Prevalence and Antimicrobial Susceptibility of Gram-negative Bacteria in Pediatric Patients with Urinary Tract Infections in Baghdad Hospitals, Iraq

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Abstract

Children commonly have a Urinary Tract Infection (UTI). To reduce the diseaserelatedto this condition, children need quick diagnosis and suitable treatment. In the present study, out of 336 samples, 122 (36.31%) were positive togrow pathogenic strains, while 214 (63.69%) were negative. Also it was found that the most of positive cultures of uropathogens were detected among female's patients (41.79%). According to the age group, the present study revealed that the age group (1-5 years) was included the highest prevalence (37.71%) followed by the group (<1year) (30.33%). Among the 122 pathogenic isolates, 91 (74.6%) were Gram-negative, 31 (25.5%) were Gram-positive. The studied data revealed that *Escherichiacoli*(43.95%)was the commonly isolated bacterium, followed by *Proteus mirabilis* (28.6%), *Klebsiellapneumoniae* (13.2%), *Enterobacter cloacae* (5.5%),*Acinetobacterbaumannii* (4.4%), *Pseudomonas aeruginosa*(4.4%).With reference to gender, the gram negative uropathogenic bacteria for women and men were 60 (65.9%) and 31 (34.1%), respectively. The results demonstrated that all gram-negative bacterial isolates exhibited a high resistance to Ampicillin (90.1%) and Cefotaxime (80.2%), while the lower resistance showed with Tigecycline (5.5%), Amikacin (15.4%), Ciprofloxacin (27.5%), Trimethoprime/sulphomethoxizole (27.5%), Levofloxacin (31.9%), Meropenem (36.2%) and Tetracycline (37.4%), which mean that Tigecycline was the effective antibiotic against the local gram-negative bacteria isolated from pediatric patients. In our study, drug resistance in these pathogens is associated to the commonlytaken antibiotics which is a troublesome finding, compromising therapeutic options in the clinical practice and lead to the use of agents with fewer effects against bacteria.

Keywords:Antibiotic resistance, Children, Gram-negative bacteria, UTI.

Introduction

Urinary Tract Infection (UTI) is one of the most common bacterial infections in childhood (Korbel*et al*. 2017). In developed countries, it is one of the most frequent infectious diseases experienced by clinicians. Every year, 150 million people are infected with UTI, leading to approximately $6 billion in health-care costs. In 1997, nearly 7 million office visits and 1 million emergency room visits were attributed to UTI, resulting in 100,000 hospital stays in the U.S. (Verrier-Jones *et al*.,2007). Urinary tract infections (UTIs) are a serious international medical issue caused by a variety of bacteria, the most prevalent of which are gram negative bacteria such as E. coli and Klebsiellapneumoniae, Proteus mirabilis. High incidence rates and rising antimicrobial tolerance among uropathogens pose a significant challenge to the financial impact of these pathogens (Flores-Mireles *et al*., 2015; Ghaima*et al*., 2018).

The virus has the potential to damage the upper urinary tract (pyelonephritis). Unfortunately, distinguishing pyelonephritis from cystitis based on clinical symptoms and signs can be complicated, if not impossible, particularly in babies and young kids (Balighian and Burke.,2018). These diseases are a major source of concern for infants, caregivers, and physicians. Prompt diagnosis and care are critical for reducing disease burden related to this disorder (Awais*et al*., 2015). *Klebsiellapneumonia*, *Escherichia coli* and other gram-negative bacteria have demonstrated the capacity to develop plasmids encoding Extended Range Beta-lactamase genes and thus become highly immune to antibacterial drugs and a broad spectrum of 3rd generation cephalosporins in hospitals and communities. (Mazzariol*et al.*, 2017).

The purpose of this study is to investigate the prevalence of gram negative bacteria among children with

urinary tract infections in some Iraqi hospitals and evaluate the antibiotic resistance patterns of the local isolates.

MATERIALS AND METHODS

Study Design and Population

This research was conducted at two major hospitals in Baghdad, Iraq, Al-Kindy and Al-Yarmook teaching hospitals, to determine the occurrence of uropathogenic Gam-negative bacteria in urine tests from children with UTIs aged 1 month to 15 years. The study contained all quasi midstream urinary (MSU) samples collected during the study time (September 2019 to August 2020).

Processing of Samples

All MSU samples were cultured using standard culture media. In short, 1μL of urine was streaked onto a MacConkey and Blood agar plate (HiMedia, India) and incubated aerobically at 37 ºC for 24 hours. According to the World Health Organization (WHO) manual, growth of 100 colonies or more, i.e. 105 colony forming units (CFU)/mL urine, was called culture positive (Vandepitte*et al*., 2003). Isolates were isolated and identified based on their morphology in Gram's staining, cultural features, and bioactive compounds, according to the Manual of Clinical Microbiology (Versalovic et al., 2011). The Vitek2 device was used to validate the recognition (bioMérieux, France).

