Antimicrobial Susceptibility Patterns Against Escherichia *Coli* and Prevalence of Extended–Spectrum β-Lactamases

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Abstract

Sixty seven samples were collected from urine, stool, pus, wounds of the patients and operating theater from Baghdad Hospitals in 2010. Sixty two isolates of *Escherichia coli* were diagnosed by bacteriological and biochemical tests. These isolates were submitted to hemolysis test and antibiotic sensitivity to 10 kinds of antibiotic disks on Mueller Hinton agar by disk diffusion method. The detection of β-lactamase production was also done as well as extended-spectrum beta-lactamase (ESBL) for all the isolates. Hemolysis test was positive for two isolates. All the isolates of *E.coli* were resistant to ampicillin and cephalothin (100%) and high resistance was observed to cephalixin (95.1%), tobramycin (90.3%), doxycycline (82.2. %) and nalidixic acid (70.9%). Both ciprofloxacin and nitrofurantoin was (67.7%). Low resistance was noticed to amikacin (11.2%) and trimethoprime (8.1%). β-lactamase test was positive for 57 isolates (91.9%), while three isolates (4.8%) showed positive result for extended-spectrum β-lactamase.

Conclusion: Isolates of *E.coli* showed high resistance to ampicillin, cephalothin and cephalixin. Low resistance was revealed to amikacin and trimethoprime. Most of the isolates were positive for β-lactamase test (91.9%) and (4.8%) of the isolates were positive for extended-spectrum beta-lactamase.

Key words: *E. coli*, antimicrobial susceptibility, β-lactamase.

الخلاصة

جمعت سبعة وستون عينة سريرية من الإدرار والخروج والقيح من المرضى وكذلك صالات العمليات و خلال العام 2010 من بعض مستشفيات بغداد .شخصت عائديه 62 عزلة لبكتريا *Escherichia Coli* بالاختبارات البكتريولوجية والبيوكيمياوية. وأخضعت العزلات لاختبار تحلل الدم على وسط أغار الدم الصلب وكذلك الحساسية للمضادات الجرثومية تجاه 10 أنواع باختبار الانتشار للأقراص على وسط مولر هنتون الصلب. اجري فحص انتاج انزيم البتالاكتاميز لكل العزلات بطريقة اليود السريعة .كما تم اجراء فحص انتاج انزيمات البتالاكتاميز واسعة الطيف. كانت نتائج اختبار تحلل الدم موجبة لعزلتين فقط .اما بالنسبة لنتائج الحساسية فقد أظهرت كل العزلات مقاومة. أظهرت العزلات مقاومة (100%) لمضادي الـــ ampicillin وcephalothin بينما أظهرت مقاومة عالية لكل من الـــ cephalixin (%95.1) , nalidixic acid (70.9%) , tobramycin (90.3%), doxycycline (82.2.%) كانت حساسية كل من nitrofurantoin و ciprofloxacin هي (67.6%). أما amikacin و trimethoprime فقد لوحظت اقل مقاومة للمضادات وكانت (11.2%) و (8.1%) على التوالي. أما اختبار إنتاج البيتالاكتاميز فقد كان موجبا لـ 57 عزلة أي بنسبة (91.9%) بينما أظهرت ثلاث عزلات ايجابيتها لأنزيمات البيتالاكتاميز الواسعة الطيف وبنسبة (4.8%) .

الأستنتاج: اظهرت العزلات قيد الدراسة مقاومة عالية للامبسيلين و السيفالوثين و السيفالكسين بينما كانت حساسة للأميكاسين و الترايمثبريم. معظم العزلات كانت منتجة للبيتالاكتاميز بنسبة (91.9%) وكانت (4.8%) من العزلات منتجة لأنزيمات البيتالاكتاميز الواسعة الطيف.

