Molecular screening of oxacillinases beta-lactamase among gentamicin-resistant *Escherichia coli* isolates

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Abstract. Expansion of β-lactamases in Gram-negative rods has been documented as most severe threat to the management of infectious diseases. The ever-increasing use of antibiotics with the evolution of intrinsic and acquired resistance has led to the development of resistance mechanism in Gram-negative rods contributing to the expansion of several multi-drug resistance epidemics in hospital environment . So this study aimed to investigate genes responsible for Oxacillinases production among gentamicin- resistant *Escherichia coli* isolates. The results showed that out of 573 specimens, 270 (56.13%) showed bacterial growth versus 264(46.07%) showed no bacterial growth 309(53.92%) , among 573 clinical specimens 102(17.80%) were male patients while 471(82.19%) were female patients. According to result of the vitek-2 system recorded 110 isolates as *E .coli* . However result of gentamicin susceptibility demonstrated that 29 (26.36%) *E. coli* isolates were resistance to gentamicin compared with 39 (35.45%) and 42 (38.18%) of isolates were intermediate and sensitive to this antibiotic respectively.. Results of antibiotic susceptibility showed that the highest bacterial resistance was Tobramycin 27(93.1%) and Ciprofloxacin 29(100 %),while Nitrofurantoin 3(10.3%) had the least resistance. Results of polymerase chain reaction (PCR) amplification showed that *OXA, OXA-1 and OXA-9,OXA-10* were 29(100%),26/(89.65%),29(100%) and 20(68.96%) respectively While OXA-2 did not detect.

1 Introduction

Escherichia coli (*E. coli*) is a gram-negative, anaerobic, facultative rod that produces catalase but not oxidase. Taxonomically, it is a member of the Gammaproteobacteria class, the Enterobacteriales order, and the Enterobacteriaceae family [1]. *E. coli* is associated with most hospital- and community-acquired infections, including a variety of intestinal and extraintestinal infections, urinary tract infections, and some fatal infections in immunocompromised patients. Self-medication and inappropriate use of antibiotics have contributed to the rise in drug resistance in clinical practice over the past few decades. Even worse, the treatment of *E. coli* infections has become exceedingly difficult due to the spread of antibiotic resistance to multiple drugs[2]. Globally, urinary tract infections (UTIs) are one

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of the most prevalent community-acquired bacterial infectious diseases. While numerous UTIs possess a relatively mild course and are effectively treatable, recurrent infections with significant long-term consequences and acute complications, such as ascending pyelonephritis or bloodstream infections, are possible. Females are afflicted more frequently than males, and comorbidities (such as diabetes) have been linked with a greater likelihood of UTIs [3]. The World Health Organization (WHO) identifies antimicrobial resistance (AMR) as one of the greatest threats to global public health and asserts that the world must alter the way it prescribes and utilizes antibiotics immediately. Even if new medicines are developed, antibiotic resistance will remain a significant threat if the behavior does not change. New mechanisms of antibiotic resistance imperil the capacity to treat prevalent bacterial infections, and if we do not take immediate action, we will enter a post-antibiotic era in which common infections and minor injuries could become deadly [4].

2 Materials and methods

2.1 Collection of the specimens

In the present investigation, 573 clinical specimens were collected at random from patients with urinary tract infections (UTIs). During the period from September to December 2022, all patients were committed to Al-Najaf City's leading medical facilities, such as the Central Public Health Laboratory, Al-Sadr Medical City, and a number of leading clinical laboratories. For sample collection, consent was obtained from all patients

2.2 Isolates and bacterial diagnosis

After cleaning the genitals of patients with urinary tract infection and collecting urine samples in sterile containers, midstream urine was centrifuged at 2000 rpm for two minutes. The sediment was incubated with a brain heart infusion broth at 37 C overnight and streaked on Blood agar and MacConkey agar surfaces, which were then incubated aerobically at 37 C overnight [5]. Using ID-GN cards and the automated Vitek-2 compact system, final identification was conducted.

