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Isolation and Identification of bacteria causing urinary tract infections in children in Kirkuk city

Nidaa wasmy Shahab[1] , Chatin Iz Al-Din Ali[2] , Sabah Mohammed Salih[3]
[1] *Department of Biology , College of Science , Tikrit University , Tikrit , Iraq*
[2] *College of Dentist , Tikrit University , Tikrit , Iraq*
[3] *College of Nursing , Kirkuk University , Kirkuk , Iraq*

Abstract
Urinary tract infection is an important infection which can cause a highly morbidity rates in boys and girls .The aim of the present study was to isolate and Identify the some types of bacterial causes of pediatric UTI in Kirkuk city. A total number amount to 105 urine samples from children suspected urinary tract infections of age group 4 days -10 years old were collected from both in Pediatric General Hospital and Kirkuk General Hospital in Kirkuk city during June to September 2015, 40 (38.09%) bacterial isolates were obtained which included *E.coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosae, Staphylococcus aureus, Staphylococcus epidermidis*. Results of the study showed that of females had UTI more than the males which accounted for 55.23% compared to 44.79%. The infection rate among younger age groups was higher than with the older groups, as well as the results of the study showed the sensitivity of Gram negative bacteria against azithromycin as well as cefataxim absolute sensitivity(100%) while the Gram positive bacteria was showed absolute sensitivity (100%) to wards Novabiocin.

Introduction
Urinary tract infections are caused by invasion of pathogenic microorganism into the urinary tract. The morbidity rate of pediatric UTI in boys and girls is 1.1%and 3% respectively. The prevalence of UTI in male and female children younger than 6 years old is 1.8% and 6.6% respective[1,2]. Urinary tract infection is classified into three types :acute pyelonephritis ,lower UTI and asymptomic bacteruria [3,4] . More than 95% of urinary tract infections are caused by a single bacterial species. *Escherichia coli* is the most frequent infecting organism in acute infection[5]. Other organisms that can be responsible for UTIs include Gram positive cocci, such as *Enterococcus faecalis, Staphylococcus aureus* and coagulase negative staphylococci (CoNS). Other Gram negative organisms responsible for causing UTIs include *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa* and *Enterobacter* species[6]. May also include fungal and viral infection, Urinary tract infection is defined as significant number ($\geq 10^5$) Colony Forming Units (CFU) ⁄ml of pathogenic organism in the urinary system[7,8]. The present study was conducted to isolate and identify the some types of bacterial cause of pediatric UTI in Kirkuk city.

Material and methods
Culture media
MacConkey agar, Blood Base agar ,Muller hinton agar, mannitol salt agar and Nutrient agar (Oxoid, England),were used for isolation, identification and determination of antibiotic sensitivity used 10 types of antibiotics.
All these media were prepared according to manufacturer instructions, sterilized by autoclaving at 121ºC for 15 minutes[9].

Study groups
Urine sample were collected from children with suspected of UTI according to signs and symptoms e.g fever, vomiting that belonged to different geographical areas in Kirkuk city were admitted to pediatric General Hospital and Kirkuk General Hospital in Kirkuk during the period from June to September 2015. They consisted 105 sample from urine collected of children where was 105 children with UTI.

Sample collection
Urine samples were collected by two ways .Those who are toilet trained midstream urine were collected into sterile wide mouth screw-cap 10ml containers, and those who are not toilet trained (infants under two years) adhesive bag were used for urine collection. One sample was taken from each children[10].

Bacterial isolates and Identificantes.
Urine samples were immediately transferred into MacConkey agar, Blood agar and mannitol salt agar which considered as selective and differential medium for the isolation, purification and identification of Enterobacteraceae and Staphylococci, and for detecting the ability of each isolate to ferment lactose and mannitol Then transferred and streaked on the blood agar , the isolates were examined for their shape, size, colour, pigments, and haemolytic activity. All plates were incubated at 37ºC for 24 hours then a single pure isolated colony was transferred to nutrient agar medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates[11,12].

Antimicrobial Susceptibility Test
This test performed by modified Kirby-Bauer method as the following
1- From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up by
sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the
turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard .

