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RESEARCH ARTICLE

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF EXTENDED SPECTRUM β-LACTAMASES- PRODUCING COLIFORM BACTERIA FROM HILLA RIVER WATERS-IRAQ

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ABSTRACT

This study aimed to determine the prevalence of coliform bacteria and their extended spectrum β-lactamase (ESBL) phenotype isolated from surface waters of Hilla river. Over the period of four months from January to April 2015,a total of 101 water samples were obtained from 10 different sites of Hilla river . Seventy-eight (77.22%) isolates were detected as coilform .Species identification revealed that 23(29.5%) were *Escherchia coli*,35(44.9%) were *Klebsiella pneumonaie*, 9 (11.5%) were *Klebsiella oxytoca* and 11 (14.1%) were *Enterobacter* spp. Phenotypic detection of ESBL was carried out using disk approximation method and confirmed in 37 (47.43%) isolates . Antimicrobial susceptibility profiles of both ESBL –producing and non ESBL-producing isolates were assessed using Kirby-Bauer disk diffusion method. High levels of resistance were observed for ESBL-producing isolates when penicillin antibiotics (ampicillin and piperacillin) recorded (94.59%) and (89.18%), respectively. The lowest rates were detected for imipenem (5.4%), meropenem (8.1%) and levofloxacin (10.81%).These findings indicated the occurrence of ESBL-producing coliforms polluted Hilla river waters.

INTRODUCTION

During recent decades, abuse and overuse of antibiotics in medicine and agriculture has led to emergence of antibiotic resistance among enteric bacteria (Chitanand *et al*.,2010). Antibiotic-resistant bacteria constitute one of the more hazardous biological contaminants found in different water bodies .Bacteria resist antibiotics by different mechanisms, production of β-lactamases being one of these. Extended-spectrum beta lactamase (ESBL) enzymes are a class of β-lactamases that mediate resistance to all β-lactam antibiotics excluding carbapenems and cephamycins, inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam (Tisseria and Lee., 2013; Korzeniewska and Harnisz, 2013). Predominantly detected in a wide range of Gram-negative bacteria, some are inhabitants of human intestine and important pathogens not only in clinical settings but the community as well (Laurent *et a*l.,2008;Kang *et al*.,2013). Infections caused by ESBL- producing organisms are difficult to treat and the therapeutic options for clinicians is limited (Kim *et al*.,2002; Liu *et al*.,2004) .ESBLs are plasmid rather than chromosomally encoded β –lactamase, associated with mobile genetic elements and conferring multi-resistant properties (Bradford,2001; Szczepanowski *et al*.,2009). They are derived from the older TEM-1,TEM-2 and SHV-1 enzymes by amino acid substitution , originally discovered in Germany, in 1983 from *Klebsiella pneumoniae*. To date, over 300 types have been detected worldwide (Bush and Jacoby ,2010). The current study aimed to evaluate the prevalence of coliform bacteria isolated from Hilla river waters, detect ESBL –producing isolates by phenotypic test and determine the susceptibility profiles of bacterial isolates.

MATERIALS AND METHODS

Samples collection

Between January and April of 2015,a total of 101 water samples (from surface layer) were collected at 10 different sites of Hilla river ,the main river in Babylon province ,Iraq. It used for agriculture and drinking water for animals. Water samples were collected in sterile glass bottles and kept on ice, then transported to the laboratory to be analyzed within 2 hrs.

Samples processing and Microbiological analysis

Each sample was filtered through a sterile 0.22 µm pore membrane (Millipore, Difco, USA). Ten-fold dilutions were

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plated by spreading 0.1 ml on plate count agar and incubated aerobically at 37 C° for 24-48 hrs (Girlich *et al*.,2010 ;Moges *et al*.,2014). After incubation, based on colony morphology suspected colonies were selected and sub-cultured on different selective and differential media such as blood agar (Himedia),MacConkey agar (Himedia) and Eosin methylen blue agar (Biolife) then identified biochemically following standard methods described by Holt *et al*.(1994),Collee *et al*. (1996) and MacFaddin (2000).

