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Detection of OxqAB Genes in ESBLs Producers of Clinical Isolates of Enterobacteriaceae

Conflict of interest: nothing to declare.
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Abstract

Introduction. OqxAB efflux pump confers resistance to various antibiotics, detergents, disinfectants, and antiseptics in addition to quinolones. There is a difficulty in treating many illnesses caused by the distribution of OqxAB among clinical isolates of ESBL-producing Enterobacteriaceae.
Purpose. To evaluate the existence of efflux pump genes (OqxA and OqxB) in these bacteria that contain ESBLs due to the dissemination of ESBLs with the establishment of resistance to quinolones in the same bacterial isolates of the Enterobacteriaceae family.
Materials and methods. One hundred and twelve of Enterobacteriaceae isolates were collected from 79, 13, 1, 5, 4, 5, 3 and two samples of urine, stool, blood, sputum, abscess, wound, vaginal swabs and bronchial, respectively, these bacteria distributed to 54, 10, 22, 14, and 10 of Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp. and Shigella spp. Respectively. All samples have been cultured on MacConkey agar and blood agar and incubated for 24 h at 37 °C. Isolates were identified based on Morphological and biochemical tests (Macfaddin, 2000), the diagnosis was confirmed using API 20E and VITEK 2 system (Biomerieux, France).
Results. The results showed 94 (83.93%) of isolates had β-lactamases, and the percentages of bacteria that produced ESBLs were 79.38% and 68.04% by screening and confirmatory tests, respectively. Our findings of PCR revealed that all (100%) isolates of ESBLs producers were carriers of efflux pump (OqxA and OqxB) genes.
Conclusion. The presence of efflux pump genes (OqxA and OqxB) in these bacteria that carry ESBLs was investigated. We concluded that, the prevalence of Enterobacteriaceae isolates that carrying ESBLs with efflux pump genes (OqxA and OqxB) in our hospitals. There were many Enterobacteriaceae isolates in our hospitals that carried ESBLs with the efflux pump genes OqxA and OqxB. Spreading of these isolates may be interfere with the treatment of different infections.
Keywords: enterobacteriaceae, efflux pump, OqxA, OqxB, β-lactamases, fluoroquinolones

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Выявление генов OxqAB в клинических изолятах Enterobacteriaceae, являющихся продуцентами ESBL

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Резюме

Введение. Эффлюксный насос OqxAB обеспечивает устойчивость бактерий к различным антибактериальным агентам, детергентам, дезинфицирующим средствам и антисептикам, а также к хинолонам. Распространенность OqxAB в клинических изолятах ESBL-продуцирующих Enterobacteriaceae является причиной затруднений в лечении многих заболеваний.

Цель. Оценить наличие генов эффлюксных насосов (OqxA и OqxB) у ESBL-продуцирующих бактерий в связи с распространением ESBL, сопровождающимся формированием устойчивости к хинолонам у одних и тех же бактериальных изолятов семейства Enterobacteriaceae.

Материалы и методы. Всего было получено 112 изолятов Enterobacteriaceae из образцов мочи (79), кала (13), крови (1), мокроты (5), содержимого абсцессов (4), ран (5), вагинальных и бронхиальных мазков (3 и 2 соответственно). Были выявлены следующие бактерии: Escherichia coli (54), Klebsiella spp. (10), Proteus spp. (22), Enterobacter spp. (14) и Shigella spp. (10). Все образцы культивировали на агаре МакКонки и кровяном агаре и инкубировали в течение 24 ч при 37 °C. Изоляты были идентифицированы на основе морфологических и биохимических тестов (Macfaddin, 2000), результаты были подтверждены с помощью тест-системы API 20E и анализатора VITEK 2 (Biomerieux, Франция).

Результаты. Согласно результатам исследования 94 (83,93%) изолята содержали β-лактамазы, а процент бактерий, продуцирующих ESBL, составил 79,38% и 68,04% по результатам скринингового и контрольного тестов соответственно. С помощью ПЦР-анализа было выявлено, что все (100%) изоляты продуцентов ESBL были носителями генов эффлюксного насоса (OqxA и OqxB).

