Academic Science Journal

Antigenic and Molecular Detection of Adenoviruses from Sewage Water in Diyala Province – Iraq

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Received: 28 January 2023 **Accepted: 20 June 2023**

DOI: https://doi.org/10.24237/ASJ.02.02.703A

Abstract

Waterborne viral illnesses are still have public health in both developed and developing. Adenovirus one of the enteroviruses are widely circulated in the environment even in the absence of associated clinical conditions in the community. Adenoviruses are the second cause of gastroenteritis after rotavirus. as they are responsible for 5-9 % of gastroenteritis cases in children. The objectives of study antigenic and molecular detection of adenovirus from water samples collected from sewage water plants (SWP) and draining canals (DC) in Diyala city (Iraq). This study was conducted in Diyala province for the period from January 2022 to August 2022. A total of 50 water samples were collected from sewage water plants and draining canals in plastic containers Immunochromatographic Assay (ICA) technique was used for direct detection of adenoviruses antigens a for molecular detection, the water samples were firstly ultra – centrifuged and the then viral nucleic acid (NA) of adenovirus were extracted Polymerase Chain Reaction (PCR) adenoviruses RNAs concentration were measured. (Sacace Biotechnologies PCR kit) technique was used for viral NAs detection, analysis was done using the statistical packages for social science version (27) and P value was considered significant. The Result using the Immunochromatographic Assay (ICA) The total positivity rate of adenovirus in sewage water plants and draining canals 18 (36%), while the Polymerase Chain Reaction (PCR) technique found that the total positivity rate was 29 (58%). The high detection rate of adenoviruses found in sewage water plants and draining canals by both immunological

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and molecular techniques may reflect the high prevalence of the virus in the community and recreating the risks public health deterioration.

Keyword: Adenovirus, Sewage Water, Draining Canals, Diyala Province.

الكشف المستضدي والجزيئي للفيروسات الغدية من مياه الصرف الصحي في محافظة ديالى – العراق

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الخلاصة

لا تزال الأمراض الفيروسية المعوية التي تنقلها المياه لها آثارًا صحية عامة اجتماعية واقتصادية في كُلّ من العالم المتقدّم والنامي، تنتشر الفيروسات المعوية البشرية بصمت في البيئة حتّى في غياب الظروف السريرية المرتبطة بها في المجتمع، تهدف هذه الدّراسة إلى الكشف عن الفيروسات الغدية في مياه الصرف الصحي باستعمال تقنية المقايسة الكروموتوكرافيا وتفاعل البلمرة المتسلسل (PCR). أجريت هذه الدّراسة في محافظة ديالى للمدة من كانون الثاني 2022 إلى آب 2022، وجُمعت (50) عينة من محطات مياه الصرف الصحي وقنوات الصرف الصحي في حاويات بلاستيكية، وضعت في صندوق مبرّد، ونقلت إلى المختبر، استعملت تقنية المقايسة المناعية الكروماتوغرافيا (ICA) (Biozek، هولندا)، للكشف المباشر المناعي عن مستضدات الفيروس الغدي، وجرى طرد عينات المياه وقياس تركيز الحمض النووي الفيروسي، ثمَّ استخلص الحمض النووي الفيروسي (China, Wondfo)، تفاعل البلمرة المتسلسل PCR (Sacace Biotechnolgies PCR Kit, Haly) تقنية قد استعملت للكشف عن الحمض النووي الفيروسي، أجريت التحليل الإحصائي باستخدام الحزم الإحصائية للعلوم الاجتماعية (إصدار 27)، وعُدّت قيمة (P) معنوية، عندما تكون متساوية أو أقل من (0.05).واظهرت النتائج باستعمال المقايسة المناعية الكروماتوغرافيا (ICA) أَنَّ عدد العينات الموجبة (18) (36%)، في حين كانت عدد العينات الموجبة باستعمال تقنية تفاعل البلمرة المتسلسل PCR (29) (58%) عينة، واستنتجت الدّراسة ارتفاع معدل الكشف عن الفيروس الغدي في محطات مياه الصرف الصحي وقنوات الصرف بوساطة استعمال التقنيات المناعية والجزيئية، وعدم معالجة هذه المياه بصورة جيدة، ولاسِيَّمَا عند استخدام مياه القنوات في سقي المزروعات، سوف تسبب مخاطر على الصحة العامة.

