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First Report of SHV-Extended Spectrum Beta Lactamase Harboring Carbapenem Resistant *Klebsiella pneumoniae* form Al-Hilla River Waters Iraq

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Abstract: The current study was sought out to describe the prevalence of bla_{SHV} producing *Klebsiella pneumoniae* isolated from surface waters of Hilla River. In the period from January-April 2015, 101 water samples were collected from 7 different locations of Hilla River. The 35 (34.6%) isolates were specified as *K. pneumoniae*. All isolates were checked for Extended Spectrum β-Lactamase (ESBL) production phenotypically using disk combination method. About 21 (60%) isolates were screen-positive. The antibiotic resistance profiles of ESBL-producing isolates was evaluated using Kirby-Bauer disk diffusion method. The highest rate of resistance was noticed for penicillin antibiotics (ampicillin and piperacillin) with 85.71% and 80.95%, respectively. Carbapenem resistance was identified in 2 isolates of *K. pneumoniae*, these were checked further by Polymerase Chian Reaction (PCR) method for detection bla_{SHV} gene, 2 (100%) isolates gave positive result.

Key words: *Klebsiella pneumoniae*, ESBL, carbapenem resistance, SHV beta lactamase, PCR, river water, producing, phenotypically

INTRODUCTION

Antibiotic resistance has become a great challenge threatening environmental and public health globally. Numerous studies suggests that aquatic environments are natural reservoirs of antibiotic resistance bacteria and their resistant genes as well as potential routes for their dissemination into surrounding microbiota (BalcAzar *et al*., 2015). The discharge of hospital, municipal and aquaculture effluents into river stream may contribute to emergence of antibiotic resistant bacteria (Sapkota *et al*., 2007; Baquero *et al*., 2008; Xi *et al*., 2009). Additionally, they could occur naturally, since, many acquired resistant mechanisms attributed to the producers of antibiotics such as actinomycetes (Martinez, 2008).

The primary producers of Extended Spectrum Beta Lactamase (ESBL) enzymes are members of the Enterobacteriaceae, mainly *Klebsiella pneumoniae* and *Escherichia* coli (Paterson and Bonomo, 2005). In recent decades, these organisms has recognized as a major nosocomial pathogens. Bacteria harboring ESBLs can confer resistances not only to oxyimino-cephalosporins but may also acquire and exhibit additional resistances to other antimicrobial classes which further limits therapeutic options (Bradford, 2001; Livermore, 2008).

The most dominant types of ESBLs is SHV enzymes, unlike the TEM-type beta-lactamases, fewer SHV type-beta-lactamases are derived from SHV-1 which was described for the first time in *K. pneumoniae*. The SHV-1 beta-lactamase is responsible for plasmid mediated ampicillin resistance in bacteria which harbour it. Recently, over 100 SHV types are known worldwide (Jacoby, 1997; Bradford, 2001). SHV beta lactamases are distributed widely among Enterobacteriaceae family, *Pseudomonas aeruginosa and Acinetobacter* spp. (El Harrif-Heraud *et al*., 1997; Huang *et al*. , 2004; Poirel *et al*., 2004).

This study is attempted to explore the prevalence of *K. pneumoniae* isolated from Al-Hilla River waters, determine ESBL-producers phenotypically and their resistance profiles, detect bla_{SHV} gene by Polymerase Chian Reaction (PCR) method among carbapenem- resistant isolates.

MATERIALS AND METHODS

Samples collection: From January to the end of April 2015, a total of 101 water samples (from surface layer) were taken from 7 different stations of Al- Hilla River, the main river in Babylon Province, Iraq. It used for agriculture and drinking water for animals. The selected sites located near by each of the following areas: Ancient Babylon City, Al-Wardia Region, Nationality Office, Bab Al-Hussein Region, Al-Attab Street, Al-Farisi Region and Al-Aifar Region. Water samples were collected using sterile glass bottles and transported in an ice-box to the laboratory and assayed within 2 h of collection. Samples processing and microbiological analysis each sample was filtered through a sterile 0.22 μm pore membrane

(Millipore, Difco, USA). Ten-fold dilutions were plated by spreading 0.1 mL on plate count agar and incubated aerobically at 37°C for 24-48 h (Girlich *et al*., 2010; Moges *et al*., 2014). After incubation, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as blood agar, MacConkey agar (Himedia, India) and eosin methylen blue agar (Biolife, Italy. Biochemical identification was performed following standard methods described by Holt *et al*. (1994), Collee (1996) and Macfaddin (2000).

Phenotypic detection of ESBLS production (recommended by CLSI, 2010): The screening test for ESBLs production was performed using a phenotypic confirmatory test, clavulanic acid disk combination method as described by (Prado *et al*., 2008).

