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First description of OXA-48 Gene among Carbapenems-Producing Enterobacteriaceae (CPE) Bacteria Isolated from Basrah Environment in Iraq

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Abstract: The widespread of carbapenemase-making Enterobacteriaceae (CPE) is a considerable issue for healthcare worldwide. The presence of the OXA-48 gene in Enterobacteriaceae in environments in the Basrah is unknown. This research targeted to check for the existence of the OXA-48 gene in swage water. From 543 isolates from different samples collected eight bacterial enterobacteriaceae strains were isolated from swag water including which give positive growth to MacConkey agar supplement with impenem and showing decreased susceptibility to carbapenem using VETK2 system. Isolates were treated for antimicrobial sensitivity examination with ChromID Carb agar RAPIDEC CARBA NP and MHT test. OXA-48 gene elements were examined via PCR amplification and sequencing. This research included eight carbapenem-resistant Enterobacteriaceae strains known as Escherichia coli Klebsiella pneumonia OXA-48 Carbapenemase genes recognized in five isolates. This study showed the existence in swage water environments of OXA-48-like-making Enterobacteriaceae and highlighted the possible function of sea environments as clinically important antimicrobic-resistant bacteria reservoirs as well as the possibility to widespread all over the population.

Keywords: OXA-48 gene, Enterobacteriaceae, Basrah environment, carbapenems, OXA

B-lactamases has the ability to hydrolyze carbapenemases.. They are associated with the lactamases of three molecular groups, Ambler type A, B, and D (Bush and Jacoby 2010) Both b-lactam antibiotics are inactivated by classic carbapenemases such as KPCs and only partially suppressed by beta-lactamase inhibitors such as boronic acid, tazobactam and clavulanic acid. All beta-lactams except aztreonam which can be hydrolyzed by Metallo-beta-lactamases (MBLs), are present in Class B enzymes and are blocked by metal chelators, such as EDTA (Meletis 2016) in vitro. The OXA (oxacillinase) family includes class D carbapenemases. Carbapenems are weakly hydrolyzed and poorly inhibited by clavulanate (Jeon et al 2015). The production of OXA-48 variants is growing and currently known are OXA-162, OXA-163, OXA-181, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-370 (Dortet et al 2015). OXA-48 hydrolyzes 1st generation like penicillin, cephalosporins, and carbapenems resist beta-lactamase suppressers and slightly effective against cephalosporins such as cefotaxime or ceftazidime of the second and third generations, and only partly hydrolyzes carbapenems (Poirelet et al 2012). Nevertheless, several enterobacteriaceae which produce OXA-48 co-produce an ESBL, which negatively impacts every b-lactam antibiotic regularly (Cantón et al 2012). In the Enterobacterales, OXA-48 is the most prevalent carbapenemase, also is one of the most dominant carbapenemases (Hamprecht et al 2019). Carbapenem-resistant gram-negative bacteria were observed as cause of human infection (Hrenovic et al 2017). There are different studies on clinically isolated in Iraq (Cantón et al 2012, Poirel et al 2012, Abas and Al-Hamdani 2017, Abas and Al-Hamdani 2017) but there is no report on the detection of the OXA-48 gene in the environment, so this is the first study in Basrah, Iraq.

MATERIAL AND METHODS

Bacterial strains and susceptibility testing: Isolates were collected from different water sections like river water, sewage water, and tape water cultured on MacConkey agar (Table 1). There were identified by using VitEK2 and antibiotic susceptibility testing was performed by VITEK 2 AST-N326 cards (BioMerieux, Inc.). Phenotypic methods for carbapenemase-producing detection Modified Hodge Test (MHT) was used. A 0.5 MacFarland turbidity mixture of the *E. coli* ATCC 25922 and *E. coli* strain was set and injected into the Muller Hinton Agar medium. The medium was dried and a

disk of meropenem (10µg) was cited in the center of the test zone. Using a straight-line swab, the test strain was distributed from the middle edge to the center and this was repeated with each of the test strains plus the control strains (positive and negative) in different directions. The samples have been incubated overnight at 35°C +/- 2°C. After incubation if bacterial growth in the form of flooding occurred at the intersection of the *E. coli* 25922 inhibition zone were observed confirmed strain. Carbapenemase-producing Detection by CHROMID®.CARBA agar the CHROMID®CARBA agar (Ref 414012) bi-plate comprises one-half specific for blaOXA-like producing organisms while the other half is specific for blaVIM, blaNDM, blaKPC, and blaIMP producing organisms.