Antimicrobial Susceptibility Testing

Kirby-Bauer's disc diffusion method was used to perform Antibiotic susceptibility testing of all Gram-negative bacterial isolates. Antibiotic discs (MAST, UK) used were Ampicillin (AM), Cefotaxime (CTX), Ceftriaxone (CRO), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Ciprofloxacin (CIP), Levofloxacin (LVX), Piperacillin (PRL), Tetracycline (TE), Tigecycline (TGC), Erythromycin (E) and Trimethoprim / Sulfamethoxazole (TS). To ensure quality control, control strain of *E. coli* ATCC 25922 was used. The results wereinterpreted as described in CLSI 2013.

Results and discussion

Isolates were isolated and identified based on their morphology in Gram's staining, cultural features, and biochemical characteristics (Versalovic*et al*., 2011). A total of 180 clinical specimens of urine collected from Septemper 2019 to August 2020, were cultured on MacConkey agar and Blood agar plates. The isolates that were obtained from these media were identified according to the microscopic examination and biochemical tests.

In this research, an experiment was conducted at 336 urinary specimens. UTIs are identifiedconstructed on patient's clinical symptoms, presence of leukocytes, and bacteria in the urine. Of 336 samples, 122 (36.31%) were positive for growth of pathogenic strains, while 214 (63.69%) were negative (Table 1).

Table 1: The distribution of patients with urinary tract infection in relation to gender.

Gender		Culture results		Total
		Positive	Negative	
Female	No.	84	117	201
	%	41.79%	58.21%	59.82%
Male	No.	38	97	135
	%	28.15%	71.85%	40.18%
Total	No.	122	214	336
	%	36.31%	63.69%	100.00%

Chi-Square	**6.500**
Contingency Coefficient	**.138**
Asymp. Sig.	**.011**

Also it was found that the most of positive cultures of uropathogens were detected among female's patients (41.79%). According to the age group, the present study revealed that the age group (1-5 years) was included the highest prevalence (37.71%) followed by the group (<1year) (30.33%) (Table 2).

Table 2: The Incidence of Urinary tract infections among patients in relation to age group.

Age group (years)	Total	
	No.	**%**
<1year	**37**	**30.33%**
1-5	**46**	**37.71%**
6-10	**22**	**18.03%**
11-15	**17**	**13.93%**
Total	**122**	**100.00%**

Chi-Square	**17.607**
Contingency Coefficient	
Asymp. Sig.	**.001**

Among the 122 pathogenic isolates, 91 (74.6%) were Gram-negative, 31 (25.5%) were Gram-positive (Table 3). The isolated bacterial species of gram negative bacteria belonging to 6 specie were isolated and identified. The studied data showed that *Escherichiacoli* was the commonly isolated bacterium (43.95%), followed by *Proteus mirabilis* (28.6%), *Klebsiellapneumoniae* (13.2%), *Enterobacter cloacae* (5.5%),*Acinetobacterbaumannii* (4.4%), *Pseudomonas aeruginosa*(4.4%)(Table 3).

Table3 : Prevalence of bacterial uropathogens isolated from patients with urinary tract infections.

Bacterial species	No.	Percentage (%)
Gram negative bacteria		
Escherichia coli	40	43.95
Proteus mirabilis	26	28.6
Klebsiellapneumoniae	12	13.2
Enterobactercloace	5	5.5
Acinetobacterbaumannii	4	4.4

Pseudomonas aeruginosa	4	4.4
Total (gram negative)	91	74.6%
Total (gram positive)	31	25.5%
Total	122	100%

Chi-Square	**72.319**
Contingency Coefficient	
Asymp. Sig.	**< .001**

With reference to gender, the gram negative uropathogenic bacteria for women and men were 60 (65.9%) and 31 (34.1%), correspondingly (Table 4). Concerning age distribution of bacterial infection, the main part of bacteria (63.8%) was found in the younger Childs (<5 years), while the lower frequency was detected in the late childhood (11-15 years) (15.4%) as showed in Table (4).

Table 4: The frequency of gram negative bacterial uropathogens among male and female in relation to age group.

Age group (years)	Gender				Total	
	Female		Male			
	No.	%	No.	%	No.	%
<1year	12	20%	10	32.3%	22	24.2%
1-5	24	40%	12	38.7%	36	39.6%
6-10	13	21.7%	6	19.3%	19	20.8%
11-15	11	18.3%	3	9.7%	14	15.4%
Total	60	65.9%	31	34.1%	91	100.00%

Chi-Square	**2.327**
Contingency Coefficient	**.158**
Asymp. Sig.	**.507**

In the present study, the antimicrobial resistance patterns of gram-negative bacterial isolates from children were recognized. Table5shows the antimicrobial resistance profile.