2.3 Detection of gentamicin resistance among *Escherichia coli* isolates and antibiotic susceptibility testing

All *Escherichia coli* isolates were initially tested against the gentamycin antibioti using gentamicin disk (10µg) (Bioanalyse,Turkey) and employed on sterile media of Mueller Hinton agar (England) The suspension of all tested isolates were achieved based on 0.5 McFarland standard. However, only gentamicin-resistant *Escherichia coli* isolates were tested on several antibiotics (Bioanalyse, Turkey), including Pipracilin (PRL, 100 µg), Amoxicillin /Clavulanate (AUG, 30 mcg), Cefatoxime(CTX 30 mg), Ceftriaxone(CRO 30 mg), Azteronam(ATM30 mg), Streptomycin(S 25µg), Tobramycin (TOB, 10 µg), Azithromycin(AZM 15), Ciprofloxacin(CIP 10mg), Gatifloxacin (GAT 30mg), Nitrofurantoin (F 300 mg)., using the disc diffusion technique according to the Kirby–Bauer [6]. The zone diameter were applied base on instructions of the Clinical and Laboratory Standards Institute (CLSI) [7].

2.4 DNA extraction and PCR assay

The instructions provided by a manufacturing company, a genomic DNA extraction micro kit (Favorgen, South Korea) was used to collect all of the nucleic acid for 29 clinical isolates of *Escherichia coli*. This was done in accordance with the manufacturer's protocol. After ensuring the integrity of the whole DNA sample by storing it in a deep freezer set to -20 degrees Celsius, a PCR analysis was carried out in order to test for the genes listed in Table 1. The equipment for gel documentation was employed for the migration of PCR amplification (bands) at 1% agarose, and then the bands were dyed with ethidium bromide at a concentration of 0.5 g/ml [8].

Table 1. Primers and condition used in the study

Primer Target	Sequence (5' to 3')	Product size	Annealing (ºC)	Reference
OXA-F	ATATCTCACTGTTGCATCTCC	618	55	[9]
OXA-R	AAACCCTTCAAACCATCC			
OXA group-I (OXA-10 group)-F	TCAACAAATCGCCAGAGAAG	276	56	[10]
OXA group-I (OXA-10 group)-R	TCC CAC ACC AGA AAA ACC AG			
OXA group –II (OXA-2 group-F	AAGAAACGCTACTCGCCTGC	478	60	[10]
OXA group –II (OXA-2 group-R	CCACTCAACCCATCCTACCC			
OXA group –III (OXA-1 group)-F	TTTTCTGTTGTTTGGGTTTT	427	51	[10]
OXA group –III (OXA-1 group)-R	TTTCTTGGCTTTTATGCTTG			
OXA-9-F	CGTCGCTCACCATATCTCCC	315	56	[11]
OXA-9-R	CCTCTCGTGCTTTAGACCCG			

3 Result and discussion

3.1 Patients and bacterial growth

Results of this study showed among 573 samples collected from urine of urinary tract infection patients which demonstrated the numbers and percentages of bacteria that grew and did not grow using different culture media was 264(46.07%)bacterial growth compared with 309(53.92%)no bacterial growth, among 573 clinical specimens 102(17.80%) were male patients while 471(82.19%) were female patients. The results of biochemical tests, Vitek-2