.Introduction

Escherichia coli is one of the commensals in the human intestinal tract. As a commensally, it contributes to the maintenance of health of a person. However, *E. coli* , when enters into unnatural sites, can cause variety of infectious diseases such as urinary tract infections, wound infections, bacteraemia, meningitis and other soft tissue infections [1]. Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Routine monitoring of antibiotic resistance provides data for antibiotic therapy and resistance control prescription programs, making policy decisions and assessing the effectiveness of both [2]. The rapid increase in bacterial resistance to antibiotics has resulted in increased morbidity and mortality among patients in hospitals, and in intensive care units in particular. Various measures have been proposed for alleviating this situation, such as increased surveillance, improved physical controls like hand washing, and the substitution of empirically employed broad-spectrum antibiotics by narrow-spectrum drugs to which the infecting organism is known to be sensitive[3]. Strains of Enterobacteriaceae that produce extended-spectrum β-lactamases (ESBLs) have emerged as significant pathogens. First reported in the mid-1980s, they were mainly found in *Klebsiella pneumoniae* and *Escherichia coli* although they can now be found in many other species [4].

The aim of the presented study was to study the prevalence of ESBL producers among *Escherichia coli*, and their susceptibility pattern to antimicrobial agents.

Materials and Methods

Isolation and Detection

Between April 2010- July 2010, a total of 67 specimens were collected (urine, stool, pus, wound and operating theater) from some of Baghdad hospitals .Clinically 62 Gram negative bacilli belonging to *E.coli.*

The isolates were identified by standard techniques, bacteriological and biochemical tests. The specimens were placed on blood agar for hemolysis test,MaCconkey agar and triple sugar iron agar .Catalase test ,oxidase test ,EPI system (Oxoid) were also used [5].

Antimicrobial agents susceptibility testing:

Susceptibility to antimicrobial agents was assessed by the disc diffusion technique on Mueller–Hinton agar.Ten kinds of antibiotic discs (Amikacin, Ampicillin, Cephalexin, Cephalothin, Ciprofloxacin, Doxycycline, Nalidixic acid, Nitrofurantoin, Tobramycin, and Trimethoprime). Antimicrobial susceptibility testing was performed by Kirby-Bauer method and interpretation of results was as recommended by NCCLS [6]. *E.coli* ATCC25922 was used as standard strain.

Beta-lactamase assay:

Production of beta-lactamase test was done for the 62 isolates by using the rapid idometric method of WHO [7].

Extended-spectrum β-lactamase Production test:

The Sixty two isolates were subjected to ESBL screening. ESBL production was tested by using cefotaxime (30µg,) ceftazidime (30µg) and augmentin (30/10µg) discs on Mueller-Hinton agar.

Organism was considered as an ESBL producer if there was a ≥5mm increase in zone diameter around ceftazidime/ augmentin disc compared to zone around ceftazidime disc alone [8].

Results and Discussion

Among 67 clinical specimens, 62 isolates (92.5%) were identified as *E.coli* and their distribution as shown in table 1 .The prevalence found in our study were similar to those found in a study conducted in *E. coli* prevalence in 2008 (92%) [9], and (63%) in Nigeria from different clinical specimens [10] .While the prevalence of *E.coli* in a recent study in Iraq from urine was (52%) [11], and the current study was (75.8%). These wide variations in the prevalence, bacterial species, and antibiotic sensitivity could be due to variation in the study methodology, agent, host and environmental factors that exist [12].

Table 1. Distribution of 62 *E.coli* isolates among clinical specimens

Specimens	Isolates Number	%
Operating theater	2	3.2
Pus	3	4.8
Stool	8	12.9
Urine	47	75.8
Wound	2	3.2

Hemolysis test was positive for the two isolates (3.2%) as shown in table 2 and these results are similar to the results demonstrated by Drews [13] who stated that a lower prevalence of beta-hemolysis among nalidixic acid or ciprofloxacin- resistant *E.coli* urine isolates compared with susceptible isolates.Fluoroquinolone resistance is linked to loss of beta-hemolysis [13,14].
In antimicrobial agents susceptibility test all the isolates of *E.coli* (100%) were resistant to ampicillin and cephalothin. High resistance was observed to cephalixin (95.1%), tobramycin (90.3%), doxycycline (82.2. %) and nalidixic acid (70.9%). Both ciprofloxacin and nitrofurantoin was (67.7%) as showed in table 3 and 4.