2- A sterile swab was dipped into the bacterial suspension, any excess fluid was expressed against the side of the tube.
3- The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows: The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.
4- By a sterile forceps the antimicrobial disc was picked up and placed on the surface of the inoculated plate. The disc was pressed gently into full contact with the agar. All plate content 5 disc.
5- The step (4) was repeated to all antimicrobial discs under the test, spaced evenly a way from each other.
6- The plates were incubated at 35ºC for 18-24 hours.
7- After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) a round the antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc. If a zone of inhibition surrounded the disc, the diameter of the zone of inhibition was measured (by millimeters) and compared their sizes with values listed in a standard chart[13].

Results

Bacterial growth of urine samples

One hundred five of the children's specimens were suspected patients with UTI according to diagnosis made by physician, where in which 40(38.09%)of specimens was confirmed bacterial infection with positive urine culture while 35(33.33%)of specimen was confirmed bacterial infection with negative urine culture and 30(28.57%)non-confirmed UTI .

Table1:Bacterial growth of urine samples

Result of urine culture	Children with UTI	
	NO.	%
Confirmed bacterial infection with positive bacterial culture	40	38.09
Confirmed bacterial infection with negative bacterial culture	35	33.33
Non-confirmed UTI	30	28.57
Total	105	100

Types of Isolated Bacteria Among The Study Groups

In the present study 40 isolates of Gram negative and Gram positive bacteria detected in urine specimens from study groups, out of 40(38.09%) specimens positive bacterial culture 9(22.5%) were Gram positive bacteria, while 31(77.5%) specimens were Gram negative. As summarized in Table 2.

Table 2: Types of isolated bacteria among study groups

Results of urine culture	Children with UTI	
	No.	%
Gram Positive Bacteria	9	22.5
Gram Negative Bacteria	31	77.5
Total	40	100

Distribution of isolated bacteria among study groups

The highest prevalence of specific bacteria was found to be *E. Coli* recorded among all the pediatric UTI to be (35%) the least percentage among the bacteria were *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosae, Staphylococcus aureus ,Saphylococcus epidermidis* which recorded 30%, 7.5%, 5%, 15% and 7.5% respectively.

Table 3: Distribution of isolated bacteria among study groups

Isolated Bacteria	Positive UTI cases	
	Children with UTI	
	NO.	%
E.coli	14	35
Klebsiella pneumonia	12	30
Proteus mirabilis	3	7.5
Pseudomonas aeruginosae	2	5
Staphylococcus aureus	6	15
Staphylococcus epidermis's	3	7.5
Total	40	100

Positive UTI Among children with UTI according to age groups

In the present study the ages of the patients ranged between 4 days-10 years old. As shown in Table 4, the highest rate of bacterial growth among children with UTI was found the age group 4 days -2 years old which constituted 29.53%(17/31). The lowest rate was within the age group of 6-8 years old which constituted 10.47% (4/11).

Table 4: Distribution of positive urine culture among children with UTI.

Age group (4days-10years)	Positive urine culture cases		
	Children with UTI		
	Total No.	Positive urine culture	%
4days-2years	31	17	29.53
2-4years	18	5	17.14
4-6years	26	8	24.76
6-8years	11	4	10.47
8-10years	19	6	18.09
Total	105	40	100

Distribution of UTI according to gender

Table(5)showed the distribution of urinary tract infection according to gender. It was found that the rate of females with UTI were higher 55.23% in comparison with males 44.79%.

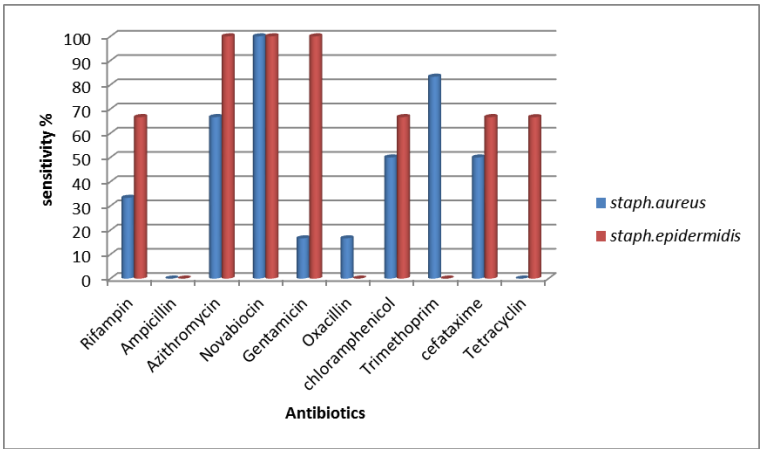
Table 5: Distribution of according to gender

Children Gender	Children with UTI	
	No.	%
Males	47	44.79
Female	58	55.23
Total	105	100

Distribution of Antibiotics Sensitivity of Gram Positive Bacteria Isolated From Children with UTI.