system showed among 573 specimens, 110 isolates were identified as *Escherichia coli* isolates. *Escherichia coli* isolates were mostly observed according to the Sex . This result is comparable to that measured in the Middle East Studies that included as the following study that conducted by Hassouna. *et al* ., [12] in Egypt and shown the study enrolled 583 patients with clinically diagnosed UTIs. Uropathogens were found in 400 urine samples (68.6%) out of which 134 *E. coli* isolates were identified. While in the Riyadh region of Saudi Arabia, a study was conducted by Abalkhail. *et al*., [13] where 2250 urine samples were collected from patients with Urinary Tract Infections (UTIs), *E. coli* accounted for 1523 isolates. Locally, several studies were conducted, including the following: A study was conducted in the city of Najaf by Najm. *et al* ., [14] it was found that the spread of *E. coli,* which causes urinary tract infections, is: It is more common among females than among females. in males. The percentage of females was 71% compared to 20% for males. As it was observed in the City of Zakho in Iraq also, the percentage was higher in females than in males (90.78% and 9.22%), respectively [15]. Many studies have been conducted by researchers from all over the world, including a study conducted by the scientist in Turkey by Caskurlu. *et al*,. [16] where a total of 9556 positive urine cultures were included, the number of *Escherichia coli* out of this total number was 6154 isolates and thus *E. coli* was ranked The first among Gram-negative bacteria, followed by *Klebsiella* and its prevalence was higher in females than males (70.6 and 53.4%, respectively). Also in Ethiopia, a study was conducted by Biset. *et al*., [17] and they was found that the rate of *E. coli* was higher compared to the rest of the bacteria that cause urinary tract infections, and the rate was (49.2%).

3.2 Antibiotic Susceptibility of gentamicin- resistance *Escherichia coli*

Results showed from among 110 (100%) Escherichia coli isolate and by using disk diffusion According to Kaurby-baur methods for gentamicin antibiotic result only 29(26.36%) Escherichia coli isolates were resistance to gentamicin, while 39 (35.45%)and 42 (38.18%) of isolates were intermediate and sensitive to this antibiotic respectively. As show table 2

Table 2. Gentamicin Susceptibility *Escherichia coli* of isolates

Bacterial name	**Total**		**Resistance**		**Intermediate**		**Sensitive**	
	110(100%)		29(26.36%)		39 (35.45%)		42 (38.18%)	
E. coli	M	F	M	F	M	F	M	F
	23 (20.90%)	87 (79.09%)	6 (5.45%)	23 (20.90%)	10 (9.09%)	28 (25.45%)	7 (6.36%)	35 (31.81%)

F, female; M, male

There were several previous studied showed elevation of gentamicin resistance among *E. coli*, however, , In a previous study conducted by Abdelwahab *et al*., [18] ,they recorded that 139 (85%) of 165 *E. coli* isolates were discovered to be resistance to gentamicin. While In a study done by Jalil, and Al Atbee, ., [19] ,they observed that 63.2% *E. coli* isolates were resistance to gentamicin drug. The rate of gentamicin resistance rates of *E. coli* isolates that cause UTIs varied in across all studies, which could be attributed to a variety of variables, including the population being studied and place of residence.

3.3 Antimicrobial susceptibility testing of gentamicin-resistant *E. coli* isolates

The results in Table 3 show that 25 (86.2%) isolates of gentamicin-resistant *E. coli* were resistance to piperacillin in a closrly study in southern Iraq by Mohammed. *et al*., [20] who found that resistance of *E . coli* to piperacillin was (92%). while the members of Cefatoxime and Ceftriaxone revealed resistance rate 25(86.2%)and 22(75.9%)respectively. Also 20(68.97%) of isolates was resistance to Aztreonam. The resistance of bacteria toward Amoxicillin-Clavulanic acid was 17(58.6%). The results showed that 12(41.4%)and 27(93.1%) of isolates was resistance Streptomycin and Tobramycin respectively. Rate of resistance Nitrofurantoin was 3(10.3%) this result is similar to the result obtained by Hussein and Saleh [21] where he also noticed a decrease in the percentage of resistance, and the reason is due to it may be related to Nitrofurantoin as it has multiple mechanisms of action, requiring organisms Living organisms develop more than one mutation in order to develop resistance. while rate of ciprofloxacin resistance was 29(100%), This result was convergent with a previous study by Daoud. *et al*., [22] in Tunisia which recorded a percentage89.2%**.** This pathogen showed that 14(48.3%)was resistance Gatifloxacin This result is less than the result obtained by Gururaju. *et al*., [23] in India who found that resistance of *E. coli* isolates Gatifloxacin was 77 %..

