Phenotype Detection of Genetic Enzymes B- Lactamase Isolation of Patients with Urinary Tract Infections Bacteria *Escherichia Coli*

Ali Hassoun Hammadi

Assistant Professor/ Faculty of Nursing, Bilad Al-Rafidain University College, Iraq

Abstract

Background*:* the main cause of multidrug resistant bacteria is the production of β-lactamase enzymes. The objective of this study was to determine the prevalence of genotype of *Escherichia coli* spp. isolated from nosocomial urinary tract infections (UTI). ***Methods*:** In a cross-sectional study from May 2019 to January 2020, mid-stream urine specimens were collected from 210 patients presented with acute urinary tract infections whom attending Al- Shahid Ghazi and Al-Hariri Hospital in Medical City/ Baghdad. Samples were cultured for E. coli isolation. Antibiotic sensitivity test and the prevalence of resistance enzyme producing gen were investigated. ***Results***: A total of 210 E. coli were isolated, multidrug resistance strain of E coli (92.85%) was reported by this study. PCR technique indicated that 88.57% of ESBL-producing isolates possessing *bla*TEM, 30% *bla*CTX-M genes and 20.48% *bla*SHV genes between isolates and E. coli still the less resistant to carbapenem due to low expression of KPC gen. ***Conclusion***: ESBLs and KPC were the higher prevalent genotype of multidrug resistant E. coli in urine sample.

Keywords: *Health; Patients; ; B- Lactamase; infections.*

Introduction

Urinary tract infections are among the most common diseases caused by *E. coli* bacteria. *E. coli* is opportunistic bacteria and therefore can become a colony of the urinary tract and cause symptomatic or asymptomatic infections[1].

Based on the presence of the gene encoding, virulence factor and types of chromosome or plasmid, Escherichia coli is classified into six classes, these includes enteropathogenic *E. coli* (EPEC), enterotoxogenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) Enterohaemoragic *E. coli* (EHEC) Diffusely adherent *E. coli* (DAEC) Enteroaggregative *E. coli* (EAggEC)[2].

Corresponding author:
Ali Hassoun Hammadi
Assistant Professor/ Faculty of Nursing, Bilad Al-Rafidain University College, Iraq
Email: ali662006@yahoo.com

It was found that about 60% of adult women have urinary tract infection and about 80% of community urinary tract infection and 30% of nosocomial urinary tract infections is due to Uropathogenic *E. coli* (UPEC) [3].

One of the bacterial mechanisms to combat beta-lactam antagonists is their production of beta-lactamase enzymes that cleaves beta-lactam ring, and prevents their association with transpeptidases[4]. Beta-lactamase enzymes produced from the gram-positive and gram-negative bacterium inactivates beta-lactam antibiotics such as penicillins and cephalosporins[5]. The production of this enzyme differs in the gram-positive and gram-negative bacteria. The negative bacteria of the gram stain produce beta-lactamase enzymes more varied compared to the gram positive one[6].

Genes of resistance beta-lactamase enzymes may be chromosomal or plasmid-carrying or transient genes[7]. They are chromosomal in most *bla*AMPC genes produced by the intestinal family[8]. Whereas,

the plasmid beta-lactamase enzymes are more common cause of resistance because of their ability to transfer from one cell to another, and widespread use of antibiotics increases the prevalence of resistance[9]. Beta-lactamase genes carried on jumping genes, it facilitates the transfer of the genetic material from one microbe to another, and some other genes that encode beta-lactamase enzymes such as *bla*VIM and *bla*IMP are present on integrin[10]. There are two dominant classification of these enzymes[2]. Classification by Ambler included four different molecular groups are A, C, and D of serine-β-lactamases (SBLs) and the class B of metallo-β-lactamases (MBLs). Class B is further divided into subclasses B1, B2, and B3, using sequence conservation data[11].

Beta-lactamase extended spectrum ESβLs were discovered in Europe in 1980 and these enzymes were isolated for the first time in 1983 from *Klebsiella* pneumonia from a patient in Germany hospital, then these enzymes became global[13]. Plasmid genes encode ESβLs, which facilitates its transmission between different bacterial species[14]. ESβLs are resistant to penicillins in addition to first, second third generation cephalosporins, and aztronam, but they are inhibited by beta-lactamase inhibitors clavulanic acid[15]. This study was aimed to investigate the isolates producing extended spectrum beta lactamase (ESBL) by conducting a sensitivity selection for beta-lactamase in accordance with WHO and CLSI recommendations.

