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ISOLATION AND IDENTIFICATION OF *E. COLI* FROM UTI OF IMMUNOCOMPROMISED CHILDREN

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ABSTRACT
A total of 125 urine samples were collected from 3 hospitals in Baghdad in the period between 1/12 2013 to 1/4/2014, from patients (Males and Females), immunocompromised children and non immunocompromised children (leukemia's and non leukemia's patient) of age range between (<5-12) years. Urine culture was working for patients the result positive for *E. coli* in urine culture. Out of 125 isolates, 43 isolates identified as *E. coli* for both group. 13 out of 95 leukemia's patient (13.7%), 30 out of 30 non leukemia's patient (100%) identified *E. coli* depending on cultural, microscopical and biochemical characteristics. Results of biochemical tests that was confirmed by using the API 20E and VITEK 2 system.
It was found higher percentage of leukemia's 41 out of 95 (43.2%) was within the age of 10-12 years and the lowest percentage 20 out of 95 (21.1%) within the age (<5) years. The results showed that higher percentage of UTI among leukemia's were 53.8 % (7 out of 13) within the age of 10-12 years and the lowest percentage was 15.38 % (2 out of 13) were within the age (< 5). Regarding leukemia's and non leukemia's patients the highest bacterial isolated was recorded for the 3th and 4th dose number (9.3 %) and 6th dose number, (7.0%) while the lowest was recorded for the 2nd dose number (2.3%). The percent of dose patients having second/ third/ fourth dose of chemotherapy have reported a decreased immune status due to decrease Absolute neutrophile count after dose of chemotherapy. Antibiotic susceptibility of *E. coli* isolates showed 100.0% resistance to cefotaxime and Gentamycin and moderate resistance to Tetracyclin 80.0%. However, a bit lower percentage of resistance was shown to Trimethoprime- Sulfamethazaxole ' All *E. coli* isolated from UTI patients with Leukemia's (13 patients) in our study were 100% resistance to cefotaxime and Gentamycin. Moderate resistance was recorded to Trimethoprime- Sulfamethazaxole.

KEY WORDS: *E. coli*, UTI, Leukemia, Neutrophil.

INTRODUCTION

Immunodeficiency is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Immunodeficiency may also decrease cancer immunosurveillance. Most cases of immune deficiency are acquired ("secondary") but some people are born with defects in their immune system, or primary immunodeficiency[1]. The cancer and chemotherapy consider as immunocompromised as one of multi factorial immunodeficiency. Leukemia is the most common malignancy in children and accounts for one-third of all childhood cancers. Approximately 3/4 of all cases of childhood leukemia are acute lymphoblastic leukemia (ALL). About 3,000 children in the United States and 5,000 children in Europe are diagnosed with ALL each year; the incidence of ALL is higher among boys than girls[2]. Approximately 30% of all childhood malignancies, with ALL being five times more common than Acute Myeloid Leukemia (AML). Successful treatment of children with ALL involves administration of a multidrug regimen that is divided into several phases (*i.e*. induction, consolidation, and maintenance) and includes therapy directed to the central nervous system (CNS). Most treatment protocols take two to three years to complete, although the specific regimen varies depending upon immune phenotype and risk category[3]. AML in children reaches approximately to 20% of leukemia's children and they represent a spectrum of hematopoietic malignancies [4]. Immune deficiencies seen in these patients, associated with the immune-suppressive effects of chemotherapy, generate major risk for infections includingurinary tract infections UTI[5]. The agents most commonly involved in the etiology of UTI in patients with hematological malignancies are Gram-negative germs mostly *Escherichia coli*[6]. Uropathoginic *E. coli* (strains encode a number of virulence factors, which enable the bacteria to colonize the urinary tract and persist in face of highly effective host defense. UPEC isolates exhibit a high degree of genetic diversity due to the possession of specialized virulence genes located on mobile genetic elements called pathogenicity islands[7,8]. Some virulence factors such as S fimbriae (sfa), afimbrial adhesion I (afaI), haemolysin (hly), cytotoxic necrotizing factor 1 (cnf-1) and aerobactin (aer) play important roles in the pathogenicity of *E. coli* strains by overcoming host defense mechanisms to cause the disease[9]. Urinary tract infections are common conditions worldwide and the pattern of antimicrobial resistance varies in different regions. In present study described the relationships between sex, age, and isolated bacterial agents and antibiotics resistance of UTIs [10]. Antibiotic resistance varies according to the geographical and regional

locations. The knowledge about the antibiotic resistance pattern is important not only for appropriate therapy but also for the prevention of resistance amongst microbes as the treatment given without considering the prevalent microbe and its antibiotic resistance pattern results in the selection of more resistant strain[11]. The antimicrobial resistance for Cefotaxime, Gentamicin, Tetracyclin, Trimethoprime-Sulfamethaxole. *E. Coli* sensitive to, Ciprofloxacin, Chloroamphincol, Amikacin and 100% sensitive to Imipenem[12].