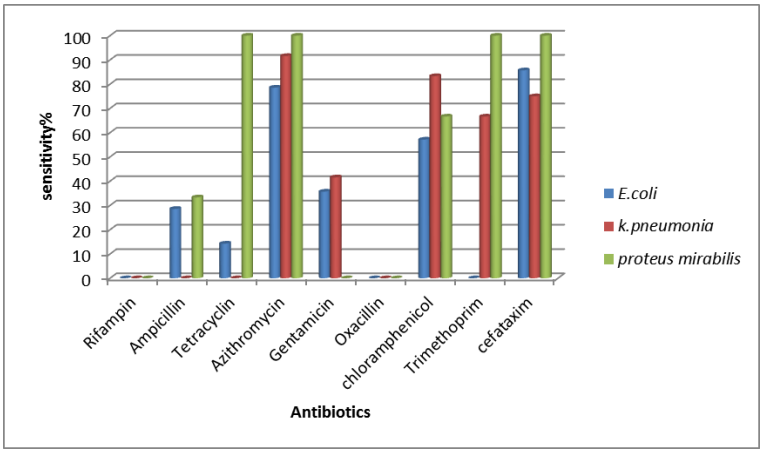
In this study *S. aureus* showed high rate of sensitivity to novabocin 100% (Figure1). *Staphylococcus*

epidermidis showed high rate of sensitivity to gentamicin, azithromycin and novabocin (100%) .



Figure(1):Antibiotics sensitivity of Gram positive bacteria isolated from children with UTI.

Distribution of Antibiotics sensitivity of Gram negative bacteria isolated from children with UTI. *Escherichia coli* showed high rate of sensitivity (85.71%) (Figure 2). *Klebsiella pneumoniae* showed high rate of sensitivity (91.6 %) to azithromycin (Figure 2). *Proteus mirabilis* showed high sensitivity rate to cefotaxim and trimethoprim and azithromycin (100%) (Figure 2).*Pseudomonas aeroginosae* showed high sensitivity rate to azithromycin(100%).



Figure(2)Distribution of Antibiotics sensitivity of Gram negative bacteria isolated from children with UTI.

Discussion

Urinary tract infection is one of the most common infection in different countries of the world and that human infects various age groups, and that 95% of these infection caused by many types of bacteria [14], the results of the current study because the prevalence in the present study groups of various bacteria was (38.09%)as shown in the Table 1 because the reasons for the emergence of the outcome of these few due to an error occurred during the sampling ,may be an error occurred during the preparation of culture media,or media inappropriate appearance of sorts isolation of different bacteria. The current search results shown in the Table 2 that the percentage of samples that gave a negative growth for culture bacteriological have been (77.5%) Gram negative bacteria which is higher than the percentage of samples positive for growth (22.5%) and the high rate of bacterial growth that emerged in this study may return to the continuous use of antibiotics indiscriminately and without consulting a specialist doctor, which led to making the strains bacterial resistance in spite of the production of new generations of antibiotics [15] as well as the failure to follow the correct methods in terms of cleanliness. Showing through the diagnostic results, which included tests species bacterial positive and negative Gram stain as shown in Table 3 in the case of infection of the urinary tract infection, the *E. coli* accounted for the highest percentage was isolated by (35%) that the result obtained by came approval of the results of a number of studies that have pointed to the sovereignty of *E. coli* in the cause of infections at the urinary tract, including the study [16] and attributed the cause of the high prevalence of this bacteria to being one of the natural bacteria present in the human gut and be infection, most often endogenous either bacteria *klebsiella pneumonia* has isolated by (12) isolation at a rate of (30%) this