Materials and Methods

Study setting and participants

This cross-sectional study was conducted between May 2019 to January 2020, which included 210 urine samples obtained from patients with acute urinary tract infection admitted to the Al- Shahid Ghazi and Al-Hariri Hospital in Medical City/ Baghdad. Midstream urine sample was collected from both males and females (M/F ratio 0.123) with age rang of 35-60 years (mean ± SD = 45.89 ± 7.61). These samples were evaluated for the presence of bacteria and/or leukocytes and cultured on blood agar and Eosin Methylene Blue agar mediums (Himedia Company, India).

Under aseptic conditions, urine cultures that produce more than 10^5 CFU/ml E. coli were included in this study. Gram stain and biochemical tests were used to identify the isolates[16].

Laboratory tests

Antibiotic susceptibility test

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of Gram negative uropathogenic isolates[17,18]. Antibiotic susceptibility testing (AST) was performed against most commonly used antibiotics (Turkey, Bioanalyse) using the Kirby-Bauer disk diffusion method on Mueller–Hinton agar (Himedia Company, India): Ampicillin (10mg/ml) Ampicillin – Sulbactam (10mg/ml+10mg/ml),, Amoxillin-Clavulanic (25mg/ml+10mg/ml), Cefalothen (10mg/ml), Imipenem (10mg/ml), Meropenem (10mg/ml), using disk diffusion method (Kirby – Bauer). AST results were interpreted as per the Clinical Laboratory Standards Guidelines (2016) guidelines (CLSI, 2016). Escherichia coli (ATCC 25922) were used as controls for AST.

B-Lactamase production test

Disk replacement method was used to detect the production of beta-lactamase enzymes by E. coli isolates according to David and Robert 2005[17].

Polymerase-chain-reaction-based identification of β-lactamase genes

Bacterial DNA was extracted using G- spin DNA extraction kit (iNtRON biotechnology, Diagnostic kit components, Korea) according to the instructions of the supplied company.

The presence of ESBL genes was detected by polymerase-chain-reaction (PCR) using the primers listed in table 1. These primers are freeze-dried and dissolved with distilled water, a final concentration of 100 Pmol / Ml as a stock solution and kept at -20 ° C to prepare a concentration of 10Pmol / ml as a starter suspension. Stock solution 10 ml is diluted at 90 ml Distilled water to become the final volume 100 Ml. (Integrated DNA Technologies company, Canada).

Table 1: ESBL genes detect

The specific primer 16 s RNA of gene		
Primer	Sequence	Product
Forward	5’ – AGAGTTTGATGGGTCAG- 3’	1450 Base pair
Reverse	5’- GGTTACCTTGTTACGCAG-3’	
The specific primer bla TEM of gene		
Forward	5’-GAGTATTCAATTTCCGTC- 3’	800 Base pair
Reverse	5’-TAATCAGAGGACCTATCTC- 3’	
The specific primer blaSHV		
Forward	5’-ATG CGT TATATT CGC CTG TG-3’	747 Base pair
Reverse	5’-TGC TTT GTT ATT CGG GCC AA-3’	
The specific primer blaCTX-M		
Forward	5’-ATG TGC AGY ACC AGT AAR GTK ATG GC-3’	593 Base pair
Reverse	5’-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3’	

Statistical Analysis

All variables were express as frequency. Chi square test was used to compare independent variables for ESBL genes positive and negative bacteria. The p-value < 0.05 was considered as statistically significant. For statistical analysis, SPSS 17.9 (SPSS, Cgicago, IL, USA) was used.

Results and Discussion

The biochemical tests indicated that all isolates were negative for the oxidase, Voges-Proskauer and Citrate utilization, while all were positive for the indole, Methyl red, and fermentation of lactose sugar. These results are consistent with Reddy et al., (2010) (Table 2)[18].

Table 2: Diagnostic biochemical properties of E. coli

Test	Result
Oxidase	Negative (100%)
Indole	Positive (100%)
Methyl red	Positive (100%)
Voges-Proskauer	Negative (100%)
Citrate utilization	Negative (100%)
CAMP Test	B- hemolytic (82%)
Lactose	Positive (100%)

Hemolysin is one of the factors that are secreted by many strains of E. coli. Hemolysin production by E. coli was studied and it was found that (82%) of isolates were able to produce hemolysin on human blood agar. This result agreed with study conducted by Ali (2012) [19] who found that more than 40% of E. coli elaborates α-hemolysin.

Antibacterial susceptibility to antibiotic susceptibility

The sensitivity of 210 isolates of E. coli was evaluated and the frequencies of susceptibilities were listed in table 3. These results were closed to many local studies conducted indicated that the resistance rate of E. coli to aminopenicillins was 95% and 86.4%[20,21]. The main cause of this resistance was explained by Al-Hamadani, (2013) as beta-lactamase enzymes producing strain of E. coli[21].