الكلمات المفتاحية: الفيروس الغدي، مياه الصرف الصحي، قنوات المبازل، محافظة ديالى.

Introduction

Adenoviruses belong to the adenoviridae family the largest Nonenveloped viruses that have a linear double - stranded (DNA) genome. infect both humans and animals [1] The spread of

adenoviruses infection is mainly by the fecal-oral and oral route, watery food and soil contaminated by infected feces are an exogenous infection source which creates many opportunities for the transfer of the infection, and cause an epidemic outbreak [2] adenoviruses are being isolated from all type of water: ground, sewage water and fresh water environments[3] these viruses are resilient organisms, able to withstand high concentrations of sodium chloride and large changes in temperature, flourishing these viruses in water environment, their natural reservoir [4]. Adenovirus infection increases in January to March, and early spring while decreased during summer July, August [5]. March detection of Enteroviruses in the water environment is performed by virus isolation in cell cultures and the use of molecular techniques for subsequent serotyping. The researchers conducted in different countries with methods mentioned above, reveal widespread environmental contamination by Enteroviruses[6]. Another studies using Nested - PCR the detection rate of Enteroviruses in sewage water was 42.5% in treated drinking water was 18.7 % in river water 28.5%. in borehole - spring and dam water was 26.7 %[9]. Although several studies were conducted in Iraq to address the prevalence of EVs in clinical samples, however no previous studies were conducted on sewage or river water were found.

Specimens and Methods:

This study was conducted in Diyala city (Iraq) for the period from January August 2022. A total of 50 water samples were collected from 6 sewage water plants and 3 draining canals, the sample volume is (250 ml) in a disposable tightly cupped plastic containers. Water samples were delivered to the laboratory by cool box. Direct detection of adenovirus antigen within 3 hours of collection. Immunochromatographic assay (ICA) Technique (Biozek, Netherlands) used for direct detection of adenoviruses antigens. (200 ml) of sewage water was placed in test tubes and centrifuged at (6000 rmp) for an hour. (80) ul were added to the collection tube, the sample was mixed, the (2) drops were placed in the circlar window. The result was read within (15) minutes Water sample, kept at 4^{o}Ce for further molecular analyses.

The molecular detection, for the sewage water samples were firstly ultra- centrifuged at 30000 RPM for 30 minutes, The viral nucleic acid were extracted, by using the wondfo kit. Then the Adenoviruses DNAs concentration were measured using thermo scientific Nano Drop (2000)

(U.S.A)., then the adenoviruses RNAs concentration were measured using thermo scientific Nano Drop 2000 (USA) DNA center at – Al-Nahrain University. The viral nucleic acids were extracted, sacace biotechnologies PCR kit (Italy) technique statistical analysis was done. Prepare the reaction mixture for each sample (50) samples ware prepared, each sample consisted of (25) ul, (10) ul of DNA and (15) ul of reaction mixture. The samples were mixed. One positive control sample and on negative sample were prepared. Put the samples in PCR – Bio Rad CFX 96 for (2) hours, then read the result. The samples considered positive if the CT is less than (4) (CT<40). The sample is considered negative if the CT greater thean (40) (CT>40). The statistical packages for social science version 27. and P value was considered significant whenever it is less than 0.05.

