Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 32, Issue 1, 2021 DOI: http://doi.org/10.23851/mjs.v32i1.948

Research Article **Open Access**

Causative Microorganisms and Antibiotics Susceptibilities in Children with Urinary Tract Infection

Thaer M. Al-Baqer[1], Samar Abdul Raheem Al-Gharrawi[2*], Noor AL Huda A. Saeed[3]

[1] Ibn Al-Balady Maternity & Children's Hospital, Ministry of Health Baghdad, IRAQ.

[2] Department of Basic Science, College of Dentistry, Mustansiriyah University, Baghdad, IRAQ.

[3] Branch of Zoology, Department of Biology, College of Science, Mustansiriyah University, Baghdad, IRAQ.

*Correspondent contact: dr.samarshaghati_bio@uomustansiriyah.edu.iq

Article Info

Received
04/11/2020

Accepted
27/12/2020

Published
20/02/2021

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.

الخلاصة

بصوره عامه ،يعد علاج عدوى المسالك البولية (UTI) لدى الاطفال مهمة صعبة ، بسبب ازياد المقاومه للمضادات الحيوية.

تهدف هذه الدراسة من التحري عن المسببات المرضية ومدى مقاومتها للمضادات الحيوية لدى الاطفال الذين يعانون من التهاب المسالك البولية في بغداد. تم جمع ٨١٠ عينه ادرار من الحالات المشتبه باصابتها بالتهابات المسالك البولية لدى الاطفال المرضى باعمار (١ يوم الى ١٢ سنه) من كلا الجنسين. تم فحص عينات الادرار وزراعتها لغرض عزل العوامل المرضية المسببة. تم الحصول على ٢٠٢ حاله موجبه للزرع البكتيري (٢٤،٣٩٪) من مجموع العينات ٨١٠ . تم عزل العديد من العوامل الممرضه والتي شملت : آ) العصيات السالبه لصبغه جرام والتي تتضمن:

Escherichia coli (41.58%), *Enterobacter spp*. (13.68%), *Proteus mirabilis* (12.78%), *Acineto-bacter 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 الموجبه لصبغه جرام : *Staphylococcus aureus (11.38%), Enterococcus faecalis (1.98%)*

and streptococcus agalactiae (1.48%).

ج) الخمائر .(0.9%) *candida albicans*

كانت معظم العزلات مقاومه لمضادات Nalidixic acid (67.05%) و cefotaxime (59.40%) و cefepime (50.49%)

واضهرت الدراسه ان البكتريا السالبة لصبغة جرام كانت الاكثر شيوعا لاصابه المسالك البولية لدى الاطفال.فيما اضهرت العزلات حساسيه لمضادات Imipenemو Amikacin و ciprofloxacine . لهذا يجب الاعتماد على اختبارات الحساسية للكائن المسبب في اختيار العلاجات الملائمة.

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 nitrites 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*., *Streptococcu*s 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 Enterobacteriacea). 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 35c°, 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 (1month-1 year) with a higher occurrence in females (Table 1).

Table1. Age and sex distribution of UTI cases of positive isolates of microorganisms (202 isolates).

Age group	Age group cases No. (%)	Females No. (%)	Males No. (%)
Newborn (< 1 month)	10(4.95)	8(3.96)	2(0.99)
Infant (1month- 1year)	56(27.72)	30(14.85)	26(12.87)
Toddler (1year – 3 year)	34(16.83)	24(11.88)	10(4.95)
Preschool (3year- 6year)	52(25.74)	42(20.79)	10(4.95)
School age child(6year– 12year)	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.

Table2. Frequency of isolated microorganisms in children with urinary tract infection (202 isolates).

Microorganism	Frequency No.	%
Gram negative bacilli	**(170) isolates**	**84.15**
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	**(30) isolates**	**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(89.60)
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.

REFERENCES

[1] Thomas K. McInery,T.K.,Adam, H.M., D.E.Campbell, T. G. Dewitt, J. M. Foy, D. M. kamat. AAP Textbook of pediatric care, 2d Ed, 2016.

[2] E. D. Balighian and M.G. Burke. Urinary tract infections in children. Pediatrics in Review, Vol .39, No 1, P. 3-12, 2018.

[3] S. L. Chang and L. D. Shortiffe. Pediatric urinary tract infections. Pediatr Clin N Am. Vol 53, No3, P 379-400, 2006.

