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Prevalence of CTX-M Gene in Klebsiella Pneumonia Isolated from Surface Water of Tigris River within Baghdad Province

Afrah F. Abd Al-Kareem[1] Kadhum H. Al-Arajy[1] Kifah A. Jassim[2]
1.Department of Biology,College of Science, University of Al-Mustansiriya
2.Central Health Laboratory, Epidemiology Section
Corresponding author's e-mail: starfarah888@yahoo.com

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Abstract
The present work was conducted to study the prevalence of *klebsiella pneumonia* in surface water which a part of feacal coliform bacteria that consider one of microbial pollution indicators in water, also determined their antibiotic resistance and ESBL encoding gene using PCR with specific primers for the detection of CTX-M gene. The results showed a total of 40 isolates of *klebsiella pneumonia* were tested for the presence of the *CTX-M* gene by PCR, 87.5% were carrying this gene, and also isolates were resistant to various classes of antibiotics. Water and sewage are the ones, which have been identified as reservoirs of enteric bacteria for spread of resistance factors, wastewater treatment plant did not show a satisfactory efficacy in removing pathogenic microorganisms, allowing for the dissemination of multiresistant bacteria into the environment and this can result in routes of dissemination of multiresistant bacteria and their genes of resistance into the environment, thus contaminating water resources, and having serious negative impact on public health.
Keywords: *CTX-M, k. pneumonia*

1. Introduction

Water is essential for all known life forms, still water pollution and the destruction of ecosystems continue to increase. Water contamination is now a major problem in the global context as a consequence of industrialization, globalization, population growth, urbanization and warfare combined with increased wealth and more extravagant lifestyles (Andersson, 2009).

Water is considered a vehicle for the propagation and dissemination of human associated bacteria. In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes. These unprotected water sources can be contaminated with microbes through rainfall runoff and agricultural inputs, mixing with sewage effluents and faeces from wild life, which render them unacceptable for human consumption (Mulamattathil *et al.,* 2014).

Klebsiella are *Enterobacteriaceae*, oxidase-negative catalase-positive non-motile straight rods, surrounded by a capsule. In humans, *K. pneumoniae* is present as commensal in the nasopharynx and in the intestinal tract. *Klebsiella* spp. can cause human diseases, ranging from asymptomatic colonization of the intestinal, urinary, or respiratory tract to fatal septicemia (Cabral, 2010).

Klebsiella are ubitiquous in the environment. They have been found in a variety of environmental situations, such as soil, vegetation, or water, and they influence many biochemical and geochemical processes. They have been recovered from aquatic environments receiving industrial wastewaters, plant products, fresh vegetables, food with a high content of sugars and acids, frozen orange juice concentrate, sugarcane wastes, living trees, and plants and plant byproducts. They are commonly associated with wood, sawdust, and waters receiving industrial effluents from pulp and paper mills and textile finishing plants. *Klebsiella* have been isolated from the root surfaces of various plants (Cabral, 2010).

It has been demonstrated that *Klebsiella* may be found on fresh market produce at levels of 10^2 to 10^5 cells per g and when vegetables are consumed uncooked in fresh salads, colonization of the intestinal tract may occur and ultimately result in a reservoir for future nosocomial infections (Knittel *et al.,* 1977).

Alarming increases in the consumption of antibiotics through human therapy and agricultural processes have been reported and this extensive usage in both human and animal medicine has resulted in the development of antibiotic-resistant bacteria which affect the treatment of infection. Antibiotic resistance has therefore become a major public health issue and its presence in waste water, surface water, and drinking water is well documented. (Mulamattathil *et al.,* 2014).

Many resistant bacteria and resistance genes have been detected in environmental samples, such as domestic sewage (Heuer *et al.,* 2002; Tennstedt *et al.,* 2003), hospital sewage (Reinthaler *et al.,* 2003; Schwartz *et al.,* 2003), sewage sludge (Guillaume *et al.,* 2000; Reinthaler *et al.,* 2003) coastal ponds (Meirelles-Pereira *et al.,* 2002), underground waters (Gallert *et al.,* 2005) and sewage-contaminated riverwaters (Costanzo *et al.,* 2005).

