Journal of ENVIRONMENTAL Treatment Techniques

J. Environ. Treat. Tech.
ISSN: 2309-1185

Journal web link: http://www.jett.dormaj.com

Bacteriological Study of Municipal Water Discharged in Al-Kufa River, Najaf, Iraq

Ahmed O.M.AL-Dahmoshi and Hazim Aziz Naji Alhadrawi*

Department of Ecology, Faculty of Science, University of Kufa, Najaf, Iraq

Received: 09/05/2020 Accepted: 17/07/2020 Published: 20/09/2020

Abstract

Aim: The present study was aimed to investigate the bacteriological aspects for monitoring of water quality of Al-Kufa River, Al-Najaf, Iraq. *Method:* Water samples were collected from three sites in frequency of four time a month (For each site the samples were collected from about 100 meter before and after the site of municipal water discharge in the month of November, 2018 and April, 2019. The bacteriological assessment of samples involves, total bacterial count. *Results and discussion*: Highest number of bacteria was recorded in Site no 3, during month of April while the lowest number was recorded in Site no 1, during month of November. Study also included isolation and identification of bacteria by using the selective culture media. An isolated bacteria includes *E.coli, K.pneumoniae, P.aeruginosa, V. cholera, S.typhi, S.aureus, E faecalis* and investigation of antibiotics on bacterial isolates was investigated. Investigation shows resistance for *E. coli* and *K. pneumonia* with antibiotic ceftazidime while *P. aeruginosa* showed high resistance for Cefotaxime and Gentamycin. *V. cholera and S.typhi* shows significantly high resistance for Beta-lactam antibiotic i.e. Amoxicillin/Clavulanic acid and Cefotaxime and Ceftazidime, while *S. aureus* and *E. faecalis* shows high resistance for Clarethromycin and for tetracycline respectively. According to the results of present study we conclude that important difference observed among the sites in terms of physical ,chemical and bacteriological determinants according to site and the period of sample collection ,The study isolates showed different high antibiotic resistance patterns and the findings reflect the importance of water as a reservoir for the dissemination of antibiotic resistance genes in the natural aquatic environment.so the identification of this kind of contamination is necessary for appropriate management practices to improve sustainable water resources.

Keywords: Municipal Water Discharged, Al-Kufa River, Resistance

1 Introduction

Water quality is determined by assessing three classes of attributes: biological, chemical, and physical [1]. There are standards of water quality set for each of these three classes of attributes. Some attributes are considered of primary importance to the quality of drinking water while others are of secondary importance [2]. Biological attributes of a waterway can be important indicators of water quality and refer to the number and types of organisms that inhabit a waterway. When assessing water quality, it is also important to look at the quality of organisms that live in a waterway [2]. The water quality monitoring has become an important topic in stream and river system that's affected by careless disposal of pollutants, where domestic and industrial effluent discharges consider the major sources of aquatic pollution [3]. It is crucial to improve our understanding of Al-Kufa habitat due to its importance for community livelihoods, so the current status of pollution in Al-Kufa River is an important factor to considered, especially due to increased human and industrial activities in Al-Kufa region [4].

2 Study area

Near the Al-Kiffil Bridge in the north of Kufa city, Iraq, the Euphrates subdivided into two parts: Al-Abassia and Al-Kufa River, the last one extends from Al- Kiffil city via Al-Najaf province to Al- qadisia province, the total length of Kufa River is about 38 km, and its capacity reach to 552 m3. The water level in this river undergoes large fluctuations, the highest level occurs during the high discharge seasons (end of March month and of early April), the lowest water level in the summer. A lot of villages and farms (animal, crop, and vegetation farms) are found along the River; there are domestic, municipal wastewater and agriculture drainage discharged to the River; in addition to the industrial wastes that which come from: the industrial region in Al-Najaf city, the leather industry, and the cement factory. All of above have affecting the water quality, distribution and diversity of microorganisms. To investigation this study, 3 Sites were chosen as clear in Table (1).

