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

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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%) bacteria isolates were obtained which included E. coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, 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 cefatimox 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% respectively[1,2]. Urinary tract infection is classified into three types: acute pyelonephritis, lower UTI and asymptomatic 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 a significant number (≥10³) 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 45° angle and streaked the whole surface again; finally the plate was rotated another 90° and streaked one 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 35°C for 18-24 hours.
7. After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) around 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.

Table 1: Bacterial growth of urine samples

<table>
<thead>
<tr>
<th>Result of urine culture</th>
<th>Children with UTI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed bacterial infection with positive bacterial culture</td>
<td>40</td>
<td>38.09</td>
</tr>
<tr>
<td>Confirmed bacterial infection with negative bacterial culture</td>
<td>35</td>
<td>33.33</td>
</tr>
<tr>
<td>Non-confirmed UTI</td>
<td>30</td>
<td>28.57</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>100</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Results of urine culture</th>
<th>Children with UTI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive Bacteria</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td>Gram Negative Bacteria</td>
<td>31</td>
<td>77.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

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 aeruginosa, Staphylococcus aureus, Saphylococcus epidermidis which recorded 30%, 7.5%, 5%, 15% and 7.5% respectively.

Table 3: Distribution of isolated bacteria among study groups

<table>
<thead>
<tr>
<th>Isolated Bacteria</th>
<th>Positive UTI cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children with UTI</td>
</tr>
<tr>
<td></td>
<td>NO.</td>
</tr>
<tr>
<td>E.coli</td>
<td>14</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>12</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

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%(17/51). 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

<table>
<thead>
<tr>
<th>Age group (4days-10years)</th>
<th>Positive urine culture cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children with UTI</td>
</tr>
<tr>
<td></td>
<td>Total No.</td>
</tr>
<tr>
<td>4days-2years</td>
<td>31</td>
</tr>
<tr>
<td>2-4years</td>
<td>18</td>
</tr>
<tr>
<td>4-6years</td>
<td>26</td>
</tr>
<tr>
<td>6-8years</td>
<td>11</td>
</tr>
<tr>
<td>8-10years</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
</tr>
</tbody>
</table>

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 UTI according to gender

<table>
<thead>
<tr>
<th>Children Gender</th>
<th>Children with UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Males</td>
<td>47</td>
</tr>
<tr>
<td>Female</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
</tr>
</tbody>
</table>

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% (Figure 1). 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 (100%) (Figure 2). Proteus mirabilis showed high sensitivity rate to cefotaxim and trimethoprim and azithromycin (100%) (Figure 2). Pseudomonas aeruginosa 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 (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 aeruginosa, staphylococcus epidermidis. From Table4, it is clear that UTI was more common first age group (4 days-2 years) since, 31 (29.53%) from 105 children who had UTI were at this age group. The distribution of UTI decreased with increasing age of 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 2 years 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 novobiocin, azithromycin and gentamicin. While the rate was lower oxacinill was (16.6%) to the antibiotic, rate higher proportion of sensitivity shown by Gram negative bacteria (100%) direction azithromycin and cefatxime and reached the lower rate (14.28%) direction tetracycline are the best and can be recommended azithromycin and novobiocin 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
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عزل وتشخيص البكتيريا المسببة لاخماج المجاري البولية في الأطفال في مدينة كركوك

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الملخص

اخماج السبيل البولي ينتمي إلى أولاد والبنات. صُوْفَّت هذه الدراسة لعزل وتشخيص بعض البكتيريا المسببة لاخماج السبيل البولي لدى الأطفال في مدينة كركوك. بلغ العدد الكلي للعينات 105 لعنة إدار ومن الأطفال مصابين بإخماج السبيل البولي وذل الامام، بين مروم 4-10 سنة، ثم جمع جميع البيانات من الأطفال المراجعين مستشفى الأطفال العام وإستشفائي كركوك العام في مدينة كركوك خلال فترة من حزيران 2015، 40% (30.03) مصابة من بينها Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, E.coli جرثومة منهما كذلك نُيِّرت نتائج الدراسة أن فئة الإناث المصابة باخماج السبيل البولي أكثر من فئة الذكور. شكلت الإناث نسبة 55.23% مقابل 44.79% للذكور كما ان معدل الإصابة لدى الفئات العمرية الصغرى كان أعلى ما هو عليه لدى الفئات العمرية الكبيرة، كما اظهرت نتائج اختبار حساسية البكتيريا السالبة لصبغة كرام اتجاه azithromycin وكذالك novabiocin في 100% عند استخدام

الإلمام