency, and lower abdominal pain. Infants with UTI have non-specific symptoms comprise fever, irritability, and vomiting, poor appetite [1, 3, 7]. The occurrence of nitrates and leukocyte esterase in urinalysis helps as indirect indication of a UTI, although it is not alternate for urine culture [8, 9].

In the current study, we aim to investigate the greatest common microorganisms, antibiotic susceptibility and resistance form and create a basis for the empiric antibiotic treatment of childhood UTI for our region.

The most common causative gram-negative bacilli of UTI are Escherichia coli, Proteus mirabilis, Klebsiella spp., Enterobacter cloacae, Morganella morganii; Pseudomonas aeruginosa (especially in kidney dialysed and in immunocompromised children patients), Providencia stuartii and Serratia spp; while Gram positive cocci are Enterococcus spp., Streptococcus group B especially among neonates and Staphylococcus aureus 10, 11, 12, 13]. Microorganisms’ resistance may progress in different methods against antibiotics such as transfer of resistance genes due to the “R " plasmids to many gram negative bacteria. Knowledge of local antibiotic resistance pattern is important for treatment and prevention of complications. Resistance is increasing because of careless and irregular use of antibiotics especially in children with recurrent UTI. Therefore, the determination of causative agent and antibiotic susceptibility are important for the proper treatment of UTI because of the increasing and changing antibiotic resistance pattern [14, 15].

MATERIALS AND METHODS

Sample collection

The American Academy of pediatrics (AAP) mentions suprapubic aspiration or urethral cauterization to found a diagnosis of UTI in neonates and young children. A clean catch mid-stream urine specimen may be obtained from older children. Culture of urine from a sterile bag attached to the perineal area has a false-positive rate too high to be suitable for diagnosis of UTI. Urine specimens (810) were examined by urinalysis for nitrite, leukocyte esterase and pus cell.

Culture:

All urine samples (810) were inoculated on blood agar as well as MacConkey agar by quantitative streaking method using a calibrated inoculating loop (holding 0.001 ml urine) and incubated at 37c° in aerobic atmosphere for 24 hours.

Diagnosis:

A specimen was considered positive for UTI if a single uropathogen was cultured at a concentration of 10^4 colony-forming unit per milliliter (CFU/ml) and ≥5 leukocytes per high power field were observed on microscopic examination of the urine [7]. The AAP criteria for the diagnosis of UTI in children (2-24 months) are the presence of pyuria on urinalysis and of at least 50,000 CFU/ml of a uropathogen from the quantitative culture of a properly collected urine
specimen; and from neonates younger than 2 months, criteria include the presence of lower amounts of a single pathogen (10,000-50,000 CFU/ml) [7, 8, 14, 15, 16, 17].

Identify of bacteria was established on standard culture and biochemical features of isolates. Gram-Negative bacteria were identified by standard biochemical tests and oxidase test, or by API 20E (Analytical Profile Index 20 tests for Enterobacteriaceae). Gram positive microorganisms were identified with the corresponding laboratory tests (catalase, coagulase, CAMP test for *Streptococcus agalactiae* and esculin agar for enterococci) or by API for *Staphylococcus* and for *Streptococcus*.

**Antimicrobial susceptibility test (AST method):**

For this test, we took well-isolated colonies of similar appearance that were considered significant. Antimicrobial susceptibility of isolates was tested by modified Kirby-Bauer disc diffusion method according to the National Committe on Clinical Laboratory Standards (NCCLS) recommendations [18, 19, 20], by using Muller – Hinton medium of pH 7.2 to 7.4 with 4 mm thickness, Antibiotic discs (Imipenem 10mg, Amikacin 30mg, Ciprofloxacin 5mg, Cefepime 30mg, Cefotaxime 30mg, Nalidixic acid 30mg, and Vancomycin 30 mg) that were stored properly. We used inoculum 0.5 MacFarland turbidity, and by dipping a sterile swab into the inoculum, then streaked the swab all over the surface of Muller-Hinton Medium, then the antimicrobial discs (seven discs) were placed on the inoculated plates by using disc dispenser or a pair of sterile forceps, after overnight incubation at 35°C, then the diameter of each inhibition zone (including the diameter of the disc ) measured and recorded in (mm) by using calipers or a ruler on the under surface of the plate, then results were interpreted according to the critical diameter in the interpretative chart of inhibition zone sizes for rapidly growing bacteria for each antimicrobial agent. [18, 19, 20].

**RESULTS AND DISCUSSION**

In our study, the total number of urine samples examined was 810 [564 (69.62%) and 246 (30.37%) for female and male children, respectively from inpatient and outpatient pediatric department. Out of these, 202 (24.39%) were positive by culture [138(68.31%) from females and 64 (31.68%) from males children]; most common age group with positive culture was the infant (1 month–1 year) with a higher occurrence in females (Table 1).

| Age group          | Age group cases No. (%) | Females No. (%) | Males No. (%) |
|--------------------|-------------------------|-----------------|---------------|
| Newborn (< 1 month)| 10(4.95)                | 8(3.96)         | 2(0.99)       |
| Infant (1 month–1 year) | 56(27.72)            | 30(14.85)       | 26(12.87)     |
| Toddler (1 year–3 year) | 34(16.83)             | 24(11.88)       | 10(4.95)      |
| Preschool (3 year–6 year) | 52(25.74)            | 42(20.79)       | 10(4.95)      |
| School age child (6 year–12 year) | 50(24.75)        | 34(16.83)       | 16(7.92)      |
| **Total cases**    | **202(100)**          | **138(68.31)**  | **64(31.68)** |