Carbapenemase-Producing Detection by RAPIDEC®.CARBA NP

The RAPIDEC®.CARBA NP is a rapid test strip and uses the concepts of the Carba NP test, a novel phenotypic carbapenem hydrolysis test. As instructed by the producer, was achieved. from overnight incubated Mueller-Hinton agar plates,. The loopful (10 loops) of a bacterial colony was selected and combined into an API combination medium (supplied in the kit) and the bacterial combination was subsequently placed into wells with a check strip and incubated at 37°C. 30 minutes and 2 hours later and if possible, the optical analysis of the test strip was done. A +ve related to a shade alteration from red to yellow-orange, while a-ve results were shown by red color (Bush and Jacoby 2010).

PCR amplification: By using a plasmid extraction mini kit as per the manufacturing instructions (Bioneer Company, Korea), DNA was extracted from the isolates. Amplification has been completed in a 20µl amount as suggested by the instruction of Promega Master mix. a monoplex-PCR have been run applying the primers OXA-48 gene (238 bp: F/"5GCTTGATCGCCCTCGATT-3' and R/"5-GATTTGCTCCGTGGCCGAAA -3') To amplify the genes encoding carbapenemases, PCR amplification (Claver. England) has been performed on a thermal cycler as follow, Denaturation at 94 ° C over 3 min, then by 35 cycles at 94 ° C for 45s, 57 ° C for 35 sec, and later 72 ° C for 35 sec with final incubation at 72 ° C for 7 min, those have been the cycle conditions for amplification. Amplified results identified by electrophoresis of agarose gel in 1% Tris-borate-EDTA (TBE) agarose (Promega, USA) and ethidium bromide staining. By using a gel documentation method (Claver, England), the electrophoresis result was observed.

DNA sequencing: DNA sequencing method was performed for genotyping and phylogenetic analysis study of bacteria isolates. The sequencing of the PCR product 238bp, for OXA-48, gene respectively, the PCR product was purified by using specialized Kit.

Table 1. Sources of different isolates

Sample sources	Number	Positive growth on Maconkey agar with Impenem	Negative growth on Maconkey agar with Impenem
River water	185	0	185
Swage water	234	8	226
Tape water	124	0	124
Total	543	8	535
Percentage		1.47	98.52

RESULTS AND DISCUSSION

MacConkey agar with 1g/L Impenem was used in selected carbapenme-resistant isolates. Several studies have reported using 1-2 mg/L imipenem-containing media might be very large for the effective recognition of carbapenemase producers. From 543 isolates from different environments samples collected eight bacterial strains were isolated from swag water including which give positive growth in these three *E. coli* and five in *K. pneumonia*.

Table 2. Antibiotic susceptibility test of eight bacterial strains

Isolates	Antibiotic														
	PRL	TPZ-TZP	CAZ	FEP	ATM	IMP	MEM	AK	CN	NTE	TOP	CIP	LVE	TE	SXT
E. coli 1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E. coli 2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E. coli 3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K. pne 1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K. pne 2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K. pne 3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K. pne 4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
K. pne 5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

For more detection of carbapenem-resistant, used three phenotic methods recommended by CLSI (Bush and Jacoby (2010) was used (Table 2). In the Priperacillin (PRL), Piperacillin/Tazobactam (TPZ-TZP) Ceftazidime (CAZ) Cefepime, (FEP) Aztreonam (ATM), imipenem (IMP) Meropenem (MEM), Amikacin (AK) Gentamicin (CN) Netilmicin (TOP), Ciprofloxacin (CIP), Levofloxa (LVE), Tetracycline (TE) Trimethoprim/ Sulfamethoxazole (SXT) all isolates were highly resistant to all types of antibiotics. In present study, best method to detect carpbenem resistant bacteria was the RAPIDEC CARBA NP test. This kit is highly sensitive and specific and provides a practical way for the early identification of multidrug-resistant Gram-negative bacteria producing carbapenemase (Garg et al 2015, Shinde et al2017, Tijet et al 2013). ChromID Carb agar and MHT are also the best method but less sensitivity and specificity in detecting carpbenem resistant bacteria gene(Gniadek et al 2016 ,Abas and Al-Hamdani 2017).