***Correspondence author:** Hazim Aziz Naji Alhadrawi, Assistant Professor, Department of Ecology, Faculty of Science, University of Kufa, Najaf, Iraq**.** Email: hazim.alhadrawi@uokufa.edu.iq

Figure 1: Google earth Image of sampling sites of Al-Kufa River

Table 1: The sampling sites and their location by GPS*

Site	N	E
Site 1	32°02'53.7" N	44°23'30.6" E
Site 2	32°02'08.8" N	44°24'45.8" E
Site 3	32°01'42.6" N	44°24'53.6" E

*GPS: Global Positioning System

The first site is located near the College of Veterinary Medicine University of Kufa This site its carry all the sewage of the northern neighborhoods of Najaf. The second site is located adjacent to the Conference Palace, at the end of Al-Ma'mal Street in Kufa city which carry the sewage of the old Kufa neighborhoods. The third site located about 2 km from the south of second site (Figure 1).

3 Materials and Methods

3.1 Sample collection

A total of (72) samples from water was collected by using 20 ml sterile containers of polyethylene that were transferred to the laboratory within half an hour. All samples were diluted with normal saline solution. From each samples 0.1 ml has been taken for culture on some selected culture media.

3.2 Antimicrobial susceptibility test by Agar Disk method

The *in vitro* antibiotic susceptibility were determined via disk diffusion method according to Clinical and Laboratory Standards Institute instructions [5]. Activation of isolates were performed using nutrient broth for 18 h at 37 °C and the growth was adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/mL) and then spread on Muller Hinton agar (MHA) with a sterile cotton swab. Antibiotic disks were placed onto MHA, gently pressed down to ensure complete contact with the agar inoculated with bacteria and then incubated for 24 h at 37 °C and then inhibition zone diameter in millimeters (mm) was recorded. Interpretation of results as a sensitive or resist were achieved according to CLSI, 2019.

4 Results and Discussion

4.1 Total Plate Count

The results of total plate count reveal high microbial content of water sample in all sites as explained in Table 2. From the results as indicated in Table 2, the highest colony forming unit was seen in the month of April as compared to month of November. Increased in high colony forming unit may be attributed by increase in temperature.

4.2 Types of bacterial isolates

According to culturing the samples on the chromogenic agar and some selective media there are different types of bacterial isolates which founded in all samples included the following types of bacteria:

- *E. coli:* Gram negative bacteria appear green metallic shine on EMB agar pink colonies on MacConkey agar and UTI chromogenic agar.
- *K.pneumonia:* Gram negative bacteria appear mucoid, deep purple colonies on EMB agar. mucoid pink colonies on MacConkey agar and dark blue colonies on UTI chromogenic agar.
- *P.aeruginosa*: Gram negative bacteria appear as blue to purple colonies on selective Pseudomonas chromogenic agar.
- *Vibrio cholera*: Gram negative bacteria appear as pink-rose colonies on selective vibrio chromogenic agar.
- *S. typhi*: gram negative bacteria appear pale yellow colonies on MacConkey agar and pink with black center because of (H_2S) on Salmonella-Shigella agar.
- *S. aureus:* gram positive bacteria appear gold yellow colonies on mannitol salt agar and gave white creamy colonies on UTI chromogenic agar.
- *E. facials*: gram positive bacteria gave light blue to green colonies on UTI chromogenic agar.

Most of the isolated bacteria have clinical importance and push a risk for human health. The result is in agreement with [6-7] who reported high coliform counts. The high coliform counts recorded from the river samples indicated the

occurrence of faecal contamination [7]. The bacteriological examination of water samples revealed the presence of bacterial indicator and other pathogenic bacteria during study period.