Table 5: The percentage of antibiotics resistance in gram negative bacteria isolated from urinary tract infections in children.

Bacterial species & No.	AM	PRL	CTX	CRO	IPM	MEM	CIP	LVX	TE	TGC	GM	AK	ERY	TS
Escherichia coli (40)	37 92.5%	25 62.5%	33 82.5%	24 60%	21 52%	14 35%	11 27.5%	11 27.5%	12 30%	2 5 %	14 35%	3 7.5%	18 45%	4 10%
Klebsiellapneumoniae (12)	12 100%	7 58.3%	10 83.3%	8 66.6%	5 41.7%	6 50%	1 8.3%	5 41. 7%	5 41.7%	0 0%	7 58.3%	2 16.7%	7 58.3%	4 33.3%
Protus mirabilis (26)	20 76.9%	15 57.7%	19 73.1%	13 50%	11 42.3%	7 26.9%	4 15.4%	9 34.6%	11 42.3%	0 0%	14 53.9%	4 15.4%	11 42.3%	9 34.6%
Enterobactercloace (5)	5 100%	4 80%	3 60%	2 40%	2 40%	2 40%	4 80%	2 40%	3 60%	1 20%	3 60%	2 40%	2 40%	2 40%
Acinetobacterbaumannii (4)	4 100%	2 50%	4 100%	3 75%	3 75%	2 50%	3 75%	1 25%	1 25%	1 25%	3 75%	0 0%	4 100%	3 75%
Pseudomonas aeruginosa (4)	4 100%	2 50%	4 100%	2 50%	3 75%	2 50%	2 50%	1 25%	2 50%	1 25%	3 75%	3 75%	4 100%	3 75%
Total	**82 (90.1%)**	55 (60.4%)	**73 (80.2%)**	52 (57.1%)	45 (49.5%)	33 (36.2%)	25 (27.5%)	29 (31.9%)	34 (37.4%)	**5 (5.5%)**	44 (48.4%)	14 (15.4%)	46 (50.5%)	25 (27.5%)

Ampicillin (AM), Cefotaxime (CTX), Ceftriaxone (CRO), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Ciprofloxacin (CIP), Levofloxacin (LVX), Piperacillin (PRL), Tetracycline (TE), Tigecycline (TGC), Erythromycin (ERY) and Trimethoprim / Sulfamethoxazole (TS). R: Resistant, S: Sensitive.

All isolated bacteria showed a modest degree of tolerance to the majority of antibiotics tested. The results demonstrated that all gram-negative bacterial isolates exhibited a high resistance (100%) to 2 antibiotics used in this study (Ampicillin (90.1%) and Cefotaxime (80.2%)), while the lower resistance showed with Tigecycline (5.5%), Amikacin (15.4%), Ciprofloxacin (27.5%), Trimethoprime/sulphomethoxizole (27.5%), Levofloxacin (31.9%), Meropenem (36.2%) and Tetracycline (37.4%), which mean that Tigecycline was the effective antibiotic against the local gram-negative bacteria isolated from UTIs patients. The data show that the three species (*Enterobacter cloacae, Acinetobacterbaumannii and Pseudomonas aeruginosa*) were more resistant than other species). The moderate resistance also recorded with the antibiotics Piperacillin, Ceftriaxone, Imipenem, Gentamicin and Erythromycin with the range (48.4- 60.4%).

This local research was performed on 2692 urine samples from patients who visited Rizgary Teaching Hospital in Erbil city; the results indicate that more than 20% of all analyzed samples were negative for bacterial growth; just 16.72 percent of them were pathogenic bacteria, of which 82.44 percent were Gram negative bacteria (GNB) and the remainder were Gram positive bacteria (GPB) (GPB).*Escherichia coli* was the most recurrent , and *Acinetobacterbaumannii* was the most resistant GNB, and GNB were highly resistant to Ticarcillin and Cefepime, and GPB were also resistant to Ticarcillin, and Tigecycline antibiotics (Al-Naqshbandi*et al*., 2019).

Many researchers from all over the globe have identified Gram negative bacteria E.coli and Klebsiella spp. as the most common species triggering UTIs, with E.coli accounting for 70-95 percent of upper and lower UTIs (Stamm, 2001). UTI health conditions include sex (distance between the anus and the urethral meatus (short in women and long in men), moisture content surrounding the urethra), and geneticpredisposition (e.g. blood group), the antibacterial function of prostatic fluid (in men), behavioural causes (such as previous sexual contact, usage of spermicidal agents and diaphragm, duration of urination, facets of personal grooming, or use of the birth control pill), urologic structural defects, diabetes, immune-suppression, breastfeeding, depression, stone forming, nosocomial acquired infections and instrumentation like catheterization (Behzadi*et al.*, 2010).

