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Genetic characterization of OXA-1 β- lactamase producing carbapenem-resistant *Klebsiella pneumoniae* from Al-Hillah river water, Babylon Province, Iraq

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Abstract

This study characterize the prevalence of *Klebsiella pneumoniae* harboring bla_{OXA-1} gene recovered from surface waters of Al-Hillah river. One hundred and one water samples were obtained from various environmental sites of Al-Hillah river between January and April 2015. Thirty five (34.6%) isolates were specified as *K.pneumoniae*. Their antimicrobial susceptibility profiles were investigated using disc diffusion method. The high level of resistance were detected for penicillin agents (ampicillin and piperacillin) with (57.14%) and (54.28%) resistance rate, respectively. Resistance to carbapenem was identified in 2 isolates of *K.pneumoniae*, these were further tested by Polymerase Chain Reaction (PCR) technique for the existence of bla_{OXA-1} gene,1 (50%) isolate with positive result.

Keywords: *Klebsiella pneumoniae*, Carbapenem resistance, OXA-1 beta lactamase, PCR, River water.

Introduction

Antimicrobial resistance is a global problem that affect human and environmental health and antibiotic resistant genes can be regarded as pollutant of the environment (Rath and Patra.,2018). The environment particularly aquatic systems have been described as reservoir for resistance genes that can be disseminated via horizontal gene transfer to clinically human pathogens (Marti *et al*.,2014; Freitas *et al*., 2019). The occurrence of antibiotic resistance bacteria in aquatic environment is alarming which arising from different sources like hospital effluents, communities, industries and farming activities (Baquero *et al*.,2008;Bouki *et al*.,2013).

Production of β- lactamases is the main contributing factor for β- lactam resistance in Gram-negative bacteria. Extended spectrum β-lactamases (ESBLs) are one type of these enzymes have become a sever challenge to chemotherapy since infections caused by ESBL- producing isolates can increased morbidity and mortality rates (Jacoby and Bush,2005;Paterson .and Bonomo, 2005; Fankhauser *et al*.,2009). OXA-1 β- lactamase has predominantly associated with other genes encoding for ESBL production and can be detected in plasmid and integron among different species of Gram-negative bacteria (Sugumar *et al*.,2014). OXA-type ESBLs were mainly described in *Pseudomanas aeroginosa* isolates, unlike TEM and SHV which are commonly prevalent in *Enterobacteriaceae* family (Rahman *et al*.,2018).

This study aimed to determine the prevalence of *K. pneumoniae* isolated from Al- Hillah river waters, detect their resistance profiles and characterize bla_{OXA-1} gene by genotypic, Polymerase Chain Reaction (PCR) method among carbapenem- resistant isolates.

Materials and Methods

Samples collection and Microbiological analysis: During a four month period (from January to April 2015), one hundred and one surface water samples were obtained from various sampling sites of Al- Hillah river, the main river in Babylon province ,Iraq. It can be used for agriculture and drinking water for animals. The sampling sites located nearby each of the following region : Ancient Babylon city, Al-Wardia region, Nationality office, Bab Al-Hussein region, Al-Attba street, Al-Farisi region and Al-Aifar region. Samples were placed on sterile glass bottles, then transported to the laboratory and processed within 2 hrs of collection. Water samples were concentrated by filtration onto a sterile 0.22 µm- pore size filter membrane (Millipore, Difco, USA). From each ten-fold dilutions, 0.1 ml was spread on plate count agar, then incubated aerobically at 37 C^{o} for 24-48 hrs (Girlich *et al*.,2010 ;Moges *et al*.,2014). Suspected colonies were picked and sub-cultured on different selective and differential agar such as Blood agar,MacConkey agar (Himedia, India) and Eosin methylen blue agar (Biolife, Italy). Species identification was determined using standard biochemical and microbiological tests as

mentioned by Holt *et al*.(1994),Collee *et al.* (1996) and MacFaddin (2000).

Antimicrobial susceptibility assay: Antimicrobial sensitivity of bacterial isolates was assessed via the standard, Kirby-Bauer disk diffusion method on Mueller- Hinton agar plates (Oxiod, England) (Bauer *et al.*, 1966). The antibiotic disks were tested : ampicillin (AMP), piperacillin (PRL), amoxicillin- clavulanic acid (AMC), cefotaxime (CTX), cefoxitin (FOX), cefaclor (CF), cefprozil (CPR), aztreonam (AZM), imipenem (IMP), meropenem (MEM) and tetracycline (TE). Inhibition zones diameters were measured and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). The *Escherichia coli* ATCC 25922 (University of Kufa, College of Medicine) was used as quality control.

Molecular characterization of *bla*$_{OXA-1}$ gene: The DNA of carbapenem – resistant *K.pneumoniae* isolates was extracted following the protocol described by Pospiech and Neuman (1995) with some modifications and kept at -20 C^{o}. Conventional PCR technique was used for amplification of *bla*$_{OXA-1}$ gene using the following sets of primers (Bioneer, Korea) OXA-1 (5^{-} ATATCTCTACTGTTGCATCTCC^{-}3^{-}) and OXA-1/R (5^{-} AAA CCC TTCAAACCATCC $^{-}$3^{-}) (619bp), in a 25 µl PCR reaction system containing 12.5 µl Go Taq Green Master Mix 2X (Promega, USA), 5 µl extracted DNA, 2.5 µl of 10 pmol/ µl of each specific primers and 2.5 µl nuclease-free water. The PCR amplification program involved: an initial denaturation at 94 C˚ for 1 min, followed by 30 cycles of denaturation at 94 C˚ for 1 min, anneling at 55 C˚ for 1 min, extension at 72 C˚ for 2 min with a final extension step at 72 C˚ for 10 min (Karami *et al*., 2008). PCR product was verified by agarose gel electrophoresis (1.5% agarose gel at 70 volts for 2-3 hrs), then visualized using UV-Transilluminator and photographed with Gel documentation system. 100 bp DNA Ladder (Bioneer, Korea) was run to assess the molecular weight of PCR product.