4.3 Antibiotic Susceptibility for Gram-negative Bacteria

The results revealed high resistance of Gram-negative bacterial isolates to different classes of antibiotic used in this study (table 3). Our records were in accordance with those gathered by many studies on antibiotics resistance among water Gram-negative pathogens [7-9] racking the spread of antibiotic-resistant bacteria in water samples, such as sewage, tap and well water, is a useful source of information that can be used by policy makers in order to create risk management strategies for water environments.

Developing of resistance to β-lactams and cephalosporins and aztreonam can be clarified as a results to carrying genes encodes for extended spectrum β-lactamases (ESBLs) like TEM-1, OXA-1, CTX-M and SHV. ESBLs genes located on bacterial chromosomes or may be exchanged among species and genus via transposable elements like plasmids. Production of extended-spectrum β-lactamases (ESBLs) is a significant resistance-mechanism that impedes the antimicrobial treatment of infections caused by *Enterobacteriaceae* and is a serious threat to the currently available antibiotic armory [11-13]. The resistance to Trimethoprim-sulfamethoxazole may be due to acquisition of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes through mobile genetic elements such as plasmids, transposons, and class 1 Integrons [14-15]. The high concentrations of different types of antibiotics that reach water by different way may be leads to emergence of antibiotics resistance among water bacteria. Classes of antibiotic residues that have frequently been detected in municipal effluents include *β*-lactam, macrolides, lincosamide, tetracyclines, sulphonamides, and fluoroquinolones [16]. Emergence and dissemination of AR is on the increase trend among enteric bacteria [17]. The transfer of resistance among microorganisms is a serious threat, contributing to the development and emergence of ARB, thereby reducing the therapeutic potential against pathogens [18].

4.4 Antibiotic Susceptibility for Gram-positive Bacteria

Resistance among Gram-positive isolates were lower than those of Gram-negative (table 4). Our results agreed with [19-20] and whom report the presence of resistance genes for tetracycline and trimethoprim-sulfamethoxazole in drinking water treatment plants (DWTPs) and finished water and report 39 antibiotics resistance genes (ARGs) for tetracycline, chloramphenicol and *β*–lactam in drinking water sources. The presence of outer membrane and negative charge of LPS in Gram negative bacteria and resistance or tolerance to high salt concentration in Gram positive bacteria may play explain the survive the water pathogen in highly polluted water especially with different types heavy metals and antibiotics [21].

Table 2: Total plate count among all sites

No.			Site 1	Site 2	Site 3
			Mean x10^6 CFU\ ml±SD		
November	1	**B**	1.25 ±0.5	1.88±0.11	1.98±0.86
		I	4.22 ±0.1	5.12±0.5	4.21±0.12
		A	3.08 ±0.09	2.33±0.1	2.28±0.45
	2	B	1.32 ±0.12	1.9±0.06	1.66±0.09
		I	5.85 ±1.1	4.82±0.2	5.22±1.46
		A	4.72 ±0.8	3.26±0.08	3.96±1.82
	3	B	1.45 ±0.6	2.1±0.05	2.44±1.02
		I	4.45 ±1	5.66±0.5	4.88±1.42
		A	3.65 ±0.4	3.3±0.4	3.02±1.24
	4	B	2.00 ±0.5	1.96±0.1	2.68±0.76
		I	4.45 ±1	4.56±0.5	5.44±1.88
		A	2.25 ±1	2.5±0.05	3.78±1.45
April	1	B	3.24 ±1.2	4.88±1.31	4.98±1.68
		I	6.35 ±2.04	6.44±2.10	6.48±2.56
		A	4.28 ±1.44	5.04±1.21	4.88±1.02
	2	B	4.66 ±3.34	4.46±1.01	5.44±1.86
		I	6.28 ±2.88	6.98±2.11	7.22±2.88
		A	4.29 ±1.66	5.22±1.12	5.88±1.88
	3	B	4.68 ±1.86	5.32±1.42	4.86±2.40
		I	6.85 ±2.64	7.42±2.44	8.24±3.54
		A	4.98 ±1.88	5.48±2.01	5.96±2.42
	4	B	5.1 ±2.44	5.98±1.11	5.89±2.12
		I	7.24 ±3.0	7.88±2.46	8.98±3.44
		A	5.88±2.22	6.02±2.34	6.26±2.46

