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RESEARCH ARTICLE OPEN ACCESS

Prevalence of Waterborne *bla*$_{NDM-1}$ Gene Producing Carbapenem-resistant *Klebsiella pneumoniae* from Al-Hillah River Water, Babylon Province, Iraq

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Abstract

The current work suggested the occurrence of blaNDM-1 gene among *Klebsiella pneumoniae* recovered from surface waters of the Al-Hillah River. Between January and April 2015, water samples (101) were taken from seven different area of the Al-Hillah River, Babylon province, Iraq. *K.pneumoniae* was reported in percentage of 35 (34.6%). The antibiotics susceptibility profile of *K.pneumoniae* was determined with disk diffusion assay. The most common resistance was detected for penicillins agents (ampicillin and cloxacillin) with 20(57.14%) and 17(48.57%) resistance rate, respectively. Two isolates of *K.pneumoniae* were carbapenem- resistant. Phenotypic screening of metallo β-lactamase detection was carried out using imipenem–EDTA double disk synergy test for carbapenem resistant isolates, 2(100%) isolates with positive result. Conventional Polymerase Chain Reaction (PCR) test was used for detection NDM-1 beta-lactamase, 1 (50%) *K.pneumoniae* isolate harboring this gene.

Keywords: Carbapenem Resistance, *Klebsiella pneumoniae,* blaNDM-1 gene, PCR, River Water

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INTRODUCTION

Resistant to antibiotics among bacteria has been recognized a universal risk to public and animal health globally. Aquatic system is vital habitats for pathogenic bacteria and a main route for circulation and transmission of antibiotic resistance genes in nature originating from different sources such as runoff from agricultural areas, hospital waste and domestic discharge.[1-3] The greater risk for human and environmental health is the migration of antibiotic resistance genes from surrounding environment to human and animal bacterial pathogens.[4-6]

New Delhi metallo-beta-lactamase (NDM) is a recently described plasmid-borne carbapenemase gene belong to molecular class B beta-lactamase which readily transferred between bacteria and can lead to extreme drug-resistant phenotypes.[7-9] First outbreak due to *K. pneumoniae* ST11producing NDM-1gene during the COVID-19 Pandemic has been particularly detected in human cases of infection in a Portuguese Hospital Centre.[10] Other report achieved by Ebomah and Okoh[11] identified *K. pneumoniae* harboring bla_{NDM-1} and bla_{KPC} from various environmental niches like farm soil in the eastern Cape Province, South Africa. Multi-drug resistant *Enterobacteriaceae* carrying NDM-1gene have been recognized worldwide, with many cases linked to international travel and tourism.[12-14]

Our study aimed to provide insights on the potential the occurrence of *Klebsiella pneumoniae* obtained from waters of Al- Hillah river, detect resistant profiles of all isolates, determine carbapenems resistance profiles, as well as to detect bla_{NDM-1} gene by available phenotypic test and by conventional Polymerase Chain Reaction (PCR) assay among bacteria which resist carbapenem antibiotics.

MATERIALS AND METHODS

Sampling

This study was employed from the beginning of January to the end of April 2015, surface water samples (101) were taken from seven selected sampling sites of the Al- Hillah River. It's the main river in Babylon province, Iraq which can be used for agricultural process and as a drinking waters for animals. The sites of this study located near by 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 unit (college of sciences for women, Babylon university) by ice box for immediate processing and analysis.

Processing of Samples and Microbiological Analysis

Water samples were concentrated by filtration onto a sterile filter membrane (0.22 µm) (Millipore, Difco, USA). From each dilution (ten-fold), 0.1 ml was spread on plate count agar, then incubated at 37°C for 24-48 hrs under aerobic conditions.[15,16] Following incubation, bacterial colonies were sub-cultured onto different selective and enrichment media. Bacterial identification was carried out using standard biochemical and microbiological tests as described previously.[17-19]

Antimicrobial Assay

The identified *K. pneumoniae* isolates were assessed for antibiotics agents using disc diffusion test (Kirby-Bauer) on plates of Mueller-Hinton agar (Oxiod, England)[20]. Twelve agents were selected: ampicillin (AMP), cloxacillin (OX), amoxicillin-clavulanic acid (AMC), cefotaxime(CTX), cefoxitin (FOX), cefaclor (CF), cefprozil (CPR), imipenem (IMP), meropenem (MEM), amikacin (AK), ciprofloxacin (CIP) and norfloxacin (NOR). Diameters of inhibition zones were measured and classified as susceptible, intermediate and resistance in accordance with guidelines of the Clinical and Laboratory Standards Institute (CLSI).[21] For quality control, standard strain, *Escherichia coli* ATCC 25922 (University of Kufa, College of Medicine) was employed.

Phenotypic Assay for Metallo Beta-Lactamase (MBL) Detection

MBLs *K. pneumoniae* producer was detected by phenotypic, imipenem – EDTA double disk synergy test.[22]

Molecular Characterization of bla_{NDM-1} Gene

The modified method of Pospiech and Neuman (1995)[23] was used to extract DNA of pure

K. pneumoniae isolates and kept at -20°C. Conventional PCR technique was used for amplification of bla_{NDM-1} gene with specific sets of primers (Bioneer, Korea) NDM-1/F (5—GGT TTG GCG ATC TGG TTTTC -3-) and NDM-1/R (5—CGGAATGGCTCATCACGATC -3-) (621bp). The final volume of 25 µl reaction mixture contained 5µl of DNA extract,12.5 µl of Go Taq Green Master Mix 2X (Promega, USA), 2.5 µl of each primer and 2.5 µl nuclease-free water. PCR conditions included: 1 min initial denaturation at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 2 min extension at 72°C followed by 10 min final extension step at 72°C[24,25]. Electrophoresis of the product was resolved on a 1.5% agarose gel stained with ethidium bromide, then the gel was observed using UV-Transilluminator for photo-documentation.