Klebsiella species contain many plasmids that differ in numbers and molecular weight, carrying

different types of genes including those encoding extended-spectrum β-lactamases (ESBLs) (Essack *et al.,* 2004). Extended spectrum β-lactamases are mostly plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactam antibiotics, including different types of penicillins and cephalosporins (Lautenbach *et al.,* 2004).

The *CTX-M* B-lactamases are one of the groups of extended spectrum B-lactamases (ESBLs) identified after the introduction of the broad-spectrum cephalosporin. These enzymes have spread worldwide and 54 different types have been identified in the past 10 years (Soge *et al.,* 2006)

The aim of this study was to determine the antibiotic sensitivity pattern of *Klebsiella pneumonia* isolated from natural surface waters of Tigris River in Baghdad City and detection of CTX-M gene by PCR for the first time in Iraq

2. Materials and Methods

2.1. Study area

The Tigris River is one of the largest rivers of the Middle East stretching for over 1,900 km, of which 1415 km are within Iraq, (Rzoska 1980). Tigris River is the only water resource for the Baghdad city, the capital of Iraq. Baghdad stretch of the Tigris River about 52 Km from Al-Tarmiyahm in the north to Al-Zafaraniah in the south (Al-Adili 1998).

Three stations were selected on Tigris River are: northern area of Ghera'att City (S1)(upstream), the area of Baghdad medical city (S2)(middle), the area near Al-Jadiriah Bridge (S3)(downstream) (fig2).

2.2. Sample collection

Water sample were collected monthly from November 2013 to October 2014.at three different sites. Samples were collected by using sterilized glass bottles, the collected samples were transport to the laboratory by ice box for analysis.

2.3. Isolation and Identification of klebsiella pneumonia

Klebsiella isolated by using membrane filtration procedure according to(APHA,2005) and used HiCrome *Klebsiella* Selective Agar Base(HiMedia), then *Klebsiella* isolates were submitted to commercial multi test system of gram negative bacteria and API 20E system (BioMrrieux, France), also the VITEK 2 system (Biomerieux,France) was used in this study to confirm the identification and antibiotic susceptibility of *Klebsiella* spp.

2.4. Antimicrobial susceptibility tests

The *K. pneumonia* strains were submitted to antimicrobial susceptibility tests by using the standard disc diffusion method according to Kirby and Bauer, (1966) and the recommendations of the National committee for clinical laboratory standards (NCCLs, 2002). The turbidity of the suspensions used for sensitivity testing was adjusted to 0.5 McFarland standards and inoculated onto Mueller-Hinton agar medium followed by incubation at 37C° for 18 to 24 h.

The following antimicrobial discs(concentrations in µg) (Bioanalyse / Turkey)were used: Ceftriaxone (CRO) (30), Ampicillin(25), Cefotaxime(30), Piperacillin+ tazobactam(100/10), Cefepime (FEP) (30), Gentamicin (10)(CN) (30), Amikacin (AK) (30), Ciprofloxacin (CIP) (5) , Trimethoprime ⁄ sulfamethoxazole (SXT) (1.25⁄23.75 lg) and Tetracycline (TE) (30).

2.5. Extraction of Plsmid DNA

Plasmid Extraction was done using a commercial plasmid isolation kit (PureYield™ Plasmid Miniprep System, Promega, U.S.A). according to the Manufacturer Company instructions.

2.6. Detection CTX –M gene by PCR

A PCR reactions with specific primer were performed to identify *CTX-M*
gene, the primer of CTX-M are F(CGCTTTGCGATGTGCAG) R(ACCGCGATATCGTTGGT) (Gröbner *et al.,*2009).PCR was carried in 25µl of PCR reaction volumes contained 1.25(10pmol/µl) for each F and R primer(Alfa DNA/Montreal), 5 µl of DNA template, nuclease free water 5 µl, Green Go *Taq* Master Mix 12.5µl pH (8) (Promega, USA) . Amplification of DNA was performed using Thermal Cycler (Thermoelectron industries, France). The PCR was carried out under the following conditions: initial denaturation at 95 C° for 5 minutes, followed by 30 cycles of denaturation at 94 C° for 30s, primer annealing at 55 C° for 30s and primer elongation at 72 C° for 1 min and final extension at 72 C° for 3 min.