Заключение. Было исследовано наличие генов эффлюксных насосов (OqxA и OqxB) у ESBL-продуцирующих энтеробактерий. По итогам исследования был сделан вывод о распространенности в стационарах нашей страны изолятов Enterobacteriaceae, продуцирующих ESBL, с генами эффлюксного насоса (OqxA и OqxB). В больничных учреждениях часто выявляют ESBL-продуцирующие изоляты Enterobacteriaceae с генами эффлюксного насоса OqxA и OqxB. Распространение энтеробактерий может затруднять лечение различных инфекционных заболеваний.

Ключевые слова: энтеробактерии, эффлюксный насос, OqxA, OqxB, β-лактамазы, фторхинолоны

■ INTRODUCTION

In Denmark, plasmid-carrying MDR-encoded Efflux Pump (OqxAB) was discovered in E. coli in 2004. In the past year, research have found that the OqxAB gene has spread more widely throughout the Enterobacteriaceae family [1]. OqxAB efflux pump confers resistance to various antibiotics, detergents, disinfectants, and antiseptics in addition to quinolones [2]. The extended-spectrum cephalosporins and fluoroquinolones have historically been regarded as the best treatments for acute gastroenteritis brought on by enteric infections [3]. The genes encoding OqxA and oqxB, are clustered together in an operon. Drug resistance, including to fluoroquinolones (FQ), is caused by the OqxAB efflux pumps [4]. The family of resistance nodulin division (RND), which includes the efflux pumps AcrAB and OqxAB, is a key factor in the development of antibiotic resistance in G-bacteria, particularly Klebsiella pneumoniae. Effectiveness pump Two domains make up OqxAB: OqxA, a periplasmic component, and OqxB, a transmembrane protein, both of whose genes are found in plasmids and chromosomes [5].

In the past year, research on the coexistence of OqxAB and ESBL genes from the same bacterial isolate with plasmid-mediated quinolone resistance (PMQR) has increased [6, 7]. The found of PMQR genes were significantly associated with extended spectrum β-lactamases genes, this may be due to plasmids that carriage these resistance in Enterobacteriaceae [8]. Seo and Lee [9] found a high prevalence of PMQR genes (OqxAB, qnrA, qnrB, qnrC, qnrD, qnrS, qepA and aac(6)-ib-cr among ESBLs producing Enterobacteriaceae hospital.

■ PURPOSE OF THE STUDY

To evaluate the existence of efflux pump genes (OqxA and OqxB) in these bacteria that contain ESBLs due to the dissemination of ESBLs with the establishment of resistance to quinolones in the same bacterial isolates of the Enterobacteriaceae family.

■ MATERIALS AND METHODS

A-Isolation and Identification of Isolates

One hundred and twelve of Enterobacteriaceae isolates were collected from 79, 13, 1, 5, 4, 5, 3 and two samples of urine, stool, blood, sputum, abscess, wound, vaginal swabs and bronchial, respectively, these bacteria distributed to 54, 10, 22, 14 and 10 of Escherichia coli, Klebsiella spp., Proteus spp., Enterobacter spp. and Shigella spp. Respectively. All samples have been cultured on MacConkey agar and blood agar and incubated for 24 h at 37 °C.

Isolates were identified based on Morphological and biochemical tests (Macfaddin, 2000), the diagnosis was confirmed using API 20E and VITEK 2 system (Biomerieux, France).

B-Detection of β-Lactamases

The test has been carried out by method iodine method of WHO (2003).

C-Phenotypic Methods for Detection of ESβLs

The procedure followed the guidelines of CLSI (2020).

1 – Secreening test.

Two discs containing ceftazidime (30 µg) and cefotaxime (30 µg) was used, if the diameter of the inhibition zone around ceftazidime was <22 mm or the zone around cefotaxime was <27 mm.

2 – Confirmation test.

The Double Disk Synergy Test (DDST) was used to detection of ESBLs. Augmentin (amoxicillin 20 mg, clavulanate acid 10 mg) was placed in the middle of an inoculated Muller Hinton agar plate and incubated with cefepime (30 µg), aztreonam (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) at 30 mm apart (center to center).