Table 3. Antibacterial agent susceptibility of gentamicin-resistance *E. coli* isolates.

Antimicrobial agent	Sensitive	Intermediate	Resistance
Pipracilin	1(3.4%)	3(10.3%)	25(86.2%)
Amoxicilllin – clavulanic acid	5(17.2%)	7(24.1%)	17(58.6%)
Cefatoxime	4(13.8%)	0(0.0%)	25(86.2%)
Ceftriaxone	7(24.1%)	0(0.0%)	22(75.9%)
Aztreonam	6(20.69%)	3(10.3%)	20(68.97%)
Streptomycin	6(20.69%)	1(3.4%)	12(41.4%)
Tobramycin	1(3.4%)	1(3.4%)	27(93.1%)
Azithromycin	9(31.0%)	2(6.9%)	18(62.1%)
Ciprofloxacin	0(0.0%)	0(0.0%)	29(100 %)
Gatifloxacin	10(34.5%)	5(17.2%)	14(48.3%)
Nitrofurantoin	23(79.3%)	3(10.3%)	3(10.3%)

3.4 Detection of *OXA* - Type genes among gentamicin-resistance *Escherichia coli* isolates

Results of PCR showed that *OXA, OXA-1 and OXA-9,OXA-10* were 29(100%),26/ (89.65%), 29(100%) and 20(68.96%) respectively (figure 1,2,3 and 4). while *OXA-2* gene was absent. locally there were several molecular studies observed and recorded distribution of *OXA* gene among different isolates of gram-negative bacteria. A local study done in Al-Najaf City/Iraq by Hayder, and Hassan, [24]they recorded the frequency rate of *OXA* gene was 22 (73.33 %) among *K. pneumoniae* isolates while Aziz (2015) mention that according to data of PCR recorded rate reached 38(71.7%) of *OXA* gene among isolates of *K. pneumoniae*. At same respect another local study done in Baghdad by Alzaidi, and Mohammed, [25]they observed (33.3%) of *Enterobacter cloacae* had been *OXA-1* gene while *OXA-2* and *OXA-10* genes not detected among this pathogen. However**,** the frequency of this gene locally among gram-negative pathogen be accelerated a local work achieved in Wasit Province/ Iraq by Al-Mayahie and Al Kuriashy, [26]observed percentage of *OXA* gene among isolates of *E. coli* was 32.9% . while another local work in Al-Najaf City done Tuwaij *et al.,* [27] they recorded *OXA* gene at high rate. This results revealed high rates of some

OXA-type genes among clinical isolates of gentamicin-resistant *E. coli* and this regard concern in Health institutes in Al-Najaf City, Iraq, therefore, appropriate strategies and solutions must be put in place to reduce the spread of these genes .

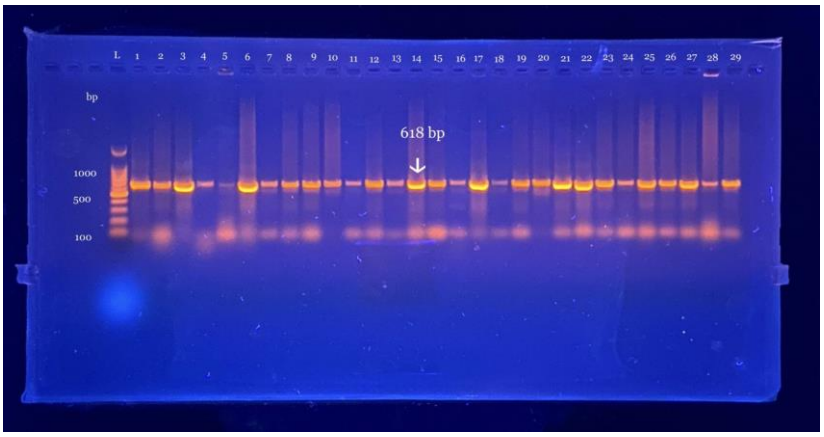

Fig.1. PCR amplification of *OXA* gene among 29 gentamicin-resistant *Escherichia coli* isolates.