METHODS

Collection of Samples

Mid-stream urine (125 samples) in sterile cup take and General urine examination to each sample done to determine the fallowing item (pus cell, RBCs, bacteria). Urine samples were centrifuged for 15 minutes at 1000rpm. And then examined under a light microscope, the presence of more than 5 WBC / HPF indicated pyuria for each samples and then make culture and sensitivity test [13].

Identification

1- Bacterial Identification Macroscopic (Culture Characteristics)

A single colony was taken from each primary positive culture. Its identification depended on the morphology properties (colony size, shape, color, translucency, edge, and elevation of texture). The colonies were then investigated by Gram stain to observe gram reaction, shape, cell arrangement [14,15].

2- **API 20E identification of *E. coli* isolates:**

The Api 20E is the identification system for identification of members of the family Enterobacteriaceae and other gram negative bacteria.

The reagents and indicators IND, TDA and VP1-VP2, which were used in API 20E system, had been prepared according to the company instructions kit of (BioMrrieux).

Antibiotics Sensitivity test for all isolates done to Antimicrobial agents

The antimicrobial susceptibility test was performed according to Kirby-Bauer (disk diffusion) technique using Muller-Hinton agar and different single antimicrobial discs supplied commercially. Results were read according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS).

All isolates were tested for antimicrobial susceptibility depending on the CLSI (2011) criteria as follows[16].

1. All isolates were tested for antimicrobial susceptibility test by transfer Few colonies (2-4) from overnight culture were transferred to 5ml of suspension medium in order to prepare the bacterial suspension and then were managed the turbidity by dinsichek adjusted to 0.5 McFarland turbidity.
2. The bacterial suspension was inoculated in Muller Hinton agar plates by using a sterile cotton swab and was left to dry before placing the antimicrobial discs.
3. Different antimicrobial discs (listed in Table 1) were placed with a maximum five discs on the surface of the medium and the plates were incubated at 37°C for 24 hours (duplicate was done for each antimicrobial).
4. The diameter of the inhibition zone of each antibiotic disc was measured by use ruler and the results were interpreted by referring to CLSI recommendation.
5. Interpretation of zone size: The diameter of inhibition zone for each individual antimicrobial agent was translated to terms of sensitive, intermediate and resistant categories by referring to an interpretation chart of the National Committee for Clinical Laboratory Standards, subcommittee on antimicrobial susceptibility testing [17].

TABLE 1: Antibiotic discs (Bioanalyses/ Turkey)

No	Antibiotics	Code	Concentration
1	Amikacin	AK	30µg
2	Cefotaxime	CTX	30µg
3	Ciprofloxacin	CIP	5µg
4	Chloramphenicol	C	30µg
5	Gentamicin	CN	30µg
5	Imipenem	IPM	10µg
7	Trimethoprime+Sulfamethazaxole	COT	75Mg
8	Tetracyclin	TE	3Oµg

Determining ANC (Absolute Neutrophil Count)

To calculate the ANC done by pediatrics hemato-oncologist manual according to the formula: ANC = Total WBC x (% "Segs" + % "Bands"), Equivalent to: WBC x ((Segs/100) + (Bands/100)) The ANC refers to the total number of neutrophil granulocytes present in the blood.

Normal value: ≥ 1500 cells/mm^3.

Mild neutropenia: ≥1000 - <1500/mm^3.

Moderate neutropenia: ≥500 - <1000/mm^3.

Severe neutropenia: < 500/mm^3[18]

Alternatively, the result was taken using automated analyzer by inserting 2ml EDTA blood tube in automated analyzer device and draw 200 µl from sample of whole blood when press the bottom (stare) and print the results include all results flag units reference interval CBC and then recorded the ANC directly from test result unit.