approach to the result obtained by the result [16] in the fact that these bacteria come in second place after any *E. coli* in its events for the infection of the urinary tract and has isolated the bacteria *Proteus* third place where the bacteria *Proteus mirabilis* isolated by (7.5%) also it isolated the bacteria *Staphylococcus aureus* (15%) by 6 isolated this is disagreement with the results that came out [17] as shown by the results a few percentages of the types of infection bacteria positive and negative to Gram stain *pseudomonans aeruginosae, staphylococcus epidermidis*. From Table4,it is clear that UTI was more common first age group(4days-2years) since, 31(29.53%) from 105 children who had UTI were at this age group .The distribution of UTI decreased with increasing of age group, being (17.14%), (24.76%), (10.47%) and (18.09%) in age groups respectively. This result agrees with that obtained by [18] who found that (48%)of children who had UTI were (under 1 year) reported that age below 1 year is more affected with UTI. Then the infection decreases above one year, to fourth year, it became less above fourth years[19] found that the higher percentage with UTI in children was in infant at age 2 month to 2 year .The main reasons due to infant born with an immature immune system that doesn`t fully develop until several years after birth. Approximately 75% of UTIs occur in the first 2years of life .The first peak of UTI is in the first year of life ,and the second peak of UTI occurs between the ages of 2 to 10 years during toilet training [20]. From Table 5 shows that the prevalence of infections urinary tract were higher in females, a rate of (55.23%) compared with males (44.79) and attributed the cause of the high prevalence in females to anatomical differences between the sexes and the short length of the urethra in females the bladders of the director makes it more prone to urinary tract infections[21]. The antibiotic is of paramount importance in the treatment of urinary tract infections, and through Sensitivity tests to antibiotics for the purpose of identifying the extent of bacterial sensitivity under study observed that negative and Gram positive bacteria to showed variation in the extent of sensitivity also indicates that the Figure[1,2], as illustrated by this figure that the highest rate of sensitivity shown by the Gram positive bacteria reached (100%) direction novabiocin, azithromycin and gentamicin. While the rate was lower oxacillin was (16.6%) to the antibiotic, rate higher proportion of sensitivity shown by Gram negative bacteria (100%) direction azithromycin and cefataxime and reached the lower rate (14.28%) direction tetracyclin are the best and can be recommended azithromycin and novabiocin these results that antibiotic using them in the treatment of urinary tract inflammation in case of infection such bacteria. why perhaps back low resistance to these bacteria to lack of exposure to resist these bacteria to lack of exposure to the two types of antibiotics on the contrary Other antibiotics used on a large scale and for a long time [22,23,24].

References

1. Hansson S, Jodal U(2004). Urinary tract infection. In: Avner ED, Harman WE, Niavdet P, editors. Pediatric nephrology. 5th ed. Philadelphia: Lippincott Williams & Wilkins:1007-25.

2. Elder JS (2011). Urinary tract infection. In: Kliegman RM, Stanton BF,St. Geme JW, Schor NF, Behrman RE, editors, Nelson textbook ofpediatrics. 19th ed. Philadelphia: Saunders:1829-38.

3. Riccabona M(2003). Urinary tract infections in children. Curr Opin Urol;13:59-62.

4. Buonsenso D, Cataldi L(2012). Urinary tract infections in children: a review.Minerva Pediatr :64:145-57.

5- Baraboutis H, Tsagalou N, Lepinski A, *et al*(2011). Primary *Staphylococcus aureus* urinary tract infection:the role of undetected hematogenous seeding of the urinary tract. *Eur J Clinical Microbiol Infect* ;12: 1095-1101.

6- Hryniewicz K, Szczypa K, Sulikowska A, Jankowski K, Betlejewska K, Hryniewicz W(2001). Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *J Antimicrob Chemother* ; 47:773—780.

7- Schnarr J, Smaill F(2008). Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. *Eur J Clin Invest* ; 38 (2): 50–57.

8- Agarwal P J(2012). Symptom-based diagnosis of urinary tract infection in women: are we over-prescribing antibiotics?. *Int J Clin Pract* ; 6:493–498.

9. Morello, J.A.; H.E. Mizer; and P.A. Granato (2006). Laboratory Manual and Work book in Microbiology Applications to Patient Care. 18th.ed. The McGraw-Hill Companies , Inc.,New York.95-99.

10-Ghanghro,A.B.,and Laghari, A.H.(2010). Urinary Tract Infection as a predictor of childhood malnutrition in southern Sindh ,Pakistan Journal of Nutrition,9:819-821.

11-Atlas, M.R. (2005). Handbook of media for Invironmental microbiology.2nd ed. Taylor& Francis publisher. New York 33-43.

12- Susan R. Heimer and Harry L.T. Mobley (2001). Intraction of protease mirabilis urease Apoenzyme and Accessory proteins Identified with yeast two Hybrid Technology" . J. Bacteriol, 183 .1423-1433 .

