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EXTENDED SPECTRUM OF B- LACTAMASE STATUS IN *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTIONS IN DUHOK CITY- IRAQ

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ABSTRACT

Objectives: To study the pattern of ESβL *E. coli* in cross sectional study from urine of patients in Azadi and Heve Paediatric Teaching Hospitals in Duhok city.

Methods: This study was conducted between June 2016 and December 2016. A study of 325 of *E.coli* isolates from urine samples in Azadi Teaching Hospital and 420 isolates from Heve Teaching Hospital. Confirmation of ESβL production was performed using double-disk synergy test and the BD Phoenix automated system..

Results: There were 175 (53.8%) confirmed strains of ESβLs *E. coli* among adults from Azadi Hospital and 163 (38.8%) strains among paediatrics from Hevi Hospital which were tested by double-disk synergy test and BD Phoenix. There was no statistical significance for ESβL strains regarding the gender of the patients. Majority of ESβL *E. coli* were among 7-9 years and 25-65 years. The rate of antibiotic resistance was > 90% for Ceftazidime, Ceftriaxone, Cefixime, Cefepime, Augmentin and Aztreonam for both adults and paediatric age groups. The rate of sensitivity for Imipenem, Amikacin and Nitrofurantoin was 4.5%, 8% and 16% respectively for adults and 17% 15.5% and 24.5% for paediatrics respectively.

Conclusion: Increasing *E.coli* from both hospitals. Exposures to antibiotics, previous hospital admission, recurrent urinary tract infection and diabetes were risk factors for ESβLs.

INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common bacterial infections and it is commonly treated in primary health care[1]. The most common cause of UTI is extraintestinal pathogenic *E. coli* affecting more than 70% of community-acquired UTIs[2].

The widespread use of third-generation cephalosporins and aztreonam has led the emergence of the extended spectrum β-lactamases (ESβLs) results from mutations in β-lactamases. ESβLs are plasmid-mediated enzymes that are inhibited in vitro by clavulanate[3]. As they are clavulanate susceptible enzymes conferring broad resistance to penicillins, aztreonam and cephalosporins and are detected most commonly in *Klebsiella pneumoniae* and *E.coli*[4]. It has been shown that recurrent UTI is one of the risk factors for ESβL-producing *E*. coli infections in hospitalized[5] and non-hospitalized patients[6, 7]. The great increase in ESβL-producing *E.coli*, found to be the reason for higher morbidity, prolonged hospitalization and increased health care costs. This is a major public health problem in both hospitals and communities [8].

There has been an interest in the epidemiological factors associated with infections caused by ESβL *E.coli*: age > 65 years, male sex, previous UTI, use of antibiotics and hospitalization as common risk factors[9, 10].

There are several ESβs genotypes. The most common of these are the SHV, TEM, and CTX-M[11]. They have emerged to be a major cause of hospital acquired infections, especially in intensive care unit[12]. These ESβL-producing strains represent a significant therapeutic challenge as they are resistant to all currently available β-lactam antibiotics but cephamycins (cefoxitin and cefotetan) and carbapenems (imipenem and ertapenem) is of a transmissible nature, i. e. plasmid-coded in some strains of nosocomial Enterobacteriaceae. [13].

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The importance of the present study in our locality is to find out the associated risk factors for ESBLs – *E.coli* compared to non- ESBLs – *E.coli* and To determine if the positive ESβLs - *E.coli* cultures are acquired from the hospital or from the community.

The aim of the current study is screening of the ESβL- *E. coli* in urine samples of patients with UTIs in Duhok city.

METHODS

Sample collection:

A total of 325 *E. coli* were collected from out-patients with UTIs whom attended the General Azadi Teaching Hospital and a total of 420 *E. coli* from Heve paediatric Teaching Hospital in Duhok city.

Socio-demographic and clinical information were obtained from medical records and included: age, gender, recent antibiotics intake within the last 3 months, diseases (Diabetes and recurrent urinary tract infection), previous admission to hospital within the last 3 months, intermittent catheterization and Long duration of prophylaxis treatment.

Bacterial cultures:

All urine samples were inoculated on blood agar, MacConkey agar plates and Kligler agar (Oxoid, UK). These plates were incubated overnight at 37 °C aerobically. All isolates were identified based on their colony, morphology, culture characteristics, and biochemical reactions according to the standard microbiological procedures[14]. Only those samples were processed for ESβLs production that showed significant growth and were identified as *E. coli* on the basis of culture and biochemical characteristics.

Identification for all the bacterial isolates were further determined using the BD Phoenix™ automated machine in General Azadi Teaching Hospital.