Antimicrobial susceptibility test

All bacterial isolates were submitted to susceptibility testing against 13 antimicrobial agents using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates (Oxiod) (Bauer *et al*.,1966) .The tested antibiotics included : Ampicillin (10µg), Piperacillin (100 µg) , Amoxicillin- Clavulanic acid (10 µg), Cefotaxime(30 µg), Ceftazidime (30µg), Ceftriaxone (30 µg), Cefoxitin (30 µg), Aztreonam (30µg), Erythromycin(15 µg) .After 18hrs of incubation at 37 C° ,the zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010). For quality control, *Escherchia coli* ATCC 25922 was used.

Phenotypic detection of extended spectrum β-lactamases

Phenotypic detection of ESBLs producing isolates was determined using disk approximation method as modified by Coudron *et al*.(1997). Briefly, antibiotics disks containing 30µg of ceftazidime, cefotaxime, ceftriaxone and aztreonam **,**placed at a distance of 15mm (edge to edge) from a central disk containing amoxicillin-clavulanic acid (20 /10 µg) on Mueller –Hinton agar plate previously inoculated with test isolate.

RESULTS AND DISCUSSION

Out of 101 water samples,78 (77.22%) isolates were recovered during this study ,*E.coli* comprised 23(29.5%) isolates ,*K.pneumoniae* 35 (44.9%),*K.oxytoca* 9(11.5%) and 11(14.1%) *Enterobacter* spp. (Table-1).In one study, Ngozi *et al*. (2010) identified 18 isolates as coliforms, 11(4.4%) isolates were *E.coli* ,4(1.6%) *Klebsiella* spp. and 3 (1.2%) *Enterobacter* spp. obtianed from sachet-water manufactured and sold in Nigeria. In another work carried out by Korzeniewska and Harnisz (2013) demonstrated that *E.coli*, *Citrobacter freundii* and *Klebsiella* spp. were the most predominant species isolates from hospital sewage in Poland ,all were ESBLs-producers.

Table 1 Types, numbers and percentages of coliform bacteria isolated from Hilla river waters.

Type of bacteria	Numbers	Percentages
Escherchia coli	23	29.5
Klebsiella pneumoniae	35	44.9
Klebsiella oxytoca	9	11.5
Enterobacter spp.	11	14.1
Total	78	100

Results of the current study revealed that 37(47.43%) isolates were identified as ESBL-producers using disk approximation method. Of which,11(29.73%) isolates belonged to *E.coli*,17 (45.95%) isolates were *K.pneumoniae* ,2(5.40%) were *K.oxytoca* and 7 (18.92%) isolates were *Enterobacter* spp. (Table-2). This result was higher than that reported in Bangladesh by Talukdar *et al*. (2013) who characterized that 22 /233 bacterial isolates were ESBL-producer by this method. Also, Nogzi *et al*. (2010) noticed that out of 18 tested coliforms,2 (18%) strains of *E.coli* were confirmed as ESBL producer by double disk synergy test.

Contamination of Hilla river waters by these agents may be related to discrete discharge of Babylon Teaching Hospital for Maternity and Pediatric sewage containing strong multi-resistant pathogenic bacteria, 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 with high doses of pathogens.

Table 2 Frequency of potential ESBL-producing isolates by disk approximation test.

Type of isolate	No. of isolates	No.(%) of ESBL-producing isolates
Escherchia coli	23	11(29.73)
Klebsiella pneumoniae	35	17(45.95)
Klebsiella oxytoca	9	2(5.40)
Enterobacter spp.	11	7(18.92)
Total	78	37(100)

Antibiotics susceptibility of ESBL-producing and non –producing isolates was estimated, table-3.

The highest rates of resistance were observed for ESBL-producing isolates with (94.59%) to ampicillin followed by (89.18%) to piperacillin, (86.48%) to amoxi-clav ,(81.08%) to cefoxitin, (75.67%) to ceftazidme, (70.27%) to cefotaxime, (62.16%) to ceftriaxone, (45.94%) to aztreonam ,(43.24%) to nalidixic acid and (40.54%) to erythromycin. Higher percentages of resistance against penicillin antibiotics among ESBL-producers were recorded by other researchers, Ngozi *et al*. (2010) in Nigeria, Tacao *et al*. (2012) in Portagal. Andersen and Sandaa (1994) stated that the presence of coliforms with higher resistance to ampicillin and other antibiotics may reflect human effect in the natural environment.