RESULTS & DISCUSSION

Incidence of *E. coli* in UTI patients with and without leukemia's

The results in the table (2) showed distribution of study groups according to Urine culture of *E. coli* in the Present study, urine culture revealed *E. coli* as the only bacteria in urine samples from leukemia's and non leukemia's patient. Out of 95 leukemia's patients, 13 found positive for *E. coli* in urine culture (13.7%).These findings are in conformity with reports by researcher[19]**.** Interestingly, *E. coli* was isolated from 30 patients (100%) without leukemia which represented the control group. This result indicates that *E.*

coli is the commonest bacteria in the patients with UTI which may be attributed to the pathogenicity of the bacteria as indicated by[20].

TABLE 2: Distribution of study groups according to Urine culture of *E. coli*

Urine culture of *E. coli*		Study group		Test of sign
		Patient (n=95)	Control (n=30)	
+Ve	No.	13	30	MCP< 0.001
	%	13.7 %	100.0 %	(HS)
-Ve	No.	82	0	
	%	86.3 %	0 %	

Distribution of leukemia's/non leukemia's based on Absolute neutrophil count and Dose of drug:

The current result Table (3) shows distribution of leukemia's / non leukemia's based on absolute neutrophil count and dose of drug. Leukemias and non leukemia's Patients were divided into three categories based on immune status which was assessed by neutrophil count. The first category represents those at a high risk of infection (neutrophil counts less than (500). The second category represents those at a moderate risk of infection (neutrophil counts 500–1000). The third category represents those at a low risk of infection (neutrophil counts 1000 - 1800+).

The first and third categories were recorded the highest for the third and fourth dose (29.6%, 24.1%; 7.9%, 7.9%) respectively. The second category was recorded the highest for the second and third dose (18.2%, 45.5%). The first category was recorded the lowest for the third dose (5.6%). The second and third categories were recorded the lowest for the fifth and sixth dose (0%, 0.3%; 0%, 0%) respectively

In conclusion, among leukemia's, those with neutrophil counts less than (500) were the highest recorded, followed by those at a moderate risk of infection (neutrophil counts 500–1000) and finally those at a low risk of infection (neutrophil counts 1000-1800+). Moreover, there is a highly significant relation (MCP< 0.001 (HS) between Absolute neutrophil count in leukemia's and non leukemia's and the dose of chemotherapy drug. The normal value of neutropenia ≥1500 cells/mm^3 [18].

This illustrates that immunocompromised patient having second/ third/ fourth dose of chemotherapy have reported a decreased immune status.

Cytotoxic chemotherapy generally suppresses the hematopoietic system, impairing host protective mechanisms and limiting the doses of chemotherapy that can be tolerated. Neutropenia, the most serious hematologic toxicity, is associated with the risk of life-threatening infections as well as chemotherapy dose reductions and delays that may compromise treatment outcomes .Moreover, the type of chemotherapy agents, the dosage and number of drugs are clearly related to the risk of neutropenia and infections. Neutropenia is the most common cause of chemotherapy dose[21]. The present studies agree with a previous study done by[22]. Explain that Neutropenia is the most common cause of chemotherapy dose. So far, there are no much information covering this issue, therefore further investigations are needed in this regard.

TABLE 3: Distribution of leukemia's / non leukemia's based on Absolute neutrophil count and Dose of drug

Absolute neutrophil count		Dose of drug							Total
		0 non dose	1st dose	2nd dose	3rd dose	4th dose	5th dose	6th dose	
High risk of infection	NO.	7	3	4	16	13	7	4	54
(Less than 500)	%	13.0%	5.6%	7.4%	29.6%	24.1%	13.0%	7.4 %	100.0%
Carries with a	NO.	4	5	6	15	2	0	1	33
moderate risk of	%	12.1%	15.2%	18.2%	45.5%	6.1%	.0 %	3.0 %	100.0%
infection (500 - 1000)									
Risk of infection	NO.	30	1	1	3	3	0	0	38
considered Low (1000	%	78.9%	2.6%	2.6%	7.9%	7.9%	.0 %	.0 %	100.0%
- 1800+)									
Total	NO.	41	9	11	34	18	7	5	125
	%	32.8%	7.2%	8.8%	27.2%	14.4%	5.6%	4.0 %	100.0%

MCP< 0.001 (HS)

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