The observation of a distinct extension of the edge of the cephalosporins disk inhibitory zone toward the disk of Augmentin, which was interpreted as synergy indicated the production of ESBLs by isolates.

D-Detection of Efflux Pump OqxAB Gene by PCR Technique

1 – DNA Extraction.

Genomic DNA was isolated using the Trans Gen Biotech (China) Easy Pure® Bacteria Genomic DNA Kit.

2 – Polymerase Chain Reaction (PCR).

Genes for the efflux pump (OqxAB) were found using PCR. Tables 1 and 2 contain lists of primers and all PCR thermal cycling settings, respectively. PCR results were placed onto a 1.5% agarose gel for electrophoresis analysis, and the result was seen under a UV-Transilluminator [10].

Table 1
Sequence of primers that used in this study

No.	Primer	Product size (bp)		Primer Sequence `(5'-3')	Reference
1	OqxA	392 bp	F	CTCGGCGCGATGATGCT	[10]
			R	CCACTCTTCACGGGAGACGA	
2	OqxB	512 bp	F	TTCTCCCCCGGCGGGAAGTAC	
			R	CTCGGCCATTTTGGCGCGTA	

Table 2
Thermocycling conditions of genes that used in the present study

	PCR Steps	Time	Temperature
1	Initial denaturation	5 min	95
2	Denaturation	30 sec	95
3	Annealing	30 sec	52
4	Extension	45 sec	72
5	Final extension	5 min	72
6	Hold	2 min	95

■ RESULTS

β-lactamases Producers of Enterobacteriaceae

The study appeared that 84.82% of enterobacterial isolates were β-lactamases producers. E. coli was showed higher percentage among β-lactamases producers. It recorded 92.59%, table 3.

Extended Spectrum B-Lactamases (Esbls) Production Enterobacteriaceae

The findings of this study showed, the enterobacterial isolates that production ESBLs by screening test registered 79.38% and 68.04% by confirmatory Test, table 4.

Table 3
Percentages of β-lactamases producers isolates

B-Lactamase producer / Bacteria	Positive		Negative		Total	
	No.	%	No.	%	No.	%
E. coli	50	92.59	4	7.41	54	48.21
Klebsiella spp.	19	86.36	3	13.63	22	19.64
Shigella spp.	6	60.00	4	40.0	10	8.93
Proteous spp.	11	78.57	3	21.43	14	12.50
Citrobacter spp.	2	100	0	0.00	2	1.79
Enterobacter spp.	7	70.00	3	30.00	10	8.93
Total	95	84.82	17	15.18	112	100

Table 4
Percentages of ESBLs producers Enterobacteriaceae by phonotypic tests

ESBLs producers / Species	Screaming Test				Confirmatory Test				Total	
	Positive		Negative		Positive		Negative			
	No. &%		No. &%		No. &%		No. &%		No.	%
E. coli	44	88.00	6	12.0	38	76.0	12	24.0	50	51.55
Klebsiella spp.	18	94.73	0	0.00	16	84.21	2	1.05	19	13.40
Shigella spp.	4	66.67	2	33.33	3	50.0	3	50.0	6	6.19
Proteous spp.	7	63.64	4	36.36	6	54.55	5	45.45	11	11.34
Citrobacter spp.	1	50.0	1	50.0	0	0.00	2	100	2	2.06
Enterobacter spp.	3	42.86	7	100	3	42.86	7	57.14	7	7.22
Total	77	79.38	20	20.62	66	68.04	31	31.96	97	100

Molecular Detection of Efflux Pump Gene (Oqxa and Oqxb) in Enterobacteriaceae

All ESBLss producers isolates of Enterobacteriaceae of this study were tested to detect efflux pump gene (OqxA and OqxB) by conventional PCR, the outcomes revealed that, all (100%) of enteric bacteria carried these genes. Table 5, fig. 1, 2 depicted the bands 392 bp and 512 bp of DNA for isolates with similarity to the OqxA and OqxB gene sequence respectively, table 5, fig. 1, 2.