Results and Discussion

Results of current investigation showed that 35(34.6%) isolates were identified as *K.pneumoniae* (Table-1), this finding correlate with the report carried out by Ebomah and Okoh (2020) who identified 32 isolates as *K.pneumoniae* isolated from surface waters in the Eastern Cape Province, South Africa. Obasi *et al*.(2019) documented 7 *K.pneumoniae* isolates from pharmaceutical wastewaters in South-western Nigeria. Other work in Hillah city determined the occurrence of *K.pneumoniae* in various clinical and environmental samples (Al-Charrakh *et al*., 2011).

Table(1):Distribution of *K.pneumoniae* isolates obtained from Al- Hillah river waters in accordance with sampling location.

Sampling location (nearby)	No. of samples	*K.pneumoniae* isolates No.(%)
Ancient Babylon city	10	0
Al-Wardia region	8	0
Nationality office	6	0
Bab Al-Hussein region	14	5(5.0%)
Al-Attba street	30	14(13.8%)
Al-Farisi region	13	7(6.9%)
Al-Aifar region	20	9(8.9%)
Total	101	35(34.6%)

The presence of *K.pneumoniae* in Al- Hillah river water can reflect the burden of river contamination which came from different sources like discharge of Babylon Teaching Hospital for Maternity and Pediatric, runoff from agricultural areas, bathing of animals and release their excretions directly into river water, industrial effluents, waste products of Hillah laboratories is discharged directly into river water which promote the spread of multidrug resistant bacteria and resistant genes and even evolve different resistant mechanisms and pathogens.

Bacterial resistance to antibiotics in environment can evolved either from accidental mutation, mutation in humans or animals under therapy or are naturally occurring environmental bacteria who under selective pressures (Seiler and Berendonk,2012). Antimicrobial susceptibility assay reveled that most *K.pneumoniae* isolates showed higher rates of resistance to penicillin agents (ampicillin and piperacillin) with 20(57.14%), (54.28%) resistance rate, respectively. Obasi *et al.*(2019) recorded (67.4%) resistance rate for penicillin antibiotic by Gram-negative bacteria including *K.pneumoniae* isolated from pharmaceutical wastewaters in South-western Nigeria. However, penem antibiotics (imipenem and meropenem) reveled the lowest rates of

resistance with 2(5.71%) each, (Table-2). Bedi *et al*.(2017) detect resistance to carbapenem among 1 *K.pneumoniae* isolate obtained from stagnant water of Delhi\NCR.

Table(2): Resistance profiles of *K.pneumoniae* isolates recovered from Al-Hillah river waters (n=35).

Antibiotic class	Antibiotic tested	Resistant *K.pneumoniae* isolates No.(%)
Penicillins	ampicillin	20(57.14%)
	piperacillin	19 (54.28)
β –lactams⁄β-lactamase inhibitor combinations	amoxicillin-clavulanic acid	18(51.42)
Cephems	cefotaxime	16(45.71%)
	cefoxitin	16(45.71)
	cefaclor	13(37.14)
	cefprozil	13(37.14)
Monobactams	aztreonam	10 (28.57)
Penems	imipenem	2(5.71)
	meropenem	2(5.71)
Tetracycline	tetracycline	7 (20)

PCR results revealed that only 1 (50%) isolate of *K.pneumoniae* with resistant to carbapenem, carrying bla_{OXA-1} gene (Fig-1). Said *et al*. (2016) detected the presence of bla_{OXA-1} gene among cefotaxime- resistant *K.pneumoniae* isolated from water samples in Tunisia. Also one report archived by Adegoke *et al.* (2020) characterized OXA-1 beta lactamase among 57.9% of *Escherichia coli* isolated from wastewater treatment plant in Natal province, South Africa.

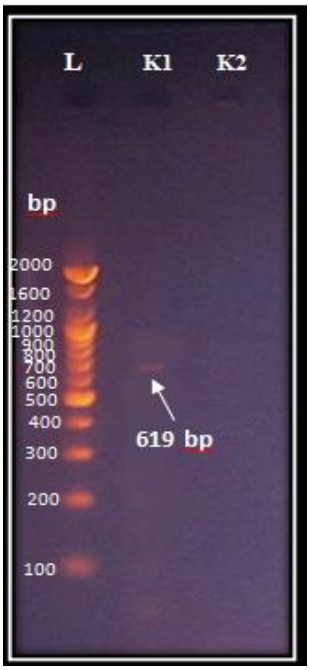

Figure (1): Conventional PCR for amplification bla_{OXA-1} gene among carbapenem-resistant *Klebsiella pneumoniae* isolates. Lane (L),100-bp DNA Ladder. Lane (1) positive result for bla_{OXA-1} gene showing a typical band size (619bp).

Conclusion

This study reflect the existence of *K.pneumoniae* carrying OXA-1 gene in surface water of Al-Hillah river .In turn its a source for ESBL producing bacteria that represent a critical threat were possibly harbored in the population The presence of such highly resistant bacteria in water samples focus attention on the need to accelerate strategies in order to better control the global problem of antibiotic resistance.

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