The PCR products were mixed with 10 µl of loading dye and analyzed by electrophoresis in 1% agarose gels (for 1 hours and half hours using 1X TBE running buffer. 1000 bp DNA ladder was included in each run, and DNA bands were viewed under UV transilliuminator and then photographed.

3. Results and discussion

In this study 40 *klebsiella pneumonia* isolates obtained from three locations on Tigris River showed highest resistance to ampicillin reach to 100% followed by piperacillin+ tazobactam 75% while these isolates had highest susceptibility to Cefepime and Ciprofloxacin as show in Table1.

A total of 40 isolates of *klebsiella pneumonia* were tested for the presence of the *CTX-M* gene by PCR, 87.5% were carrying this gene after amplification of *CTX-M* forward(F) and reverse(R), the amplified DNA with the *CTX-M* primers resulting in PCR product with band of molecular size about 551 bp as showed in (Fig 1)

The *CTX-M* enzymes are known as an increasingly serious public health concern worldwide and have been noted to be the cause of outbreaks as reported (Bonnet, 2004). The spread of *CTX-M* has also been described through prospective studies in industrialized countries such as Canada, France, and the United Kingdom (Ruppé *et al.,* 2009).

In study of two hundred sixty-six strains of *Klebsiella pneumoniae* isolated from natural water sources in US were analyzed to determine antimicrobial susceptibility differences between natural strains and human *Klebsiella* isolates found that 79% and 84% of environmental isolates were resistant to ampicillin and carbenicilin respectively (Matsen *et al.,*1974).

The increasing acquisition of extended-spectrum β-lactamase plasmids among *K. pneumoniae* isolates has led to a dramatic increase in antibiotic-resistant outbreaks (Lawlor *et al.,* 2007) .

Within the past 20 years, *Klebsiella pneumoniae* has become the most commonly reported bacterial species responsible for wide range of infection. *K. pneumoniae* is known worldwide for its rapid acquisition of resistance to traditional and new antimicrobial drugs, including resistance to third-generation cephalosporins, fluoroquinolones, carbapenem, and extended-spectrum β- lactamases(Wiskur *et al.,* 2008).

The increasing number of *Klebsiella* strains producing ESBLs leads to limitation of therapeutic options and calls for enhancing surveillance and control measures of hospital acquired infections. (Damian *et al.,* 2009)

Water and sewage are the ones, which have been identified as reservoirs of enteric bacteria for spread of resistance factors. The widespread use of antibiotics has helped to fester a remarkable type of resistance in bacteria. Emergence of resistance to third generation cephalosporins has been reported on strains of *Klebsiella* and transferable resistance to cefotaxime was demonstrated already. Resistance to β-lactam antibiotics among the *Enterobacteriaceae* members is the most commonly expressed by the production of β lactamases . The mechanism of resistance involved is the transferable plasmid mediated β-lactamases has been described, in multi-resistant *K. pneumonia*. Natural reservoirs of resistance genes may also provide a source of transferable traits for emerging pathogens (Arikan and Aygan, 2009).

In conclusion, the results of this study underline the importance of the surface water for dissemination of the antibiotic resistance between the microorganism and such a screening of antibiotic resistance may be reflecting the consequence of the drug using habits and would help to address the contribution spread of resistant bacteria to the environment, thus promoting prevention measures to protect public health.

Table.1 Susceptibility of the 40 isolates of Klebsiella pneumoniae to 10 Antibiotics.

