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Research Article

Presence of Extended-Spectrum β-Lactamases Genes in *E. coli* Isolated from Farm Workers in the South of London

Mohammed Fadhil Abo-Ksour

Department of Microbiology / college of science / Al-Mustansiriyah University / Iraq

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ABSTRACT

Sixty - four isolates of *E. coli* were isolated from urine, stool, and skin samples of farm workers in south of London. Antibiotic susceptibilities were tested by using the agar dilution protocol by using amikacin, ampicillin, aztreonam, meropenem, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamycin, imipenem, ofloxacin, and cefpodoxime. Determination of ESBL of *E. coli* isolates was performed according to CLSI by using two discs method. PCR was used to detect of CTX-M, TEM, and SHV genes and genotyped *E. coli* isolates which were reported as ESBL producers in both two disks and E-test methods. The results illustrated that thirty-eight isolates (n=64, 59 %) had multi drug resistance, and the resistance results recorded as the follows; imipenem (0%), meropenem (0%), ofloxacin (7%), ciprofloxacin (10%), ampicillin (89%), cefpodoxime (86%), aztreonam (82%), gentamycin (70%), ceftriaxone (31%), ceftazidime (37%), cefotaxime (62%), and amikacin (65%). Twelve of *E. coli* isolates (n=64, 18.7%) were phenotypically ESBL producers. eleven isolated of ESBL phenotype-positive *E. coli* carried *bla* genes (n=12, 91.7 %), *bla*CTX-M was found in eleven isolates (91.6 %), *bla*TEM in eight isolates (66.6 %), and *bla*SHV in one isolate (8.3 %).

Keywords: *E. coli*, ESBL, CTX-M, TEM, SHV, β-lactamases.

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) represent one of the most critical point in the bacterial resistance, and they play a significant role in the rapid spreading of resistance in the entire world[1]. These enzymes are produced gram negative bacteria and have the ability to hydrolyze cephalosporins and penicillins antibiotics[2]. In 1961, TEM is the first plasmid-mediated β-lactamases gene was discovered, followed by SHV gene, then the more important one (CTX-M) was described[3]. In 2003, the first discovered of CTX-M family in the United Kingdom as a result of repeat test of samples collected in 2000, and since that date the ESBL *E. coli* represent a rapidly developing problem in UK[4]. Generally, CTX-M gene becomes the dominant in Europe and Asia countries, as well as in most other countries over the world as it has caused outbreaks or appeared[1,4].

Due to the wide spread and random using of most β-lactam antibiotics, it's very important to determine ESBL producers according to health and economic losses which caused by them around the world[5]. Farms, groves, orchards and other placed that allow to direct contact between human and animals have high interest because they play a significant role in spreading the resistance between human to the farm animals and then create a serious way of antibiotic resistance, and for that reason, many researches and study were done yearly in the entire world are performed to record prevalence of ESBL producing bacteria in both farm animal and workers[6,7].

MATERIALS AND METHODS

Sample collection

Sixty - four isolates of *E. coli* were isolated from routinic collection of urine and stool specimens of farm workers in south of London in the previous study, the samples were collected in the period between 2nd till 28th of April 2010.

Antimicrobial Susceptibility testing

Antibiotic susceptibilities of the bacterial isolates were tested by using the agar dilution protocol according to CLSI guidelines[8]. The following antibiotic discs were used: amikacin, ampicillin, aztreonam, meropenem, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamycin, imipenem, ofloxacin, and cefpodoxime

The phenotypic detection of ESBLs

Determination of ESBL of *E. coli* isolates was performed according to CLSI by using two discs method, which based on the bacterial response to ceftazidime (30µg)/ceftazidime (µg) + clavulanic acid (10 µg) as β-lactamase inhibitor and cefotaxime (30µg)/cefotaxime (30µg) + clavulanic acid (10 µg)[9]. Standard strain *E. coli* (ATCC-25922) was used as a control in this study. The results were recorded as ESBL producers if the clear zone of the used antibiotics with clavulanic acid was 5 mm larger than its value without clavulanic acid. E-test strip with two sides (ceftazidime + clavulanic acid on the first side and ceftazidime on the other side) was used to confirm the positive results[10].

Molecular Detection of β-Lactamase Genes

PCR was used to detect of CTX-M, TEM, and SHV genes and genotyped *E. coli* isolates which were reported as

**Author for Correspondence: mohammadfadhil@trientedu.edu*

Table 1: The designed primers of ESBL genes.

Target	Primer	Primer sequence (5'→3')	Size	Temp
Bla$_{TEM}$	TEM-F	TCCGCTCATGAGACAATAACC	428	58°
	TEM-R	TTGGTCTGACAGTTACCAATGC		
Bla$_{SHV}$	SHV-F	TGGTTATGCGTTATATTCGCC	316	56°
	SHV-R	GGTTAGCGTTGCCAGTGCT		
Bla$_{CTX-M}$	CTX-F	TCTTCCAGAATAAGGAATCCC	562	56°
	CTX-R	CCGTTTCCGCTATTACAAAC		

ESBL producers in both two disks and E-test methods. The bacterial DNA of the collected isolates was extracted according to QIAamp® DNA Mini Kit[11]. Specific primers for PCR analysis of the studied genes were designed and then synthesized by Sigma company and used after suspending from lyophilized shape as a recommended by Sigma protocol, see table 1. QIAgen Company master mix was used in this study and their PCR protocol was performed, the reaction programed to contain four stages; 96° for 4 min as a primary denaturation stage, thirty-five cycles as an amplification stage each cycle contains denaturation step (96°C for 30 sec), annealing step (62°C for 30 sec), and extension step (72°C for 5 sec), followed by 72°C for 10 min the as a continuous extension stage. After that, the products were analyzed by gel electrophoresis and then examined by 336 nm UV light[11].