Processing of samples:

The bacterial isolates were phenotypically investigated in Microbiology Laboratory in General Azadi Teaching Hospital.

Screening for ESβLs

The ESβLs screening was performed according to National Committee for Clinical Laboratory Standards (NCCLS): ESβL *E. coli* producers were identified first by disc diffusion technique using Ceftazidime (≤ 22mm), Cefotaxime (≤ 27mm), Ceftriaxone (≤ 25mm) and Aztreonam (≤ 27mm). If the isolates are resistant to any of these drugs, they are considered as suspect of ESβL producers[15]. Both Double Disc Synergy Test (DDST) and Phoenix™ automated machine were performed for confirmation.

Confirmation of ESβLs

All isolates that have been found to be resistant to cefotaxime, ceftazidime, ceftriaxone and Aztreonam were subjected to confirmatory tests by the double disc diffusion methods, following the CLSI guidelines[16]. Mueller Hinton agar plates were classified according to 0.5 McFarland tube). Three 3rd generation cephalosporin with one monobactam (aztreonam 30µg) discs were placed at 20 mm distance from augmentin disc.

Susceptibility test

All the isolates were screened for antibacterial sensitivity using "Kirby-Bauer method" according to CLSI standards[17]. This test was performed on Muellar Hinton agar medium (Oxoid Ltd, England). 17 AST discs (Bioanalyse) were used: Amoxicillin/Clavulanic acid: 20/ 10µg (AMC 30), Cotrimoxazole 1.25/23.75µg (SXT), Ciprofloxacin 10 µg (CIP), Nitrofurantoin 100µg (F), Nalidixic acid 30µg (Na), Ceftriaxone 10µg (CRO), Gentamicin 10µg (CN), Ceftazidime 30µg (CAZ), Cefotaxime 30µg (CTX), Amikacin 10µg (Ak), Aztreonam 10µg (AZT), Imipenem 10µg (IMP), Cefixime 5µg (CFM), Piperacillin 30µg (PRL), Cephalothin 30 µg (Kf), Meropenim 10 µg (MEM), Ampicillin 25µg (Am), Trimethoprim 10µg (TMP) and Metronidazole 30µg (MET).

Statistical analysis:

A descriptive analysis was applied to the study sample and expressed as means ± standard deviation, frequencies and percentages. Data were analyzed using the SPSS v16.0 statistical package (SPSS Inc, Chicago, IL, USA).

RESULTS

During the study period between June 2016 and December 2016, out of 325 *E. coli* isolates 200 isolates among adults from General Azadi Hospital and 200 isolates out of 420 among children from Heve Hospital were identified as ESβLs by Phoenix and included in the study.

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Regarding to gender of patients, 47% of the ESβL isolates were isolated from males and 53% of the isolates were isolated from females in Heve Teaching Hospital, while in General Azadi Hospital 52.5 % were males and 47.5% were females. During this study, the distribution of ESβL in both paediatric and adult is shown in Table 1.

Table(1):-Frequencies and percentages of ESβL *E. coli* among different age groups

Age group (years)	Number (No.) positive ESβLs (200)	Percentages (%) of positive ESβLs (200)
(Paediatrics)		
1-3	108	54
4-6	33	16.5
7-9	44	22
10-14	15	7.5
(Adults)		
15-24	61	30.5
25-65	130	65.5
66 and more	9	4.5

The pattern of ESβL,AmpC and Carbapenemase among patients from both hospitals are shown in Table 2.

There were high percentage of ESβLs producing *E. coli* among adult patients (53.8%) and paediatric patients (38.8%), when compared with AmpC and Carbapenemase types.

Table(2):- Pattern of ESβL, AmpC and Carbapenemase isolates of *E. coli* among patients

Types of ESBL	Frequency (Mean) for adults No.=200	Frequency (Mean) for paediatrics No. = 200
ESβL	175 (53.8%)	163 (38.8%)
AmpC	21 (6.46%)	33 (7.86%)
Carbapenemase	4 (1.23%)	4 (0.95%)

Regarding to the risk factors, it was obvious from (table 3), there were (73.5%) among adult and (67.0%) in paediatric patients related to previous hospital admission, while in diabetic patients the risk factor for paediatric patients (63.5%)

and adult patients (42%) at the same time, the adult patients with recurrent UTIs had 18% ESβLs producing *E. coli* and paediatric patients with recurrent UTIs had 54%. Treatment with amoxiclave was (62.5%) and treatment with Ciprofloxacin was (27.0%) of ESβLs producing *E. coli* during the last 3 months.