However, lower rates of resistance were noticed for imipenem , meropenem and levofloxacin with (5.4%,8.1% and 10.81%) resistance rate, respectively. Table-3. The lower values of resistance toward imipenem and meropenem has been reported among ESBL-producing *E.coli* and *K.pneumoniae* isolated from effluent wastewater in Eygpt (Amine,2013).

In a Malaysian study ,Tissera and Lee (2013) recorded 19 isolates as ESBL-producers from urban surface waters, all were sensitive to imipenem and meropenem antibiotics. Moreover, non-ESBL-producer isolates showed less resistant rates against tested antibiotics, table -3. This result in agreement with the findings of Prado *et al*. (2008) in Brazil who recorded highest levels of resistance among ESBL-producing *K.pneumoniae* strains.

Table 3 Susceptibility profiles of ESBL and non ESBL-producing coliform isolates against different antibiotics(n=78).

Antibiotic	No.(%) of resistant isolates		Total (%)
	ESBL-positive (n=37)	ESBL-negative (n=41)	
Ampicillin	35(94.59)	24(58.35)	59(75.64)
Piperacillin	33(89.18)	15(36.58)	48(61.53)
Amoxicillin-clavulanic acid	32(86.48)	18(43.90)	50(64.10)
Cefotaxime	26(70.27)	3(7.3)	29(37.17)
Ceftazidme	28(75.76)	3(7.3)	31(39.74)
Cefriaxone	23(62.16)	4(9.75)	27(34.61)
Cefoxitin	30(81.08)	11(26.82)	41(52.56)
Aztreonam	17(45.94)	9(21.95)	26(33.33)
Imipenem	2(5.4)	0	2(2.56)
Meropenem	3(8.1)	0	3(3.84)
Nalidixic acid	16(43.24)	15(36.58)	31(39.74)
Levofloxacin	4(10.81)	4(9.75)	8(10.25)
Erythomycin	15(40.54)	8(19.51)	23(29.48)

CONCLUSION

Results of the present work have shown that Hilla river waters are contaminated with ESBL-producing coliform bacteria .The presence of these agents may provide an indication of water borne diseases and poses a major risk to exposed human populations.

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References

Amine, A .E. K. (2013). Extended spectrum beta- lactamase producing bacteria in waste water Alexandria, Egypt. Inter. J.. Biosc., Biochem .Bioin form. 3 (6):605-608.

Andersen, S. R and Sandaa, R.A.(1994). Distribution of tetracycline resistance among gram-negative bacteria isolated from polluted and unpolluted marine sediments. Appl. Environmen. Microbiol. 60(3): 908-912.

Bauer, A. W.; Kirby, W.M.M.; Sherris, J.C and Track, M. (1966). Antibiotic susceptibility testing by standardized single disc method .Am.J. Clin.Pathol.45: 493-496.

Bush, K. and Jacoby, G. A. (2010). Updated functional classification of - lactamases. Antimicrob. Agents Chemother. 54(3): 969-976.

Chitanand ,M.P.; Kadam ,T.A.; Gyananath ,G.; Totewad,N.D. and Balhal,D.K.(2010). Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. Indian J Microbiol .50:216–220

Clinical and Laboratory Standards Institute (CLSI). (2010). Performance standards for antimicrobial susceptibility testing; 20th. Informational Supplement. Approved standard M07-A8. Clinical and Laboratory Standards Institute.

Collee, J. G.; Fraser, A. G.; Marmiom, B. P.; and Simmon, A. (1996). Mackie and McCarteny Practical Medical Microbiology. 4th ed. Churchill Livingstone Inc., USA.

Coudron, P. E.; Moland, E. S.; and Sanders, C. C. (1997). Occurence and detection of extended -spectrum β-lactamases in members of the family *Enterobacteriacea* at a Veterans Medical Center :seek and you may find. J. Clin. Microbiol. 35: 2593-2597.