[4] E. J. S. Nelson. Textbook of pediatrics. In: Behrman RE, Kleigman RM., Jenson HB, editors. Urinary tract infection and vesicoureteral reflux 19th ed. Philadelphia: Saunders. Elsevier. PP.1829-1830, 2011

[5] K. Tullus and N. Shaikh. Urinary tract infections in children. Lancet.,Vol 395, No10237, P.1659-1668, 2020.

[6] N. Shaikh and A. Hoberman. Urinary tract infections in children: Epidermiology and risk factors. In: Post T.W, editor. Up-to-date. Waltham.AM. 2010

[7] K. B. Roberts. Revised AAP Guide line on Urinary Tract Infections in Febrile infants and young children. Am Fam Physician. Vol 86, No10, P. 940-946, 2012.

[8] World Health Organization. Urinary tract infections in infants and children in developing countries in the context of IMCI. WHO-FCH-CAH-05.11.pdf.

[9] K.B. Roberts. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics. Vol 128, No3, P.595-610, 2011.

[10] B.R. Raghubanshi, D. Shrestha, M. Chaudhary, B.M.S.Karki and A.K. Dhakal .Bacteriology of urinary tract infection in pediatric patients at KIST medical college teaching hospital. J. Kathmandu Med. Coll., vol. 3, no. 1, P. 21-25, 2014.

[11] L. B. Shrestha, R. Baral and B. Khanal. Clinical, etiological and antimicrobial susceptibility Profile of

Pediatric urinary tract infections in a tertiary care hospital of Nepal. BMC Pediatrics, Vol 19, No 1, P. 36, 2019.

[12] M. Abuhandan, B. Guzel and H. Ciftci. Antibiotic sensitivity and resistance in children with urinary tract infection in Sanliurfa. Turkish Journal of Urology. Vol 39, No. 2, P.106-110, 2013.

[13] J. E. Yoon, W. K. Kim, J. S. Lee, K. Shin and T. HaAntibiotic susceptibility and imaging findings of the causative microorganisms responsible for acute urinary tract infection: a five –year center study. Pediatr. Vol. 54, No. 2, P. 79-85, 2013.

[14] A.A. Paschke, T. Zaoutis, P.H. Conway, D. Xie and R. Keren. Previous antimicro-ial exposure is associated with drug-resistant urinary tract infections in children. Pediatrics. Vol. 125, No. 4, P.664-672,2010.

[15] S. Farshad, R, Ranjbar and M. Anvarinejad. Emergence of multidrug resistant strains of Esherichia coli isolated from urinary tract infections. The open conference Proceedings: Journal 1 (1) P.192-196, 2010.

[16] J. L. Robinson, J. C. Finlay, M. E. Lang, R. Bortolussi. Urinary tract infections in infants and children: Diagnosis and management. Paediatr. Child Health. Vol 19, No 6, P.315-325, 2014.

[17] J.J Zorc, D.A. Kiddoo, K. N. Shaw. Diagnosis and management of Pediatric Urinary Tract Infections. Clin. Microbiol. Rev. Vol 18, No 2 P. 417-422, 2005.

[18] J. Vandepitte, J. Verhaegem, K. Engbaek, P. Rohner, P. Piot, C. C Heuck. World Health Organization Geneva. Basic Laboratory Procedures in clinical Bacteriology 2nd edition P 103-120.2003.

[19] National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial susceptibility testing; Twelfth Informational Supplement. Approved Standared M100-S12.Wayne PA: NCCLS; 2002.

[20] V. Bhat and H. Vira. (2018). Quality Control Issues in Antibiotic Susceptibility Testing by Disc Diffusion Technique. Clin Infect Dis 2: 104.2018.

[21] G.M Stephens, S. Akers, H. Nguyenand H. Woxland. Evaluation and management of urinary tract infections in the school-aged child. Prim Care, Vol. 42, No.1, P.33-41, 2015.

[22] H.D. Marston, D.M. Dixon, J.M. Knisely, T.N. Palmore and A.S.Fauci. Antimicrobial Resistance. Jama, Vol 316, P. 1193-1204, 2016.

[23] A. Leung, A. Wong, A. Leung and K.L. Hon. Urinary Tract Infection in Children. Recent Pat Inflamm Allergy Drug Discov. Vol 13, No 1, P.2-18, 2019.