The connection between sexual contact and UTI is well known in younger women, but it is less evident in older women. During sex, vaginal bacteria enter the urinary tract by conquering the periurethral mucosa and advancing through the urethra to the bladder through the urethra (Hooton, 2012). In an earlier study, 61 273 successive urine tests were collected over a 22-month cycle from routine clinics in an urban region of north Italy, and data inequity by age and gender revealed that E. coli exclusion concentrations were lower in males aged $\geq$60 years (52.2 %), with E. faecalis and P. aeruginosa being more prevalent in this population (11.6% and 7.8%, respectively), as well as in those under 14 years (51.3 %), where the incidence of P. mirabilis was found to be as high as 21.2%. Streptococcus agalactiae was shown to have a 2.3% total incidence, although it was found to be more common in women aged 15 to 59 years (4.1 %) (Magliano et al., 2012).

The isolates retrieved from UTIs in this sample exhibited high tolerance. As a result, the majority of them exhibited susceptibility to at least three types of antibiotics (multiple-drug resistant bacteria). The greatly improved uropathogen tolerance to cephalosporins, methoprime, and ciprofloxacin demonstrates that drug resistance is a serious concern in pediatric urology.This dilemma is exacerbated by poor empiric prescription procedures, a lack of urine monitoring, and the nonselective use of prophylaxis (Edlin and Copp, 2014). Hospitalization and empirical parenteral antibiotherapy are expected in children with severe clinical manifestations. Uropathogens, as previously mentioned, are more prone to most antimicrobial agents (amoxicillin-clavulanate, cephalosporin, nitrofurantoin and amikacin);as a result, empirical antibiotic treatment could not always be effective. Increased Klebsiella spp. cases can result in increased hospitalization and parenteral antibiotherapy, as well as increased costs. (Marcus *et al*., 2005).

A number of 126 distinct gram-negative bacterial strains were identified in the local sample in Al-Najaf, Iraq. The most common bacterium was Escherichia coli (49 isolates), followed by Klebsiella pneumonia (35 isolates), Pseudomonas aeruginosa (18 isolates), Citrobacterfreundii (12 isolates), Enterobocteraerogenes (8 isolates), and Proteus mirabilis (2 isolates) (4 isolates). Antibiotic resistance was found in all bacterial isolates from UTI patients, as well as the Bla-TEM, Bla-SHV, and Bla-CTX-M genes (Majeed and Aljanaby ,2019). The research of Ponce de Leon et al (2018) Both E. coli (57%) and K. pneumoniae (12%) were the most commonly identified species, as well as the ones with the largest prevalence of Extended spectrum Beta-lactamase generating isolates (54 and 39 %, respectively). In nosocomial infections, the average incidence of ESBL-producing pathogens was higher than in society infections (21 % vs. 27 %). In the Urinary Tract Infections, the ESBL prevalence was 37%. Furthermore, ertapenem, imipenem, and amikacin were the antibiotics that retained the most bacterial resistance.

The research on UTI in Children was conducted in a single hospital in Ankara, Turkey, which contained 850 positive urinary cultures, 588 (69.2%) of which were from girls and 262 (30.8%) from boys. Their average age was 36.5 45.0 months. Escherichia coli was the most prevalent causative agent (64.2 %of cases), followed by Klebsiellapneumoniae (14.9 %). There was substantial tolerance to ampicillin (62.6 percent), cephalothin (44.2 percent), co-trimoxazole (29.8 percent), and cefuroxime (28.7 percent). There was no evidence of imipenem tolerance in the isolates. Amikacin, ceftriaxone, ciprofloxacin, and cefepime have the least resistance (0.1, 2.4, 7.5 and 8.3 percent , respectively). Imipenem was the most effective anti-E.

coli agent, followed by amikacin (0.2 percent) and ceftriaxone(2.7%) and nitrofurantoin (5.1%) (Gunduz and Uludağ, 2018).

Conclusion

The Gram-negative bacteria represent high prevalence as the causative agents of urinary tract infections among children in Baghdad hospitals. The present findings exhibited that *E. coli* were the most common isolates, followed by *Proteus mirabilis.*Also it was found that the youngest Childs suffered from urinary tract infections more than the other age groups. Most of the gram-negative bacteria revealed high resistance to penicillins and cephalosporins, while Tigecycline was the effective antibiotic against the local isolates. *Enterobacter cloacae, Acinetobacterbaumannii and Pseudomonas aeruginosa* were more resistant than other species.

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