Table 3: Antibiotic Resistance percentage among Gram-negative bacterial isolates

Isolates		Antibiotics resistant (N)%								
(N) %		AMC	CTX	CAZ	ATM	IPM	AK	CN	CIP	STX
E.coli	**B(33)**	(20)60.6	(18)54.5	(30)90.9	(23)69.6	(11)33.3	(25)75.7	(29)87.8	(19)57.5	(20)60.6
	I(56)	(50)89.1	(43)76.7	(40)71.4	(42)75	(22)39.2	(39)69.6	(43)76.7	(35)62.5	(31)55.3
	A(40)	(36)90	(33)82.5	(38)95	(31)77.5	(12)03	(32)80	(36)90	(25)62.5	(23)57.5
K. Pneumonia	**B(20)**	(15)75	(18)90	(19)95	(13)65	(9)45	(11)55	(18)90	(12)60	(10)50
	I(30(	(25)83.3	(26)86.6	(25)83.3	(23)76.6	(11)36.6	(23)76.6	(27)90	(25)83.3	(19)63.3
	A(24)	(20)83.3	(22)91.6	(20)83.3	(10)41.6	(13)54.1	(20)83.3	(19)79.1	(16)66.6	(18)75
P. Aeruginosa	**B(38)**	(28)73.6	(30)78.9	(33)86.8	(29)76.3	(15)39.4	(30)78.9	(32)84.2	(19)50	(20)52.6
	I(50(	(40)80	(48)96	(45)90	(43)86	(30)60	(37)74	(48)96	(41)82	(38)76
	A(44)	(36)81.8	(40)90.9	(39)88.6	(42)95.4	(23)52.2	(35)79.5	(39) 88.6	(39) 88.6	(43)97.7
S.typhi	**B(5)**	(4)80	(4)80	(3)60	(3)60	(1)20	(1)20	(4)80	(2)40	(2)40
	I(12)	(12)100	(9)75	(10)83.3	(11)91.6	(5)41.6	(9)75	(8)66.6	(9)75	(8)66.6
	A(7)	(5)71.4	(5)71.4	(6)85.7	(7)100	(3)42.8	(4)57.1	(4)57.1	(3)42.8	(3)42.8
V.cholera	**B(0)**	(0)0	(0)0	(0)0	(0) 0	(0)0	(0)0	(0)0	(0)0	(0) 0
	I(2)	(2)100	(2)100	(2)100	(2)100	(1)50	(1)50	(1)50	(1)50	(1)50
	A(0)	(0) 0%	(0) 0	(0)	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0

AMC= Amoxicillin/Clavulanic acid, CTX= Cefotaxime, CAZ= Ceftazidime, ATM= Aztreonam, IPM= Imipenem, AK=Amikacin, CN=Gentamicin, CIP= Ciprofloxacin, SXT= Trimethoprim-sulfamethoxazole.

Table 4: Antibiotic Resistance percentage among Gram-positive bacterial isolates

Isolates		Antibiotics resistant (N)%								
(N) %		VAN	AK	CN	CLR	TE	CIP	NOR	F	SXT
S. aureus	**B(20)**	(16)80	(15)75	(8)40	(20)100	(17)85	(12)60	(16)80	(14)70	(20)100
	I(32)	(25)78.1	(30)93.7	(27)84.3	(26)81.2	(30)93.7	(21)65.6	(28)87.5	(32)100	(26)81.3
	A(24)	(16)66.6	(20)83.3	(20)83.3	(22)91.6	(21)87.5	(15)62.5	(20)83.3	(20)83.3	(15)62.5
E. faecalis	**B(36)**	(26)72.2	NA	NA	NA	(28)77.7	(12)33.3	(10)27.7	(16)44.4	NA
	I((40	(32)80	NA	NA	NA	(38)95	(34)85	(35)87.5	(32)80	NA
	A(32)	(30)93.7	NA	NA	NA	(32)100	(30)93.7	(31)96.8	(28)87.5	NA