Table 5
Numbers and percentages of bacterial isolates with OqxA and OqxBgenes

Type of bacteria	Total Number	No.(%) of isolates with OqxA	No. (%) of isolates with OqxB
E. coli	38	38 (100%)	38 (100%)
Klebsiella spp.	16	16 (100%)	16 (100%)
Enterobacter spp.	3	3 (100%)	3 (100%)
Proteus spp.	6	6 (100%)	6 (100%)
Shigella spp.	3	3 (100%)	3 (100%)
Total	66	66 (100%)	66 (100%)

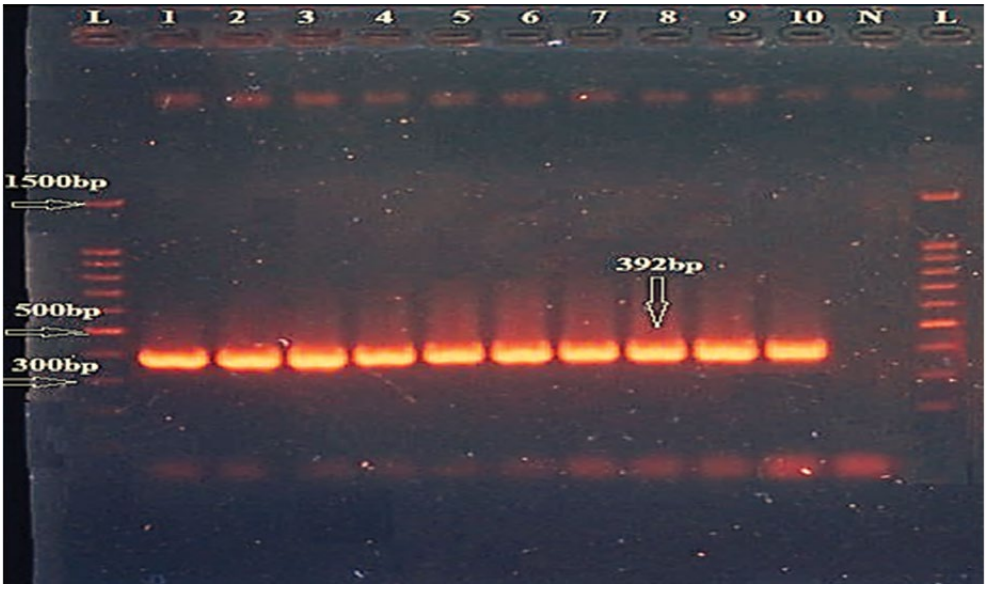

Fig. 1. A 392 bp PCR product using the OqxA primer was electrophoresed on a gel at 59°C using agarose 1.5% for 10 minutes at 100 volts, followed by 60 minutes at 75 volts. Lane L: DNA ladder (100–1500 bp), Lanes (1–10) indicated positive results, and Lane (N) represented negative control when visualized under ultraviolet light following staining with ethidium bromide