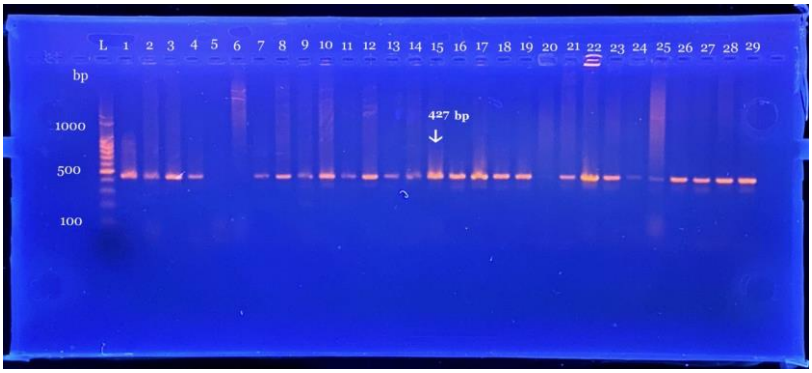

Fig. 2. PCR amplification of *OXA-1* gene among 29 gentamicin-resistant *Escherichia coli* isolates.

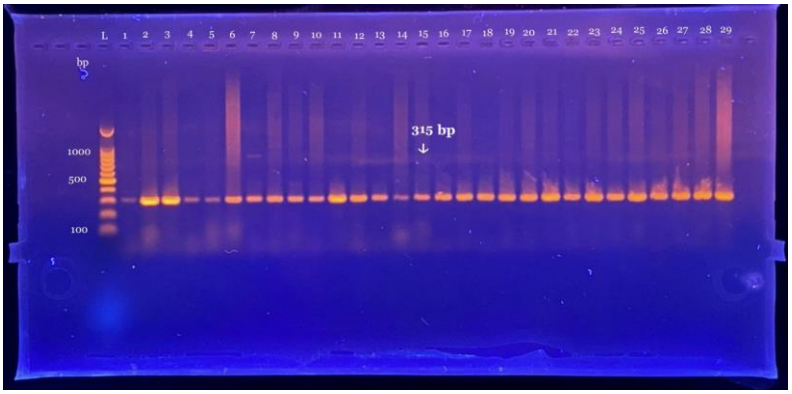

Fig.3. PCR amplification of *OXA-9* gene among 29 gentamicin-resistant *Escherichia coli* isolates.

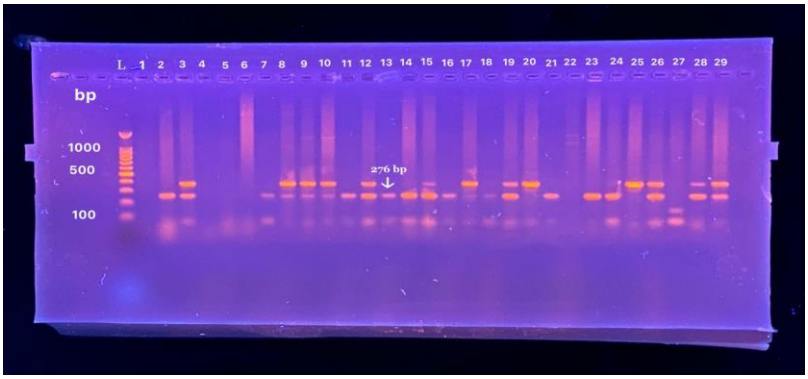

Fig. 4. PCR amplification of *OXA-10* gene among 29 gentamicin-resistant *Escherichia coli* isolates.

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