Antimicrobial susceptibility assay: The susceptibility testing was evaluated against 13 antibiotics from 6 classes by the standard, Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates (Oxiod, England). The following antibiotic disks were tested: ampicillin (10 μg), piperacillin (100 μg), amoxicillin-clavulanic acid (10 μg), cefprozil (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg) cefepime (30 μg), cefoxitin (30 μg), aztreonam (30 μg), imipenem (10 μg), meropenem (10 μg), nalidixic acid (30 μg) and levofloxacin (5 μg). After 18 h of incubation at 37 °C, the zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI., 2010). Escherchia coli ATCC 25922 (College of Medicine, University of Kufa) was used as quality control.

Molecular detection of bla_{shv} gene

DNA preparation: DNA of bacterial isolates was prepared following the protocol described by Pospiech and Neuman with some modifications and used directly for PCR as DNA template.

PCR amplification: PCR amplification of bla_{SHV} gene was performed using the following sets of primers (Bioneer, Korea) SHV/F (5-ATGCGTTATATTCGCCTGTG-3) and SHV/R (5—TGCTTTGTTATT CGGGCCAA-3) (753bp) in a 25 μL reaction volume using 12.5 μL Go Taq Green Master Mix 2X (Promega, USA), 5 μL DNA template, 2.5 μL of 10 pmol/ μL of specific up stream primers and 2.5 μL of 10 pmol/ μL of specific down stream primers and 2.5 μL nuclease-free water. The PCR amplification conditions were as follows: an initial denaturation at 94°C for 30 sec, followed by 35 cycles of denaturation at 94°C for 30 sec, anneling at 60 °C for 1 min, extension at 72 °C for 1 min and a final extension step of 72 at 10 min (Paterson *et al*., 2003; Hujer *et al*., 2006). The reaction product was separated on 1.5% agarose gel at 70 Volts for 2-3 h, after staining with ethidium bromide, the product was visualized under UV-Transilluminator, then photographed with gel documentation system. The 100 bp DNA Ladder (Bioneer, Korea) was used to assess PCR product size.

RESULTS AND DISCUSSION

Among 101 water samples, 35(34.6%) isolates were detected as *K. pneumoniae*, this observation correlate with the results obtained by Prado *et al*. (2008) who identified 43 *K. pneumoniae* strain isolated from effluents and sludge of a hospital sewage treatment plant in Brazil. Also, Saleem *et al*. (2011) identified (6.71%) *K. pneumoniae* isolated from Dal Lake waters, Kashmir, India (Table 1).

Pollution of Al-Hilla River waters by these agents may be related to the fact that this river receiving contaminants and bacteria from different sources such as Babylon Teaching Hospital for Maternity and Pediatric sewage, runoff from agricultural areas, bathing of animals and release their excretions directly into river water, industrial effluents, waste products of Hilla laboratories is discharged directly into river water which promote the spread of antibiotic resistant bacteria and resistant genes and even evolve different resistant mechanisms and pathogens.

ESBL production was assayed phenotypically by disk combination method, results revealed that 21(60%) isolates were screen positive. Lu *et al*. (2010) identified 250 beta-lactam resistant isolates including *Klebsiella* spp. from a single sediment sample of urban river in China, 75 isolates were ESBL-producers using this method. Prado *et al*. (2008) characterized ESBL production in 20 (46.5%) analysed samples isolated from effluents and sludge of sewage treatment process in Brazil.

Bacterial resistance to antimicrobial drugs is a natural phenomenon under selective pressure. However, due to the extensive use and misuse of antibiotics in medical therapy, agriculture and aquaculture, resistance has become a major problem (Oliveira *et al*., 2015). In this investigation, antibiotics resistant profiles of ESBLs producing isolates revealed a higher resistance for penicillin antibiotics (ampicillin and piperacillin) with (85.71%) , (80.95%) resistance rate, respectively. Imipenem and meropenem antibiotics displayed the lowest rates of resistance with 2 (9.52%) each, (Table 2). Similar trends were observed by Lu *et al*. (2010) who demonstrated

Table 1: Numbers and percentages of *K. pneumoniae* isolates recovered from Al-Hilla River waters according to sampling location

Sampling site (near by)	No. of water samples	No. of *K. pneumoniae* isolates (%)
Ancient Babylon City	10	0
Al-Wardia Region	80	0
Nationality Office	60	0
Bab Al-Hussein Region	14	5(5.0%)
Al-Attab Street	30	14(13.8%)
Al-Farisi Region	13	7(6.9%)
Al-Aifar Region	20	9(8.9%)
Total	101	35(34.6%)

Table 2: Frequency of extended spectrum beta lactamase producing *K. pneumoniae* using disk combination method