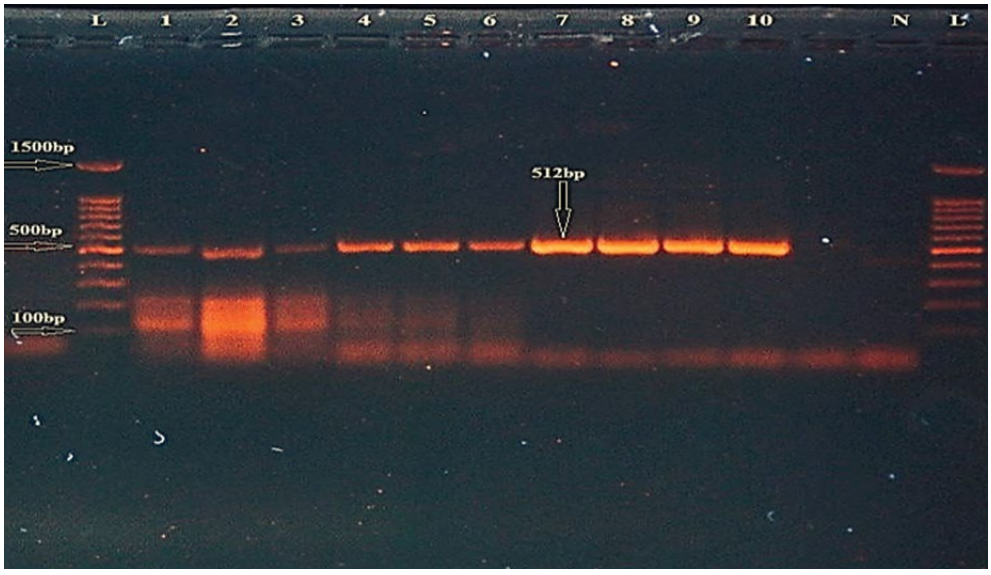

Fig. 2. A 512 bp are visible at 61 C in the gel electrophoresis for the PCR product of the (OqxB primer) (Agarose 1.5%, 10 min. at 100 volts, then decreased to 75 volts, 60 min.). Lane L: DNA ladder (100–1500 bp), Lanes (1–10) indicated positive results, and Lane (N) represented negative control when visualized under ultraviolet light following staining with ethidium bromide

■ DISCUSSION

ESBL-E bacteria cause various infections in respiratory infections, urinary tract infections, and infections of the central nervous system [11]. The phenotypic results of this study appeared 79.38% and 68.04% of Enterobacteriaceae isolates had ESBLs by screening and confirmatory tests, respectively. These finding were partial similar with The results of Sahel and Abid, [12] that showed 55 (80.88%) of isolates were resistant to cefotaxime and ceftazidime and ESBL positive during the initial screening and 17 (25%) isolates producing ESBLs, with confirmatory test (DDST). Some previous studies on some hospitals in our cities showed the prevalence of these enzymes among Gram-negative bacteria, Flayyih and Abid, [13] found 90% and 35% of Acinetobacter baumannii produced ESBLs by screaning and confirmatory tests, respectively, the study of Abid and Fayad, [14] showed 50% of E. coli that isolated from different clinical specimens had ESBLs Plasmid-encoded Extended-Spectrum beta-lactamases (ESBLs) reduce the efficacy of third generation cephalosporins, which are routinely used as empirical treatments for Gram negative bacterial infections [15]. Over the past ten years, the OqxAB efflux pump, a plasmid-mediated quinolone resistance (PMQR) element, has spread widely among Enterobacteriaceae [16]. The study of Viana et al. [17] Since the spread of PMQR genes found in Brazilian clinical isolates, and their coexistence with ESBL genes highlights the complexity of plasmid-mediated resistance determinants in Enterobacteriaceae. The results of the current study showed that a high percentage of isolates producing ESBLs were carriers of both genes (OqxA and OqxB). These findings were agreement with Azargun et al. [18] They reported a significant number of PMQR determinants among the ESBL producing Enterobacteriaceae isolates from their hospital, somewhat in agreement with earlier investigations, Perez and Van Duin. [19] quinolone resistant and quinolone-susceptible K. pneumoniae collected from northeast Ohio had 100% of their oqxAB genes identified,` Rodríguez-Martínez et al. [20]. They discovered that 87 (76.3%) and 85 (74.6%) of 114 clinical isolates of K. pneumoniae that produced ESBL were tentatively categorized as positive for oqxA and oqxB, respectively. There was a significant correlation between PMQR genes and the ESBL genes blaTEM-116, blaCTX-M-15, and others [19]. Our results were in conflict with other studies [21]. Albornoz et al [2] found that 42% of clinical resistant E. coli isolates were positive for both genes (OqxA and OqxB) and study of Kim et al. [22] 0.4% of E. coli isolates and 4.6% of E. cloacae isolates tested positive for both the oqxB and oqxB genes, according to a study that looked at the incidence of plasmid-encoded multidrug efflux pumps in clinical isolates of the Enterobacteriaceae family. The different outcomes could be a result of the different geographic regions, bacterial types, sample types, and antibiotic administration techniques [23].

■ CONCLUSION

There were many Enterobacteriaceae isolates in our hospitals that carried ESBLs with the efflux pump genes OqxA and OqxB. Spreading of these isolates may be interfere with the treatment of different infections.

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