Out of these 202 positive cultures, there were 170 (84.15%) of Gram-Negative Bacilli and 30 (14.85%) of Gram-Positive Cocci. The most prevalent Gram-Negative bacilli isolated was *Echerichia coli* 84 (41.58%) followed by *Enterobacter spp.* 28 (13.68%) and *Proteus mirabilis* 26 (12.78%); While the most prevalent Gram-Positive cocci isolated was *staphylococcus aureus* 23(11.38%) as shown in Table 2.
Table 2. Frequency of isolated microorganisms in children with urinary tract infection (202 isolates).

| Microorganism                  | Frequency No. | %    |
|-------------------------------|---------------|------|
| *Escherichia coli*            | 84            | 41.58|
| *Enterobacter* spp.           | 28            | 13.68|
| *Proteus mirabilis*           | 26            | 12.78|
| *Acinetobacter* spp.          | 9             | 4.45 |
| *Providencia* spp.            | 9             | 4.45 |
| *Pseudomonas aeruginosa*      | 7             | 3.46 |
| *Klebsiella pneumoniae*       | 5             | 2.47 |
| *Citrobacter* spp.            | 1             | 0.49 |
| *Salmonella* spp.             | 1             | 0.49 |
| **Gram positive cocci**        |               | 14.85|
| *Staphylococcus aureus*       | 23            | 11.38|
| *Enterococcus* faecalis       | 4             | 1.98 |
| *Streptococcus agalactiae*    | 3             | 1.48 |
| **Candida albicans**          | 2             | 0.9  |

According to Antibiotic susceptibility Test; mostly the isolates were resistant to Nalidixic acid (67.05%), cefotaxime (59.40%), and Cefepime (50.49%); while Imipenem, Amikacin and Ciprofloxacin have the least resistance. (Table 3).

Table 3. Distribution of Antibiotics resistance pattern

| Tested Antibiotics | Bacteria No. | Resistant No.(%) | Intermediate No.(% ) | Susceptible No.(%) |
|--------------------|--------------|------------------|----------------------|-------------------|
| Imipenem (10Mg)    | 202          | 4(1.98)          | -                    | 198(98.01)        |
| Amikacin (30Mg)    | 202          | 21(10.39)        | -                    | 181(90.60)        |
| Ciprofloxacin (5Mg)| 202          | 50(24.75)        | 8(3.96)              | 144(71.28)        |
| Cefepime (30Mg)    | 202          | 102(50.49)       | 12(5.94)             | 88(43.56)         |
| Cefotaxime (30Mg)  | 202          | 120(59.40)       | 16(7.92)             | 66(32.67)         |
| Nalidixic acid (30Mg) | 170      | 114(67.05)       | 12(7.05)             | 44(25.88)         |
| Vancomycin (30Mg) | 30           | 2(6.66)          | -                    | 28(93.33)         |

**DISCUSSION**

**Correlation of urinalysis:**

Sometimes, we found pus cells present in urinalysis with negative culture, this could be found in patient already on antibiotics, or in a tuberculosis patient (a common cause of sterile pyuria), in patient who has a cute glomerulonephritis (autoimmune disease), urinary tract calculi and trauma secondary to urinary tract instrumentation.

On contrary: presence of positive culture without pus cells in urinalysis could be found in Proteus infection that liquefies the pus cells (Alkaline), or in immunosuppressed patients and unreliable sample contamination. In urinalysis nitrite negative sample is not always a reliable sign of absence of UTI because only Gram- Negative bacilli can give positive nitrite test. Gram- positive cocci do not produce nitrite.
Also leukocyte esterase in urinalysis is not always indicative of UTI; it may be due to vaginitis and vulvitis.

Culturing urine remains the gold methods for diagnosing UTI infections [21]. Some time, the routine culture appears negative, but the child got symptoms or the gram stain showed bacteria. In this case, an anaerobic culture should be done [22]. In our study, we got a negative culture in about half of our isolates; this could be belonging to anaerobic bacteria, which is out of our research interest. Therefore, further studies should be done to investigate.

If the urine culture yields unusual bacteria or multiple bacteria in an uncontaminated urine specimen, immunodeficiency and malformation of the kidney and urinary tract should be excluded. The appearance of Streptococcus agalactiae (group B) in neonate's urine culture is mostly due to intrapartum infection from the birth canal; while Candida albicans may cause UTI especially after instrumentation of the urinary tract. Pseudomonas aeruginosa could be isolated from urine culture of kidney- dialysed or in immunocompromised children [3]. Salmonella spp. could be isolated from urine of children with typhoid fever infection.

In addition, we must keep in mind the technical errors that influence our AST results (i.e the size of inhibition zone) that can be avoided as:

a. Inoculum density.

b. Composition and depth of agar medium.

c. Potency and spacing of the antimicrobial discs.

d. Temperature and time of incubation [19, 20].

Naldixic acid antimicrobial disc used for Gram- Negative bacilli isolates only, while vancomycin used for Gram- Positive cocci only. Increasing antibiotic resistance occurs because of irrational and irregular use of antibiotics, not waiting for the result of culture and sensitivity in such a low socioeconomic status area [23].

CONCLUSIONS

In conclusion, Gram- Negative bacilli were mainly responsible for UTI, and the most frequent causative agent was slant; and the highest resistance rates were against naldixic acid, cefotaxime and cefepime in children with UTI that were tested in this study. Fewest rates of resistance were found against Imipenem, Amikacin and ciprofloxacin.

The most striking finding in our study is the alarming prevalence of multidrug resistant strains of Escherichia coli. The finding is similar to study which was don [15, 23]. Therefore, the choice of antibiotic therapy in UTI should depend on the local sensitivity pattern of the infecting organism. We recommend more researches on ESBL (Extended Spectram B- Lactamase) producing organisms. Ultrasound or CT scan is recommended for patients who are not responding to treatment to exclude urinary tract abnormalities.

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