13. Vandepitte, J.; J. Verhaegen; K. Engbaek; P. Rohner; P. Piot; and C.C. Heuck. 2003.Basic Laboratory Procedures in Clinical Bacteriology. 2nd.ed. World Health Organization, Geneva.

14- Cynthia C(2001).General Description of Urinary tract infection" NidusInformation services , Inc. 41 East 11 the street , 11 the floor .

15.Nasciment,G.G.F.;Locatelli,J.Freitas,p.c.andsilva, G.L.(2000).Antibacterial activity of plant extracts and

phytochemicals on antibiotic – resistant bacteria, Braz. J. Microbiol., 31(4).
16-Sonavane, A.; Mathur, M.; Turbadkar, D. and Baradkar v. (2008). Antimicrobial susceptibility pattern in urinary bacterial isolates. Bombay Hospital Journal, 50(2):240-244.
17-Al-Geborry, G.M. (2009). Etiology of bacterial pathogens caused urinary tract infections in children of AL-Nasseria city .Al-Nassiriya Technical Institute.
18-Rupinder, C.S.; Mc Neeley D.F., and Mehta, M.(2012). Prevalence of urinary tract infections in children and their sensitivity to various antibiotics . J. Acad. Indus.Res.1(4)161:2278-5213.
19-Herr, S.M.; Wald, E.R., Pitetti, R.D., and Choi, S.S. (2004). Enhanced urinalysis improves identification of febrile infants ages 60 days and younger at low risk serious bacterial illness. pediatrics. 108.866-879.
20-Keren, R.; Carpenter, M.A., and Hoberman A., etal. (2008). Rationale and design issues of the randomized intervention for children with vesicoureteral Reflux (RIVUR) study. Pediatrics.; 122, 240-250.
21-Inabo, H.I. and Obanib, H.B. (2006). Antimicrobial susceptibility of urinary tract clinical isolates to commonly used antibiotic. African Journal of Biotechnology,(5):487-489.
22- Naylor GH(1998).A16 month analysis of Urinary tract infection in children" Journalof Medical Microbiology, 17 (1). 31-36 .
23- Forbes, B.A; Sahm, D.F. and Weissfeld, A.S., Bailey and Scotts (1998). Diagnostic Microbiology, 10 the ed Mosby.
24- Najah A. Mohammed, Abdalaa L. Jiad (2004).A bacteriological study of urinary tract infection in Baquba city" , J. Technology, 7 (4) .

عزل وتشخيص البكتريا المسببة لاخماج المجاري البولية في الاطفال في مدينة كركوك

نداء وسمي شهاب[1] ، جتين عز الدين علي[2] ، صباح محمد صالح[3]

[1]قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق
[2]كلية طب الاسنان ، جامعة تكريت ، تكريت ، العراق
[3]كلية التمريض ، جامعة كركوك ، كركوك ، العراق

الملخص

اخماج السبيل البولي هي من الاصابات المهمة التي باستطاعتها ان تسبب معدل عالي اعتلالات عند الاولاد والبنات .هدفت هذه الدراسة الى عزل وتشخيص بعض انواع البكتريا المسببة لاخماج السبيل البولي لدى الاطفال في مدينة كركوك بلغ العدد الكلي للعينات 105عينة ادرار من الاطفال مصابين باخماج السبيل البولي والذي تراوحت اعمارهم بين 4 يوم–10 سنة تم جمع العينات من الاطفال المراجعين مستشفى الاطفال العام ومستشفى كركوك العام في مدينة كركوك خلال فترة من حزيران الى ايلول عام 2015 ,وتم الحصول على 40(38.09%) عزلة جرثومية منها جرثومة *E.coli* ,*Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* , *Staphylococcus aureus* , *Staphylococcus epidermidis* .كذلك اظهرت نتائج الدراسة ان فئة الاناث المصابات باخماج السبيل البولي اكثر من فئة الذكور اذ شكلت الاناث نسبة 55.23% مقابل 44.79% للذكور كما ان معدل الاصابة لدى الفئات العمرية الصغيرة كان اعلى مما هو عليه لدى الفئات العمرية الكبيرة , كما اظهرت نتائج اختبار حساسية البكتريا السالبة لصبغة كرام اتجاه azithromycin وكذلك cefataxim بشكل مطلق (100%) في حين كانت الجراثيم الموجبة لصبغة كرام حساسة بشكل مطلق (100%) عند استخدام novabiocin .