Table (3):- Developing an infection with ESβL by different effects

Risk factors	Paediatric patients ESBLs positive (200)	Adult patients ESBLs positive (200)
Diabetes	127 (63.5%)	84 (42.0%)
Recurrent UTIs	108 (54.0%)	36 (18.0%)
Previous hospital admission	134 (67.0%)	147 (73.5%)
Taking Amoxiclave within the last 3 months	/	125 (62.5%)
Taking Ciprofloxacin within the last 3 months		54/200 (27.0%)

All isolates that had been resistant to third generation cephalosporin were found to show positive synergy by the double disc diffusion test indicating the presence of ESβs

All the ESβL producing *E. coli* among adults were resistant to ampicillin and Amoxiclave but it was 71.5% and 91% respectively among paediatric patients. It was 98.5% resistant for both Cefixim and Ceftazidim and 98% for both

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Ceftriaxone and Aztreonam among adults and they were 79%, 94.5%, 905 and 95% respectively among children. The patterns of the other antimicrobials tests for both adults and paediatrics are shown in Table 4.

Table(4):- Antibiotic Resistance patterns of ESβLs

	Resistance among paediatrics (200)	Resistance among adults (200)
Amikacin (AK, 10 µg)	31 (15.5%)	16 (8.0%)
Gentamicin (CN, 10 µg)	97 (48.5%)	117 (58.5%)
Emipenem (IMP, 10 µg)	34 (17.0%)	9 (4.5%)
Cifixime (CFM, 5 µg)	158 (79.0%)	197 (98.5%)
Ceftazidime (CAZ, 30 µg)	189 (94.5%)	197 (98.5%)
Ceftriaxone (CRO, 10 µg)	180 (90.0%)	198 (98.0%)
Cefepime (CFM, 30 µg)	163/200 (81.5%)	190/200 (95.0%)
Aztreonam (AZT, 10 µg)	190/200 (95.0%)	196/200 (98.0%)
Ampicillin (AM, 25 µg)	143/200 (71.5%)	200/200 (100%)
Co-Amoxiclave: Amoxicillin-Clavulanate (AMC 20/10 µg)	190/200 (95.0%)	200/200 (100%)
Pipracillin (PIP, 30 µg)	67/200 (33.5%)	117/200 (58.5%)
Trimethoprim (TMP,10 µg)	140/200 (70.0%)	149/200 (74.5%)
Nitrofurantoin (F, 100 µg)	49/200 (24.5%)	32/200 (16.0%)
Ciprofloxacin (CIP, 10 µg)	89/200 (44.5%)	165/200 (82.5%)
Norfloxacin	70/200 (35.0%)	172/200 (86.0%)
Metronidazole (MET, 30 µg)	102/200 (51.0%)	93/200 (46.5%)
Nalidixic Acid (N, 30 µg)	58/200 (29.0%)	81/200 (40.5%)

DISCUSSION

This study revealed that the overall ratio of male to female UTI patients in < 14 years of age was 0.89 however the ratio was 1.11 in adults of General Azadi Teaching Hospital. These ratios were not statistically significant. This is in agreement with a study done by[18]. While, a study done by[19], among the ESβLs producing isolates of *E coli* found 100/484 (20.7 %)

were infecting males while 384/484 (79.3 %) were infecting females. Moreover a study done by[20] illustrates a relation of ESβLs for both sexes. Nevertheless, found male sex is an independent predictive risk factor for ESβLs.

The current study found that the frequencies of ESβL was prevalent among all age groups but the highest percentage was for those less than 3 years of age and this can be attributed to low immunity among the neonates. A study done by[21], found that age > 65 years was independently predictive of ESβL positivity.

A study done by[22] has shown the risk factors for UTIs to be previous infections and exposure to antibiotics also posing a major risk for UTIs with resistant bacteria. The same researcher has found recent hospitalization, administration of antibiotics effects on ESβLs.

More generally, different studies have shown conflicting relations for sexes and infection due to ESβL-producing Enterobacteriaceae [23-26].

Different ESβLs exhibit different levels of resistance to third generation cephalosporins[27]. A wide range of activity of antimicrobial resistance pattern to the antimicrobial agents was observed in ESβL *E. coli*. A high degree of resistance (> 90%) was seen in 3rd and 4th generation Cephalosporin (Ceftriaxone, Ceftazidime, Cefepime, Cefixime) and Aztreonam. Also, Amoxicillin-Clavulanate. Intermediate level of resistance (40% - 70%) was observed in Gentamicin, Pipracillin,Trimethoprim, Metronidazole and Nalidixic Acid. The least resistance was observed among Emipenem, Amikacin and Nitrofurantoin[27].

In conclusion, the results of the present study revealed that there is high distribution of ESβLs among *E. coli* isolates from both hospitals. Exposures to antibiotics, previous hospital admission, recurrent urinary tract infection and diabetes were risk factors for ESβLs. This study

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has important implications for public health, especially in relation to UTIs in women.