No. of *K. pneumoniae* isolates (%)	No. of ESBL-positive isolates (%)	No. of ESBL-negative isolates (%)
35	21(60%)	14 (40%)

Table 3: Antibiotics resistance profiles of ESBL-producing *K.pneumoiae* isolates against various antibiotics (n = 21)

Antibiotic class	Agent used	No.of resistant ESBL-producing isolates (%)
Penicillins	Ampicillin	18(85.71)
	Piperacillin	17(80.95)
β-lactams/β-lactamase inhibitor combinations	Amoxicillin-clavulanic acid	16(76.19)
Cephems	Cefotaxime	14(66.66)
	Ceftazidme	11(52.38)
	Cefriaxone	12(57.14)
	Cefepime	12(57.14)
	Cefoxitin	13(61.90)
Monobactams	Aztreonam	10(47.61)
Penems	Imipenem	2(9.52)
	Meropenem	2(9.52)
Quinolones	Nalidixic acid	7(33.33)
	Levofloxacin	3(14.28)

higher resistance to beta-lactam antibiotics including, penicillin, monobactam and narrow-spectrum to fourth generation cephalosporins among ESBL-producing Enterobacteriaceae isolated from the sediment habitat of urban river, China. Khosravi *et al*. (2013) reported a wide range (96.36%) of resistance to ampicillin antibiotics among ESBL producing *K. pneumoniae* from Iranian clinical settings. The observation of increased resistance to penicillin may be related to widespread use of these antibiotics in Hilla clinical settings (Table 3).

According to PCR results, bla_{SHV} gene was identified in 2 (100%) carbapenem-resistant isolates (Fig. 1). Machado *et al*. (2008) identified two *K. pneumoniae* carrying SHV-12 and SHV-27 from public hospital waste waters in Portugal. Another study characterized bla_{SHV} in 48 ESBL producers, the most common isolates were *K. pneumoniae* obtained from hospital wastewater in Riode Janeiro city, Brazil (Chagas *et al*., 2011). Bla_{SHV}gene was also recorded previously in clinical studies in Iran (Khosravi *et al*., 2013), in India (Fouzia and Damle, 2015) indicating the possible dissemination of ESBLs genes into

Fig. 1: PCR detection of bla_{SHV} gene (753bp) in carbapenem-resistant *Klebsiella pneumoniae* isolates. Lane (L), DNA molecular size marker (100-bp Ladder). Lane (1, 2) of *K. pneumoniae* isolates showing positive result with bla SHV gene

the surrounding environments. However, to our knowledge this the first research recorded ESBL producing *K. pneumoniae* strain harboring SHV-β lactamase from Hilla River waters.

The presence of ESBL producing isolates in Hilla River waters should be of great concern, since, genes encoding ESBL enzymes can be easily transferred to other pathogens by conjugative plasmid giving the bacteria multiresistance pattern.

CONCLUSION

The present research confirms that Al-Hilla River waters are contaminated with ESBL producing *K. pneumoniae* carrying SHV-β lactamases that are common to clinical strains. The presence of antibiotic resistant bacteria and their genes poses potential risks of outbreaks of human infection and highlighting the need to control and management of these cases in terms of funding and healthcare. Effective prevention measurements are required to minimize the rise and circulation of these bacteria in the community and reduce emergence of antibiotic resistance enteric pathogens.

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REFERENCES

BalcAzar, J.L., J. Subirats and C.M. Borrego, 2015. The role of biofilms as environmental reservoirs of antibiotic resistance. Front. Microbiol., 6: 1-9.

Baquero, F., J.L. Martinez and R. Canton, 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol., 19: 260-265.

Bradford, P.A., 2001. Extended spectrum ß-lactamase in the 21st century: Characterization epidemiology and detections of this important resistant threat. Clin. Microb. Rev., 48: 933-951.

CLSI., 2010. Performance standards for antimicrobial susceptibility testing: Twentieth informational supplement. Document M100-S20, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA. USA.

Chagas, T.P.G., L.M. Seki, J.C. Cury, J.A.L. Oliveira and A.M.R. Davila *et al*., 2011. Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, Brazil. J. Appl. Microbiol., 111: 572-581.

Collee, J.G., 1996. Mackie and Mccarteny Practical Medical Microbiology. 14th Edn., Elsevier, Amsterdam, Netherlands, ISBN: 9788131203934, Pages: 978.

El Harrif-Heraud, Z., C. Arpin, S. Benliman and C. Quentin, 1997. Molecular epidemiology of a nosocomial outbreak due to SHV-4-producing strains of Citrobacter diversus. J. Clin. Microbiol., 35: 2561-2567.