The results of the current study also showed that 83.81% of the isolates of E. coli bacteria are resistant to Augmentin, a combination of amoxicillin and clavulonic acid acid. Penicillin salbactam was close to Augmentin, as it reached 82.38%, all are due to beta-lactamase enzymes producing isolate that confirmed by Karlowsky et al. 2002[22].

The resistance to amoxicillin-clavulanic acid combination was reflecting the origin of the *bla*TEM which is mediated by plasmids and is responsible for the multiple resistance of antagonists[23]. The results of this study came close to the results of Subha et al. (2003) who found that 90% of their isolates were resistant to Augmentin caused by beta-lactamase enzymes production[24].

For cephalosporin antibiotics, a high resistance rate against cephalothine (91.90%) was founded due to the fact that the high affinity for protein binding (PBPs) and changes in this protein lead to resistance to these antibiotics. This result was agreed with other studies that confirm more than 85% of E coli was resistant to cephalothine[25]. Inconsistently, studies conducted by Corvec et al, who found that the resistance was 45%[26].

It is not surprising that all uropathogens isolated from patient, including ESBL-producers and non-ESBL producers, were 100% susceptible to imipenem. Earlier reports have described imipenem as the drug of choice for complicated bacterial infections such as UTI. This antibiotic has a broad-spectrum action against wide group of pathogens such as Gram positive and Gram-negative bacteria. Because of its safety and effectiveness for treatment of bacterial infection, we recommend continue use of imipenem as a therapy for severe infections [27].

Table 3: Antibacterial susceptibility test

Antibiotic	Antibacterial susceptibility		
	Susceptible	Intermediate	Resistant
Ampicillin	6 (2.86%)	3 (1.43%	201 (95.71%)
Ampicillin -Sulbactam	21(10.00%)	16 (7.62%)	173 (82.38%)
Amoxillin-Clavulanic	23 (10.95%)	11 (5.24%)	176 (83.81%)
Cefalothen	9 (4.29%)	8 (3.81%)	193 (91.90%)
Imipenem	193 (91.90%)	17 (8.10%)	0 (0.00%)
Meropenem	194 (92.38%)	3 (1.43%)	13 (6.19%)

The current study evidence that the pathological conditions were dependent on the type of agent that the microorganism possesses which is a reflection of the expression. Watts et al. (2010) demonstrated in their research that geographic regions influence the type of genotype and genes of the pathogen microscope[28]

E coli Genotypes

This study showed the presence of different genotypes that are associated with bacterial resistance. Genotype tests confirm the presence of ESBL-producing isolates in 182 (86.67%). These isolates were shown to be highly resistant to pencilins-clavulonic acid and cephalosporin antibiotics. The higher prevalence of multidrug resistance strain of E coli (92.85%) was reported by this study is agreed with many global studies[29,30]. The susceptibility pattern of the ESBL-producing isolates in this study indicates a cross resistance of these ESBLs to many other common antibiotics[31]. David and Robert, 2005 recognized poor outcome when ESBL-producing bacteria infect patients and treated by penicillin or cephalosporin[17]. Conversely, some infections due to organisms testing resistant to ceftazidime but susceptible to cefotaxime or ceftriaxone have responded to treatment with these alternate cephalosporins[32].

Research agreed studies that the prevalent genes were 86.7% and 72.2% on the sequence was CTX-M2 responsible for resistance in the ceftazidime antagonists[33,34]. However, it did not correspond to others[20,21] whose found that the dominant genes encoded on plasmids contained the TEM gene. Also, it was found that the TEM gene, which is found on most isolates plasma, is the most common in Gram-negative bacteria in Baghdad city hospitals.

Assay of genotype using PCR technique indicated that 88.57% of ESBL-producing isolates possessing *bla*TEM, 30%, *bla*CTX-M and 20.48% *bla*SHV genes between isolates. In addition, the presence of Carbapenemase-producing bacteria such as KPC and MBLs producing bacteria, represents a major threat to human health because of fails in most antibiotic therapy, were less frequent present in isolates. KPC was present in 8% of isolate and mainly associated with carbapenem resistance comparing to 1% of MBLs bearing bacteria. In parallel, we have found that the most active antimicrobial agents in vitro remained to be the carbapenems[33].

Conclusion

Result of our study showed high prevalence of ESBLs and KPC but low prevalence of MBLs in cultured bacteria from urine samples of patients with acute UTI. In addition, KPC was the main carbapenem resistance mechanism in *Klebsiella* and *E. coli* isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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