Table 2. Hemolysis positive *E.coli* isolates

Specimens	Hemolysis positive isolates	(%)
Operating theater	1	1.6
Pus	-	0
Stool	-	0
Urine	1	1.6
Wound	-	0
Total	2	3.2

Table3. Antimicrobials susceptibility patterns of 62 *E.coli* isolates

Antibiotics	Resistant isolates	Sensitive isolates
Amikacin(30 µg)	7	35
Ampicillin (10µg)	62	-
Cephalexin(30µg)	59	3
Cephalothin(30µg)	62	-
Ciprofloxacin(5µg)	42	10
Doxycycline(30µg)	51	10
Nalidixic acid(30µg)	44	9
Nitrofurantion(300µg)	42	12
Tobramycin(10µg)	56	4
Trimethoprime(5µg)	5	11

Alhelfi study in Iraq [12] and another study in Oman [13] mentioned a high resistance rate in *E. coli* to ampicillin (99%) which is similar to our results (100%). Trimethprim and amikacin showed the lowest resistance among the rest antimicrobials in this study, a new study in Kuwait showed the similar result [15].
The *E.coli* isolates in this study showed multiple resistances to more than 6 kinds of antimicrobials as displayced in table 5.
β-lactamase test was positive for 57 isolates (91.9%),while three isolates (4.8%) showed positive result for extended-spectrum beta-lactamase as in table 6. The prevalence of β-lactamase isolates found in our study was not similar to those found in a study conducted in 1997 in Iraq

investigating *E. coli* producing β-lactamase prevalence ,as the percentage was (60%) of the isolates [16].

Table 4. The resistant *E.coli* isolates and their percentage

Antimicrobial agents	**Resistant isolates**	**(%)**
Amikacin(30 µg)	7	11.2
Ampicillin (10µg)	62	100
Cephalexin(30µg)	59	95.1
Cephalothin(30µg)	62	100
Ciprofloxacin(5µg)	42	67.7
Doxycycline(30µg)	51	82.2
Nalidixic acid(30µg)	44	70.9
Nitrofurantion(300µg)	42	67.7
Tobramycin(10µg)	56	90.3
Trimethoprime(5µg)	5	8.1

Table 5. Multiple resistance among *E.coli* isolates.

Number of antimicrobial agents	**Number of resistant isolates**
5	5
6	7
7	8
8	12
9	12
10	6

Table 6. Prevalence of β-lactamase and ESBL producing isolates

Specimens	**β-lactamase positive isolates**		**ESBL positive isolates**	
	No.	**(%)**	**No.**	**(%)**
Operating theater	2	3.2	-	-
Pus	3	4.8	-	-
Stool	8	12.9	-	-
Urine	42	67.7	3	4.8
Wound	2	3.2	-	-
Total	57	91.9	3	4.8

These wide variations in the prevalence could be due to variation in the study methodology, agent, host and environmental factors that exists.

An ESBL-producing *E.coli* prevalence of 4.8% was observed in this study.

AL-Helfi in a recent study in Iraq has reported that the prevalence of ESBL producing *E.coli* was (18%).ESBL producing E.coli was varied from (62.3%) as in mentioned by Shivaprakasha [17] and (57%) in Ståle study [18].Direct comparisons are difficult due to selective sampling and different handling of the samples in the clinical laboratories. In UK the prevalence of ESBL producing isolates was (1.3%) by using the same technique [19].In this study ESBL producing isolates were resistant to more than 6 kinds of antimicrobial agents.

The available therapeutic options for the treatment of ESBL-associated infections are limited by drug resistance conferred by the ESBLs, along with frequently observed co-resistance to various antibiotic classes, including cephamycins, fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim /sulfamethoxazole [20].

The extended spectrum beta lactamase (ESBL) enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of beta lactams, including third generation cephalosporins, penicillins and aztreonam.

Plasmids responsible for ESBL production carry resistance to many antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and co-trimoxazole. The ESBL producing organisms are reported in increasing numbers worldwide. [21].

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