Fouzia, B. and A.S. Damle, 2015. Prevalence and characterization of extended spectrum beta-lactamase production in clinical isolates of Klebsiella pneumonia. J. Med. Microb. Diagn., 4: 1-6.

Girlich, D., L. Poirel and P. Nordmann, 2010. Novel ambler class a carbapenem-hydrolyzing ß-lactamase from a Pseudomonas fluorescens isolate from the Seine River, Paris, France. Antimicrob. Agents Chemother., 54: 328-332.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, 1994. Bergeys Manual of Determinative Bacteriology. 9th Edn., Williams and Wilkins, Baltimore, Maryland.

Huang, Z.M., P.H. Mao, Y. Chen, L. Wu and J. Wu, 2004. Study on the molecular epidemiology of SHV type beta-lactamase-encoding genes of multiple-drug-resistant Acinetobacter baumannii. Zhonghua Liu Xing Bing Xue Zhi, 25: 425-427.

Hujer, K.M., A.M. Hujer, E.A. Hulten, S. Bajaksouzian and J.M. Adams *et al*., 2006. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob. Agents Chemother., 50: 4114-4123.

Jacoby, G.A., 1997. Extended-spectrum ß-lactamases and other enzymes providing resistance to oxyimino-ß-lactams. Infect. Dis. Clinics, 11: 875-887.

Khosravi, A.D., H. Hoveizavi and M. Mehdinejad, 2013. Prevalence of Klebsiella pneumonia encoding genes for CTX-M-1, TEM-1 and SHV-1 extended-spectrum beta lactamases (ESBL) enzymes in clinical specimens. Jundishapur J. Microbiol., 6: 1-5.

Livermore, D.M., 2008. Defining an extended-spectrum ß-lactamase. Clin. Microbiol. Infect., 14: 3-10.

Lu, S.Y., Y.L. Zhang, S.N. Geng, T.Y. Li and Z.M. Ye *et al*., 2010. High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. Appl. Environ. Microbiol., 76: 5972-5976.

Macfaddin, J.F., 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd Edn., Lippincott Williams and Wilkins, Philadelphia, pp: 412-423.

Machado, E., T.M. Coque, R. Canton, J.C. Sousa and D. Silva *et al*., 2008. Leakage into portuguese aquatic environments of extended-spectrum -ß-lactamase-producing Enterobacteriaceae. J. Antimicrob. Chemother., 63: 616-618.

Martinez, J.L., 2008. Antibiotics and antibiotic resistance genes in natural environments. Science, 321 : 365-367.

Moges, F., M. Endris, Y. Belyhun and W. Worku, 2014. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. BMC. Res. Notes, 7: 215-221.

Oliveira, D., T. Carvalho, A. Medeiros, A.P. Frazzon and S. Van Der Sand, 2015. ß-Lactam resistance genes in Gram-negative bacteria isolated from a stream in Porto Alegre. J. Adv. Scient. Res., 6: 19-24.

Paterson, D.L. and R.A. Bonomo, 2005. Extended-spectrum ß-lactamases: A clinical update. Clin. Microbiol. Rev., 18: 657-686.

Paterson, D.L., K.M. Hujer, A.M. Hujer, B. Yeiser and M.D. Bonomo *et al*., 2003. Extended-spectrum ß-lactamases in Klebsiella pneumonia bloodstream isolates from seven countries: Dominance and widespread prevalence of SHV-and CTX-M-type ß-lactamases. Antimicrob. Agents Chemother., 47: 3554-3560.

Poirel, L., E. Lebessi, M. Castro, C. Fevre and M. Foustoukou *et al*., 2004. Nosocomial outbreak of extended-spectrum ß-lactamase SHV-5-producing isolates of Pseudomonas aeruginosa in Athens, Greece. Antimicrob. Agents Chemother., 48: 2277-2279.

Prado, T., W.D.C. Pereira, D.M. Silva, L.M. Seki and A.D.A. Carvalho *et al*., 2008. Detection of extended-spectrum β-lactamase-producing Klebsiella pneumonia in effluents and sludge of a hospital sewage treatment plant. Lett. Appl. Microbiol., 46: 136-141.

Saleem, S., A.N. Kamili, D.K. Kakru, S.A. Bandh and B.A. Ganai, 2011. Isolation, identification and seasonal dis-tribution of bacteria in Dal Lake, Kashmir. Int. J. Environ. Sci., 2: 185-193.

Sapkota, A.R., F.C. Curriero, K.E. Gibson and K.J. Schwab, 2007. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. Environ. Health Perspect., 115: 1040-1045.

Xi, C., Y. Zhang, C.F. Marrs, W. Ye and C. Simon *et al*., 2009. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl. Environ. Microbiol., 75: 5714-5718.