RESULTS AND DISCUSSION

E. coli isolates which isolated from routine collection of urine, stool, and skin specimens of farm workers in the south of London of previous study were chosen. The range of workers ages was ranging from 21 to 54 years with mean equal to 36 years, the male: female ratio of the workers was 2.3:1.

Twelve antibiotics were chosen in this study as members of the common antibiotic families that may use in treatment of *E. coli* infections according to WHO protocol[12] The results illustrated that thirty-eight isolates (n=64) had high resistance to three or more of the chosen antibiotics with percentage of multi drug resistance equal to 59.3%. Antibiotic susceptibility results showed that all *E. coli* isolates (n=64) were susceptible to imipenem and meropenem, as well as both ofloxacin (7%) and ciprofloxacin (10%) had a good activity against bacterial isolates. On the other hand, high resistance values were observed against ampicillin (89%), cefpodoxime (86%), aztreonam (82%), and gentamycin (70%). The results also recorded a moderate bacterial resistance against ceftriaxone (31%), ceftazidime (37%), cefotaxime (62%), and amikacin (65%). The statistical analysis illustrated a high significant difference of resistance values of many antibiotics among the ESBL and non-ESBL producers, like amikacin, gentamycin, ciprofloxacin and ofloxacin ($P \leq 0.001$).

Many researchers indicated that there were no significant activity of ampicillin, cefpodoxime, and gentamycin against *E. coli* isolates, the results which were carried out by the previous studies showed high resistance of *E. coli* isolates against these antibiotics[13,14]. Based on our and many other results, these antibiotics can't considered as good drugs in the treatment of *E. coli* infections although considering them as a first antimicrobial agent in the routine treatment of *E. coli* infections[14,15]. The present results of imipenem and meropenem agreed with many other previous results, most of them recorded a best activity against *E. coli* and other bacterial isolates, that good activity may come from their ability to high resistance of degradation by β-lactamase or cephalosporinases[15]. Bacterial susceptibility to fluoroquinolones (ciprofloxacin and ofloxacin) may come from difficult preventing these antibiotics to enter the bacterial cell and then binding with the target side[16]. ESBL detection results showed that twelve *E. coli* isolates (n=64, 18.7%) were phenotypically ESBL producers, they showed high resistant to ceftazidime in a level reaching to and more than nine-fold reduction in ceftazidime + clavulanic acid value, as well to cefotaxime in the same way. Out of the twelve ESBL isolates, nine of them were recovered from urine (75 %), two were recovered from stool (16.7 %) while the last one was recovered from skin (8.3 %). The results showed that seven isolates (58.3 %) came from male specimens, while the other five isolates (41.7 %) came from female specimens.

Genetic detection results of the ESBL phenotype-positive *E. coli* isolated showed that eleven isolated carried *bla* genes (n=12, 91.7 %). Among the twelve ESBL producer bacteria, *bla*CTX-M gene was found in eleven *E. coli* isolates (91.6 %), *bla*TEM gene was found in eight isolates (66.6 %), and one isolate (8.3 %) harboured *bla*SHV. The results also showed that one *E. coli* isolate (8.3 %) carried all the three tested *bla* genes (TEM, CTX-M, and SHV), eight isolates (66.6 %) carried two bla genes (TEM and CTX-M), two isolates (16.6 %) harboured one bla gene (TEM), and one of the phonotypical ESBL producer *E. coli* (8.3 %) didn't contain any *bla* genes which chosen in this study, as shown in table 2.

The genotypic results illustrated that bla TEM was the main domain gene comparing with other tested bla gene, appearance of bla CTX-M gene in eight isolates (66.6 %) agree many previous study which mentioned a high occurrence of this gene in many ESBL-producers[16,17]. It is important to detect ESBL producers in order to know the ESBL prevalence in the farm animal area and then to figure out the best way to limit the multi-drug resistance bacteria[18].

Many researches elucidated that increasing and random using of the expanded-spectrum cephalosporins in bacterial infections gave rise to prevalence of ESBL-producer bacteria[17,19]. In addition, presences of *bla* genes on bacterial plasmids play a significant role in the resistance increasing due to the ability of plasmids to transfer from one genus to other[20]. Day by day, ESBL-

Table 2: bacterial Susceptibilities of ESBL-producing isolates against antibiotics.

				Antibiotic resistance		*bla* genes		
Isolate	Source	Gender	Age	Antibiotic No	%	CTX-M	TEM	SHV
1	U	F	36	7	53.3	+	+	
2	U	F	34	6	50.0	+	+	
3	U	M	49	9	73.3	+	+	+
4	U	F	27	10	83.3	+		
5	U	M	22	8	66.6	+	+	
6	U	M	41	7	53.3	+	+	
7	St	M	40	6	50.0	+	+	
8	U	M	28	9	73.3	+	+	
9	St	M	34	5	41.6	+		
10	U	F	31	8	66.6	+	+	
11	Sk	M	50	8	66.6	+		
12	U	F	21	6	50.0			

U= urine, St= stool, Sk= skin, F= female, M= male.

producer *E. coli* become a critical case in the entire world due to decreasing therapeutic options for their infections. Absence of all detected *bla* genes in one phenotypically ESBL-producer *E. coli* isolate may come from having other *bla* gene that we didn't tested in this study, and / or developing another resistance mechanism by mutation in the target side or efflux system[21].

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