VAN=Vancomycicn, AK=Amikacin, CN=Gentamicin, CLR=Clarethromycin, TE=Tetracyclin, CIP=Ciprofloxacin, NOR=Norfloxacin, F=Nitrofurantion, SXT= Trimethoprim-Sulfamethoxazole, C=Chloramphenicol

It is very important to mention that many earlier studies observed that resistant bacteria to many antibiotics and other toxic chemicals by virtue of carrying plasmids and or transposons encoding genetically linked metal and antibiotic resistance. Besides that, several studies presented evidence that in waters habitats there is a high potential for horizontal gene transfer, mediated by plasmids and facilitated by integrons [22-23]. Plasmids carrying resistance genes have been identified in pathogenic bacteria of the genus *Escherichia, Salmonella, Klebsiella,* and *Pseudomonas.* These plasmids carry determinants for resistance to drugs of different groups [Tetracyclines, quinolones, aminoglycosides, sulfonamides, β-lactams and chemotherapeutics. [24-26]. Therefore, human activities, especially discharge of wastewater can aggravate antibiotic resistance leading to the wide dissemination of resistance genes in the aquatic environment.

5 Conclusion

According to the results of present study we conclude that, important difference observed among the sites in terms of physical, chemical and bacteriological determinants according to site and the period of sample collection. The study isolates showed different high antibiotic resistance patterns and the findings reflect the importance of water as a reservoir for the dissemination of antibiotic resistance genes in the natural aquatic environment.so the identification of this kind of contamination is necessary for appropriate management practices to improve sustainable water resources.

Competing interests

The authors declare that there is no any conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is origin.