No.	Antibiotics	Resistant (%)
1	Ampicillin	100%
2	Amikacin	1%
3	Cefotaxime	52%
4	Cefepime	0%
5	Ceftriaxone	62%
6	Ciprofloxacin	0%
7	Gentamicin	9%
8	Piperacillin/ tazobactam	75%
9	Tetracycline	10%
10	Trimethoprime／sulfamethoxazole	27%

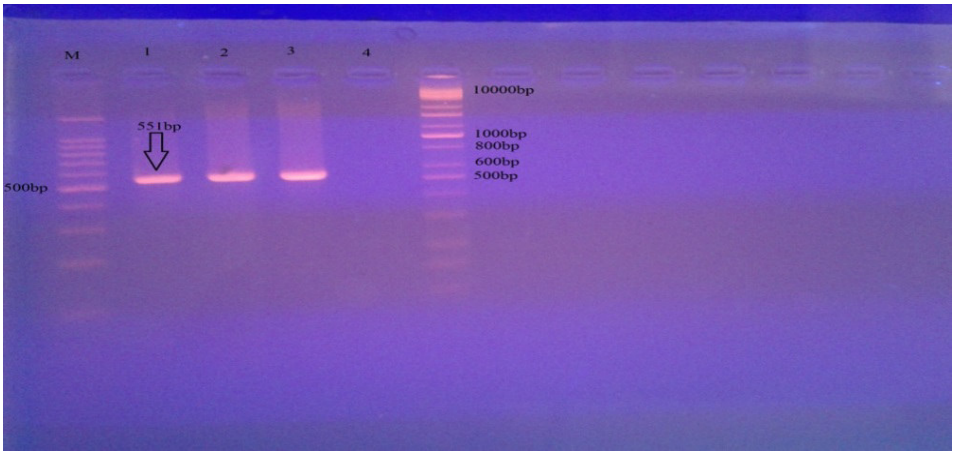

Figure1. CTX-M gene after PCR on 1% Agarose gel electrophoresis. Lane M: 100-bp DNA ladder; lanes1, 2, 3, positive CTX-M gene at (551bp), Lane 4 negative controle, Lane5 10kbp DNA ladder .

Figure2. Tigris River map and the location of sample station

References

Al-Adili, A. S., 1998 "Geotechnical Evaluation of Baghdad Soil Subsidence and their Treatments," Ph.D. Thesis, University of Baghdad, Iraq.

Andersson, S.(2009). Characterization of Bacterial Biofilms for Wastewater Treatment. School of Biotechnology, Royal Institute of Technology (KTH), Sweden.

APHA, AWWA and WFF. (2005). Standard Methods for the Examination of Water and wastewater, 21th ed., edited by Eaton, A. D. ; L. S. Clesceri; E. W. Rice, and A. E. Greenberg. American Water Work Association and Water Environment Federation, USA.

Arikan, B. and Aygan,A.(2009). Resistance Variations of Third Generation of Cephalosporins in Some of the Enterobacteriaceae Members in Hospital Sewage. *Int. J. Agri. Biol*., 11: 93–96

Cabral, J.P.S.(2010). Water Microbiology. Bacterial Pathogens and Water. *Int. J. Environ. Res. Public Health* , *7*, 3657-3703.

Costanzo, S.D., Murby, J. and Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environmental. Mar Pollut Bull 51, 218–223.

Damian, M., Usein,C.R., Palade, A.M., Ceciu,S. and Cosman,M. (2009). Molecular Epidemiology and Virulence Characteristics of *Klebsiella pneumoniae* Strains Isolated from Hospital-Associated Infections. *The Open Epidemiology Journal*, *2,* 69-78

Essack, S.Y., L.M.C. Hall and D.M. Livermore, 2004. Klebsiella pneumoniaeisolate from South Africa with multiple TEM, SHV and AmpC beta-lactamases. Int. J. Antimicrob Agents, 23: 398-400.