RESULTS AND DISCUSSION

Results identified 35/101(34.6%) isolates positive for *K. pneumoniae* in river waters (Table 1). Abd Al-Kareem et al.[26] proved the occurrence of 40 *K. pneumoniae* isolates from surface water of Tigris River in Baghdad city, Iraq. Our finding also consistent with the report detected by Ebomah and Okoh[11] who document 32 isolates as *K. pneumoniae* recovered from surface waters in the Eastern Cape Province, South Africa. Prevalence of *K. pneumoniae* in river water, Malaysia was (69%).[27] Another study in Hillah city recorded the occurrence of same bacteria in various clinical and environmental specimens.[28,29]

The existence of *K. pneumoniae* in surface river water may be attributed to contamination of Al- Hillah River by different sources like discharge of hospitals mainly Babylon Teaching Hospital for Maternity and Pediatric, swimming of animals and discharge their wastes directly into river stream, runoff from agricultural areas, industrial effluents. Additionally, Hillah laboratories released their waste products into these waters which stimulate the proliferation and dissemination of pan-resistant strains and even evolve various mechanisms of resistance and pathogens.

Table 1. Frequency of *K.pneumoniae* obtained from Al- Hillah river waters according to sampling sites

Sampling location (near by)	Samples No.	No.(%) of isolates positive for *K.pneumoniae*
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%)

Bacterial resistance to antimicrobial agents was ancient which can occur under selective pressure. However, due to the inappropriate prescription and massive use of antibiotics in medical therapy, agriculture and aquaculture, resistant bacteria have become a serious threat worldwide.[30,31] Susceptibility testing of bacterial isolates showed that most resistant agent was the penicillins antibiotics (ampicillin and cloxacillin) with 20(57.14%), 17(48.57%) resistance rate, respectively. Lihan et al.[32] recorded (31.6%) resistance rate for penicillin antibiotic by bacteria including *K. pneumoniae* in recreational river water of a community resort in Baram, Sarawak, Malysian, Barneo.

However, penems (meropenem and imipenem) antibiotics displayed lower rates of resistant with 2(5.71%) for each, (Table 2). Bedi et al.[33] detect resistance to carbapenem among

Table 2. Resistance profile of all 35 *K.pneumoniae* isolated from surface river waters.

Antibiotic class	Agent tested	Resistant *K.pneumoniae* isolates No.(%)
Penicillins	ampicillin	20(57.14)
	cloxacillin	17(48.57)
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)
Aminoglycosides	amikacin	9(25.71)
Penems	imipenem	2(5.71)
	meropenem	2(5.71)
Quinolones	ciprofloxacin	8(22.85)
	norfloxacin	14(40)

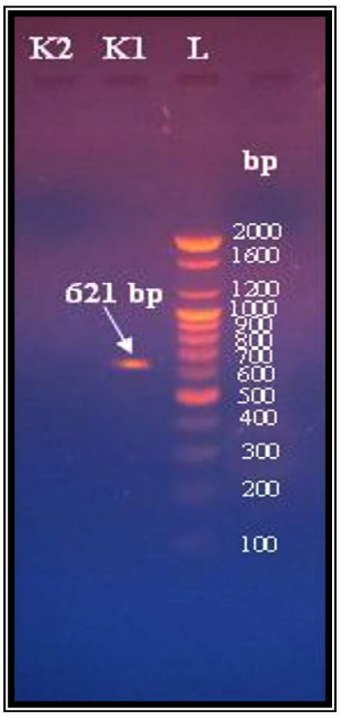

Figure. Results of PCR amplification for bla_{NDM-1} gene in carbapenem resistant isolates. Lane (1) positive result for NDM-1 gene (621bp), Lane (L), DNA Ladder (100-bp).

1 *K. pneumoniae* isolate obtained from stagnant water of Delhi\NCR.

All 2(100%) *K. pneumoniae* with resistant to carbapenem were positive for phenotypic MBL assay using the imipenem – EDTA double disk synergy test. Local study on Hillah River waters achieved by Abbas[34] proved 2(66.7%) *K. pneumoniae* as MBL producers by this method. Shah and Zharh[2] identified all (100%) meropenem resistant *K. pneumoniae* isolates as MBL producers.

The molecular screening of NDM-1 gene was performed using PCR technique on the two *K. pneumoniae* with resistant to carbapenem, the bla_{NDM-1} gene was detected in 1(50%) isolate only (Figure). Ahammad et al.[35] documented the presence of bla_{NDM-1} gene among coliform bacteria from Ganges river. Shah and Zahra[2] characterized 43 meropenem resistant bacteria from different water samples of which 3 *K. pneumoniae* isolates were harbored this gene in Islamabad, Pakistan.

CONCLUSION

This study report the finding of metallo beta lactamase of NDM-1 type containing *K. pneumoniae* in water samples from the Al-Hillah River. The presence of such highly resistant bacteria in water samples focus attention on the need to accelerate strategies to limit the emergence and spread of resistant organisms.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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