References

1. Gorde S, Jadhav, M. Assessment of Water Quality Parameters. *A Review Journal of Engineering Research and Applications*. 2013;3(6): 2029-2035
2. Akoteyon IS, Omotayo AO, Soladoye O and Olaoye, HO. Determination of Water Quality Index and Suitability of Urban River for Municipal Water Supply in Lagos-Nigeria. *Europ J Scientific Res*. 2011; 54(2): 263-271.
3. Campbell LM. Mercury in Lake Victoria (East Africa). (2001). Another emerging issue for a Beleaguered Lake. PhD, Thesis. Waterloo, Ontario, Canada.
4. Mulamattathil G, Bezuidenhout C and Ateba CN. Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles. *Journal of Pathogens*. 2011. Volume 2014, Article ID 371208, p.11
5. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing. (2019). Twenty-Fifth Informational Supplement. CLSI Document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA. Accessed from https://clsi.org/standards/products/microbiology/documents/m100/.
6. Shittu OB, Olaitan JO, Amusa TS. Physio-chemical and Bacteriological Analysis of Water used for Drinking and Swimming Purpose. *Afr. J. Biol. Res*. 2008; 11:285–290.
7. Sanders E, Yuan Y, Pitchford A. Fecal coliform and E. coli concentrations in effluent-dominated streams of the upper Santa Cruz Watershed. *Water*. 2013; 5: 243–261.
8. Blaak H, Lynch G, Italiaander R, Hamidjaja RA, Schets FM and de Roda Husman AM. Multidrug-Resistant and Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* in Dutch Surface Water and Wastewater. *Plos One*. 2015;10(6): e0127752.
9. Cornejova T, Venglovsky J, Gregova G, Kmetova M and Kmet V. Extended-Spectrum Beta-lactamases in *Escherichia coli* from Municipal Wastewater. *Ann Agric Environ Med*. 2015, 22(3), p. 447-450.
10. Baquero F, Martinez JL and Canton R. Antibiotics and Antibiotic Resistance in water environments. *Curr Opin in Biotechnol*. 2008; 19(3): 260–265.
11. Rupinder B, Geeta W and Shikha J. Prevalence of Extended Spectrum β-lactamases in Multi-drug resistant Strains of Gram-negative Bacilli. *J Acad Indus Res*. 2005; 1(9): 558-560.
12. Majda Q, Najma A. and Summyia B. Evaluation of extended Spectrum beta-lactamase Mediated Resistance in *Escherichia coli* and *Klebsiella* in Urinary Tract Infection at a Tertiary Care Hospital. *Biomedica*. 2013; 29: 78-81.
13. Kritu P, Prakash, Shiba KR, Reena KM, Ram NS and Ganesh R. Antibiogram typing of gram-negative isolates in different clinical samples of a tertiary hospital. *Asian J Pharm Clin Res*. 2013; 6(1):153-156.
14. White PA, McIver, CJ and Rawlinson WD. Integrons and Gene Cassettes in the Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*. 2001; 45(9): 2658-2661.
15. Singh R, Schroeder CM, Meng J, White DG, McDermott PF, et al. Identification of Antimicrobial Resistance and class 1 Integrons in Shiga-toxin-producing *Escherichia coli* recovered from humans and food animals. *Journal of Antimicrobial Chemotherapy*. 2005; 56(1): 216-219.
16. Rahube TO, Viana LS, Koraimann G and Yost CK. Characterization and Comparative Analysis of Antibiotic Resistance Plasmids Isolated from a Wastewater Treatment Plant. Front. *Microbiol*. 2014; 5: 558.
17. Von Wintersdorff, CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, and Wolffs PFG. Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Frontiers in Microbiol*. 2016; 7: 173.
18. Hawkey PM, & Jones AM. The Changing Epidemiology of Resistance. *Journal of Antimicrobial Chemotherapy*. 2009; 64(1): i3-i10.
19. Guo X, Li J, Yang F, Yang J and Yin D. Prevalence of Sulfonamide and Tetracycline Resistance genes in Drinking Water Treatment Plants in the Yangtze river Delta, China, *Science of the Total Environment*. 2014; 493: 626–631.
20. Moges F, Endris M, Belyhun Y and Worku W. Isolation and Characterization of Multiple Drug Resistance Bacterial Pathogens from Wastewater in The Hospital and Non-Hospital Environments. *Northwest Ethiopia*. 2014: 7.
21. Kafilzadeh F, Moghtaderi Y and Jahromi AR. Isolation and Identification of Cadmium-Resistant Bacteria in Soltan Abad River Sediments and Determination of Tolerance of Bacteria through MIC and MBC. *European Journal of Experimental Biology*. 2013; 3: 268-273.
22. Devika L, Rajaram R and Mathivanan K. Multiple Heavy Metal and Antibiotic Tolerance Bacteria Isolated from Equatorial Indian Ocean. *International Journal of Microbiological Research*. 2013; 4: 212-218.
23. Novo A, André S, Viana P, Nunes OC and Manaia CM. Antibiotic Resistance, Antimicrobial Residues and Bacterial Community Composition in Urban Wastewater. *Water Research*. 2013; 47: 1875-1887.
24. Ceylan O. and Uğur A. Bio-Monitoring of Heavy Metal Resistance in Pseudomonas and Pseudomonas Related Genus. *Journal of Biological Environmental Science*. 2012; 6: 233-242.
25. Shamim S. and Rehman, A. Cadmium Resistance and Accumulation Potential of *Klebsiella pneumoniae* Strain CBL-1 Isolated from Industrial Wastewater. *Pakistan Journal Zoology*. 2012; 44: 203-208.
26. Jafari JME, Shakibaie MR and Poormasoomi L. Isolation of a Novel Plasmid from Hospital Isolate of *Pseudomonas aeruginosa*. *Journal Clinical and Experimental Pathology*. 2013; 3: 1-5.