Gallert, C., Fund, K.L. and Winter, J. (2005) Antibiotic resistance of bacteria in raw and biologically treated

sewage and in groundwater below leaking sewers. Appl. Microbiol. Biotechnol 69, 106–112.
Guillaume, G., Verbrugge, D., Chasseur-Libotte, M.L., Moens, W. and Collard, J.M. (2000) PCR typing of tetracycline resistance determinants (Tet A–E) in Salmonella enterica serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. FEMS Microbiol. Ecol. 32, 77–85.
Gröbner,S., Linke, D.,Schütz,W, Fladerer, C., Madlung,J.,3 Ingo B. Autenrieth,1 Wolfgang Witte,W. and Yvonne Pfeifer,Y.(2009). Emergence of carbapenem-non-susceptible extended-spectrum b-lactamase-producing Klebsiella pneumoniae isolates at the university hospital of Tübingen, Germany. Journal of Medical Microbiology, 58, 912–922.
Heuer, H., Krogerrecklenfort, E., Wellington, E.M.H., Egan, S., Van Elsas, J.D., Van Overbeek, L., Collard, J.M., Guillaume, G. et al. (2002) Gentamicin resistance genes in environmental bacteria: prevalence and transfer. FEMS Microbiol Ecol 42, 289–302.
Knittel, M.D., Seidler,R.J., Eby,C. and Cabe, L.M.(1977). Colonization of the Botanical Environment by *Klebsiella* Isolates of Pathogenic Origin.J.*Appl. Environ. Microbiol.* 34(5):557.
Lautenbach, E., Strom, B.L., Bilker, W.B., Patel, J.B., Edelstein, P.H. and Fishman, N.O.(2004). Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Clinical Infectious Diseases, 33: 1288-1294.
Lawlor,M.S., O'Connor,C. and Miller,V.L.(2007). Yersiniabactin Is a Virulence Factor for *Klebsiella pneumoniae* during Pulmonary Infection.J.Infec.Immun.75(3): p. 1463–1472.
Matsen, J. M.,Spindler, J.A. and Blosser, R.(1974). Characterization of Klebsiella Isolates from Natural Receiving Waters and Comparison with Human Isolates. J.Applied. Microbiology,28(4): 672-678.
Meirelles-Pereira, F., Pereira, A.M.S., Silva, M.C.G., Gonc¸alves, V.D., Brum, P.R., Castro, E.A.R., Pereira, A.A. and Pereira, J.A.A. (2002) Ecological aspects of the antimicrobial resistance in bacteria of importance to human infections. Braz J Microbiol 33, 287–293.
Mulamattathil,S.G., Bezuidenhout,C., Mbewe,M.1 and Ateba,C.N.(2014). Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles. Journal of Pathogens.Volume 2014
National committee for clinical laboratory standards (NCCLs). (2002). performance standards for antimicrobial susceptibility testing. Villanova P.A.U.S.A.
Reinthaler, F.F., Posch, J., Feierl, G., Wust, G., Hass, D., Ruckenbauer, G., Mascher, F. and Marth, E. (2003). Antibiotic resistance of E.coli in sewage and sludge. Water Res 37, 1685–1690.
Ruppé, E., Hem, S., Lath, S., Gautier, V., Ariey, F., Sarthou, J.L.,Monchy, D., Arlet,G.(2009). CTX-M β-lactamases in E. coli from communityacquired urinary tractinfections.Cambodia.EmergInfect Dis.15(5):741–748.
Rzoska, J.,(1980). "*Euphrates and Tigris, Mesopotamia Ecology and Destiny*," The Hague, Boston, London, p. 122.
Schwartz, T., Kohnen, W., Jansen, B. and Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiol Ecol 43, 325–335.
Soge,O.O., Adeniyi, B.A. and Marilyn, C. Roberts, M.C.(2006). New antibiotic resistance genes associated with CTX-M plasmids from uropathogenic Nigerian Klebsiella pneumoniae. Journal of Antimicrobial Chemotherapy. 58, 1048–1053
Tennstedt, T., Szczepanowski, R., Braun, S., Pu¨hler, A. and Schlu¨ter, A. (2003). Occurrence of integron-associated resistance gene cassetes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. FEMS Microbiol Ecol 45, 239–252.
Wiskur,B.J., Hunt,J.J. and Callegan,M.C.(2008). Hypermucoviscosity as a Virulence Factor in Experimental *Klebsiella pneumoniae* Endophthalmitis.J. IOVS.Vol. 49, No. 11.

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