Grilich,D; Poirel, L. and Nordman, P.(2010).Novel ambler class A carbapenem-hydrolyzing β-lactamase from a *Pseudomonas fluorescens* isolate from the Seine River, Paris, France. Antimicrob. Agents.Chemother.54(1) :328-332.

Holt, J. G.; Krieg, N. R.; Sneath, H. A. Stanley, J. T. and Williams, S. T. (1994). Bergeys manual of determinative bacteriology. 9th. Ed., Baltimore; Wiliams and Wilkins, USA.

Kang,C.I.,Wi,Y.M.,Ko,K.S., Chung,D.R., Peck, K.R., Lee, N.Y.,*et al.* (2013). Outcomes and risk factors for mortality in community-onset bacteremia caused by extended-spectrum b-lactamase-producing *Escherichia coli*, with a special emphasis on antimicrobial therapy. Scand.J.Infect.Dis. 45, 519–525.

Kim, Y.K.; Pai ,H.; Lee, H.j.; Park, S.E.; Choi, E.H.; Kim, J.; Kim, J.H.and Kim, E.C. (2002). Bloodstream infections by extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella Pneumoniae* in children: epidemiology and clinical outcome. Antimicrob. Agents Chemother. 46: 1481-1491.

Korzeniewska,E.; Harnisz,M.(2013). Beta-lactamase-producing *Enterobacteriaceae* in hospital effluents. J. Environ. Manag.123 :1-7.

Laurent,C.,Rodriguez-Villalobos, H., Rost, F., Strale, H., Vincent,J.L., Deplano, A.,*etal*. (2008). Intensive care unit outbreak of extended-spectrum β-lactamase–producing *Klebsiella pneumoniae* controlled by cohorting patients and reinforcing infection control Measures. Infect. Control Hosp. Epidemiol. 29, 517–524.

Liu ,C.P.; Wang, N.Y.; Lee, C.M.; Weng, L.C.; Tesng, H. K.; Liu, C.W.; Chiang, C.S.and Huang, F.Y. (2004). Nosocomial and community-acquired *Enterbacter cloacae* bloodstream infections: risk factors for and prevalence of SHV-12 in multi-resistant isolates in a medical center. J. Hosp. Infect. 58: 63-77.

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams and Wilkins, USA.

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

Ngozi,A.F.; Romanus,I.I.; Azubuike, A.C. ;Ayogu, ,A.E.; Oji Anthonia Egwu,O.A.; Collins, O.N..(2010). Presence of coliform producing extended spectrum beta lactamase in sachet-water manufactured and sold in Abakaliki, Ebonyi state, Nigeria.Inter.Res.J.Microbiol.1(2):032-036.

Prado,T.; Pereira1,W.C.; Silva, D.M.; Seki, L. M..;. Carvalho, A.P.D'A. and Asensi, M.D.(2008). Detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in effluents and sludge of a hospital sewage treatment plant Lett. Appl. Microbiol. 46 :136–141.

Tacao,M.; Correia, A. and Henriques, I.(2012). Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the dissemination of *bla*CTX-M-like genes .App. Environ. Microbiol. 78 (12): 4134-4140.

Talukdar,P.K.; Rahman,M.; Rahman, M. ;Nabi,A. ;Islam,Z.; M. Mahfuzul Hoque,M.; Endtz,H.P.; and Islam1,M.A. (2013). Antimicrobial Resistance, Virulence Factors and Genetic Diversity of Escherichia coli Isolates from Household Water Supply in Dhaka, Bangladesh. Plos one.8:e61096.

Tissera, S.and Lee,S.M. (2013). Isolation of extended spectrum ß-lactamase (ESBL) producing bacteria from urban surface waters in Malaysia. Malays. J .Med .Sci. 20(3): 14-22.

Zhang,H. Zhou,Y.; Guo,S. and Chang,W . (2015). Multidrug resistance found in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from rural water reservoirs in Guantao, China.Front.Microbiol.6:267-270.

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