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Frequency, Risk Factors and Antibiotic Sensitivity Pattern of Extended-Spectrum Beta-Lactamase Producing *E. coli and K. pneumoniae* Causing Urinary Tract Infection: Experience from a Tertiary Care Hospital of Bangladesh. Birdem Medical Journal.

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پوختە

ئامانجەکان

زانینی و ناسینەوەی جۆرەکانی **E.coli ESBL** پشکنینی دژە میکرۆب **E.coli ESBL**ی هینە وەرگرتن ژ میزا نەخۆشێت خە ستا ئازادی و خەستا هێڤی لە باژێری دهۆك.

ڕێگاکان:

لەماوەی 6 مانگ **(6 \2016 تاکو 12\ 2016) E.coli325** هاتنە دیاری کرن لە میزا نخۆشێت خەستا ئازادی و **E.coli 420** ژ خەستا هێڤی . پشکنینی دژە میکرۆب **E.coli** کرا بەڕێگەی **Kirby Bauer Disc diffusion test** و دووبارە پشکنین بە ڕێگای **BD Phoenix**

ئەنجام:

ڕێژەی **E.coli ESBL 53.8** % لە سەرجەم **E.coli** شخەستا ئازادی و ڕێژەی **38.8%** لە خەستا هێڤی.ریژەی دژەمیکرۆبی **E.coli** بەرزبوو **90%**<بۆ **ceftriaxone, ceftazidim,cefixim, cefepim, augmentin and Aztreonam** و ریژەی کەم بوو **4.5% Imipenem** و **8%** بۆ **Amikacin** و **16%** بۆ **Nitrofurantion**

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دەرئەنجام:
ڕیژەی ESBL E.coli دژی زۆربەی دژەمیکرۆبەکان بەرزبوو لە هەردوو خەستا لباژێرێ دهۆك.پێشتر وەرگرتنی Antibiotics لەلایەن نە خۆش و پێشتر هەبوونی خەوتنی نەخۆش لە نەخۆشخانە هۆکاری سەرەکین بۆ دروست بوونی ESBL.

الخلاصه
دراسه ESBLعند بكتيريا *E. Coli* المعزوله من التهابات المجاري البوليه لمدينه دهوك- العراق.

الاهداف:
معرفه انتشار بكتريا *E.coli* والمقاومه لعدد من المضادات الحيويه والمعروفه ب (ESβL) في ادرار مرضى مستشفى ازادي التعليمي ومستشفى هيفي التعليمي في مدينه دهوك- عراق .

الطريقة :
تمت الدراسه على 325 عزله *E.coli* من مرضى مستشفى ازادي و420 عزله من مستشفى هيفي للفتره من حزيران 2016 ولغايه كانون الاول 2016. في البدايه تم عزل بكتيريا ال *E.coli* المقاومه لمعظم المضادات الحيويه بطريقه Kirby Bauer Disc Diffusion ومن ثم تم اجراء فحوص تاكيديه باجراء Double Disc Diffusion ومن ثم بجهاز ال Phoenix للتعرف على نوعها ومدى استجابتها للمضادات الحيويه.

النتائج:
كانت هناك 175 عزله *E.coli* ESβL (53.8%) من عزلات ال *E.coli* مستشفى ازادي وكذلك 163 عزله *E.coli* ESβL (38.8 %) من مستشفى هيفي. معظم الحالات كانت بين الاعمار 25–65 سنه.كانت هناك نسب المقاومه للمضادات الحيويه عاليه (اكثر من 90%) للمضادات : Ceftazidime, Ceftriaxone, Cefixim, Cefepime, Augmentin, and Aztreonam. ولكن نسب المقاومه قليله لكل من Imipenem 4.5%) و (8%) Amikacin وكذلك Nitrofurantoin (16%).

الاستنتاجات:
اشارت الدراسه ان هناك نسبه عاليه ل *E.coli* ESβL المقاومه لمعظم المضادات الحيويه من ادرار مرضى مستشفى ازادي ومستشفى هيفي في مدينه دهوك. استعمال المضادات الحيويه مسبقا والتهاب المجاري البوليه المتكرره كانت من العوامل المرافقه للبكتريا. الدراسه تؤكد على ضروره الحكمه في استعمال المضادات الحيويه وخاصه في التهابات المجاري البوليه.

***KEYWORDS*: *Escherichia coli* (*E. coli*); Extended Spectrum β-Lactamase (ESβL); Cephalosporines; Carbapenems; BD Phoenix and ESBLs genotypes SHV, TEM, and CTX-M.**