Plasmid DNA was obtained from the isolates by applying a plasmid extraction mini kit according to the manufacture's guidelines (Bioneer Company, Korea). The eight isolates were harbing plasmid DNA because they show high resistance to antibiotics and plasmids play a vital function in the development resistance of bacterial antibiotics, distributing resistance genes along with the highly worrisome clinical pathogens (Alekshun and Levy2007). The monoplex-PCR have been run applying the primers OXA-48 gene (238 bp: F/ "5GCTTGATCGCCCTCGATT-3' and R/"5-GATTTGCTCCGTGGCCGAAA -3') to amplify the genes encoding carbapenemases,

OXA-48 gene founded in five isolates (fFgure 1). Out of the five samples that were harbor with resistance genes (OXA-48), three are *K. pneumoniae* and two are *E. coli*. In *K. pneumoniae and E. coli* the most common detection is of OXA-48., but other Enterobacterial coli may also occur (Khajuria et al 2013, Manageiro et al 2014). The presence of this gene in environmental samples maybe because of the

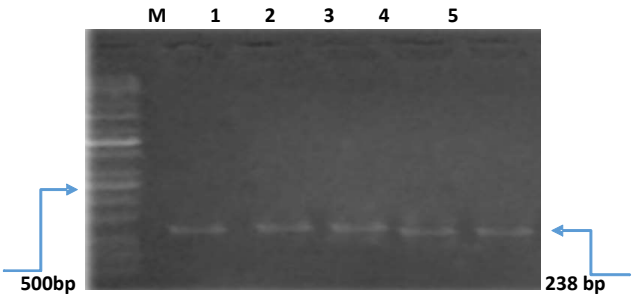

Fig. 1. Detection of OXA-48 gene by PCR amplification fragments

Table 3. Detection of carbapenem-resistant, used three phenotic methods recommended by CLSI

Isolates	Test		
	Rapidec Carba NP test	ChromID Carb agar	MHT
E. coli 1	+	+	+
E. coli 2	+	-	+
E. coli 3	+	+	-
E 3. *K. pneumia* 1	+	+	-
K. pneumia 2	+	+	+
K. pneumia 3	+	+	-
K. pneumia 4	+	+	+
K. pneumia 5	+	+	+
	100%	87.5%	62.5%

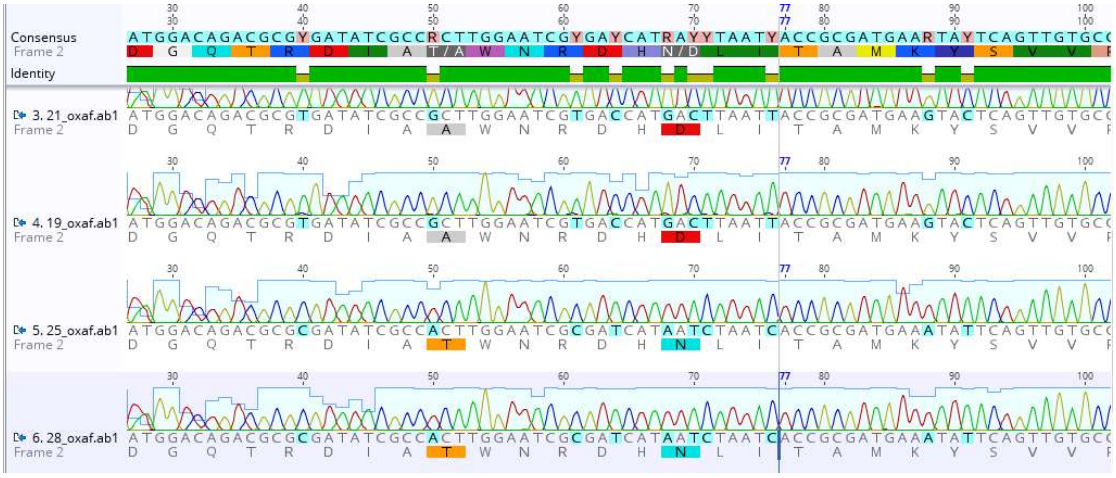

Fig. 2.

origin of the gene which, seems that the aquatic, environmental, and non-human-pathogenic *Shewanella* spp. are the predecessors of the blaOXA-48-like genes and it may be speculated that those genes have been mobilized in the environment (Cantón et al 2012).

All of the members of the family *Enterobacteriaceae* has it. on the other hand located usually in isolates of *K. pneumoniae* (largely of nosocomial source) and *Escherichia coli* (mostly of community origin) (Cantón et al 2012).

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