Table 1: Programs reaction of PCR

CYCLE	MIS	TEMPERATURE	PCR
1	05:00	95	Initial Denaturation
30	00:30	95	Denaturation
	00:30	55	Annealing
	01:00	72	Extension
1	07:00	72	Final Extension
	10:00	10	Hold

Results

Using the direct Immunochromatographic Assay (ICA) table (1) revealed that out of 50 samples, the total rate of adenoviruses in sewage water was 18 (36%). The results also showed that out of 50 water samples submitted for PCR, the total positivity rate for adenoviruses was 29 (58%).

Table 2: Adenoviruses total positivity rate by ICA and, PCR technique.

TEST USED	NO. SAMPLES	POSITIVE		NEGATIVE	
		no	%	no	%
ICA results	50	18	36	32	64
PCR results	50	29	58	21	42

Table (3) Showed the adenoviruses detection rate according to the location of sample collection. Using the ICA technique, the positive detection rate of adenoviruses. in the districts areas was

significantly higher compared to that of sub - districts areas (24 % vs 12 %, p = 0.037*) the positive detection rate of adenoviruses in the districts areas was significantly compared to that of sub- districts areas (36 % vs 28 %, p = 0.024*) using the PCR.

Table 3: Adenoviruses positivity rate by ICA and PCR according to location

DETECTION TECHNIQUE		LOCATION				
		Districts		Sub – Districts		P value
		No	%	No	%	
ICA Results	Positive	12	24	6	12	0.037*
	Negative	38	76	44	88	
	Total	50	100	50	100	
PCR Results	Positive	32	64	39	78	0.024*
	Negative	32	64	39	78	
	Total	50	100	50	50	

The adenoviruses positivity rate according to the type of water sample was revealed in table (4) by immunochromatographic Assay (ICA) technique, the detection rate in sewage water was insignificantly than in draining canals (20 % vs 16 %, p = 0.293) using the Polymerase Chain Reaction PCR technique, the detection rate in sewage water was insignificantly compared to that of draining canals water (32 % vs. 26 %, p = 0.0 291).

Table 4: Adenoviruses positivity rate by ICA and PCR according to sample type.

DETECTION TECHNIQUE		WATER SAMPLE TYPE				
		Sewage Water Plants		Draining Canals		P value
		No	%	No	%	
ICA Results	Positive	10	20	8	16	0.029
	Negative	40	80	42	84	
	Total	50	100	50	100	
PCR Results	Positive	16	32	13	26	0.029
	Negative	34	68	37	74	
	Total	50	100	50	100	

The results found that the adenoviruses detection rate during cold months was significantly compared to the hot months (26 % vs 10 %, p = 0.036*) by Immunochromatographic Assay (ICA) The results also found that the adenoviruses detection rate during cold months was significantly compared to the hot months (38% vs 20% p= 0.28*) by polymerase chain reaction (PCR) technique, table (5)

Table 5: Adenoviruses positivity rate in by ICA and PCR according to climate.

DETECTION TECHNIQUE		CLIMATE CHANGE				
		Cold Months		Hot Month		P value
		No	%	No	%	
ICA Results	Positive	13	26	5	10	0.036*
	Negative	37	74	45	90	
	Total	50	100	50	100	
PCR Results	Positive	19	38	10	20	0.028*
	Negative	31	62	40	80	
	Total	50	100	50	100	

Table (6): Showed the adenoviruses detection rate recording to the months of the years of sample collection using the Immunochromatographic Assay (ICA) technique, the positive detection rate of adenoviruses in the January month was significantly higher 8 (16%) compared to other months of the year, march 4 (8%) May 2 (96%) and July 2 (96%) using the polymerase chain reaction (PCR) technique, The results also found that the adenoviruses detection rate in the January was significantly 13 (26%). compared to other month of the year, March 8 (16%) May 5 (10%) and July 3 (6%).

Table 6: Adenoviruses positivity rate in by ICA and PCR according to the months of the years.

DETECTION TECHNIQUE		MONTH OF THE YEARS								
		January		March		May		July		P value
		No	%	No	%	No	%	No	%	
ICA Results	Positive	8	16	4	8	2	4	2	4	0.033*
	Negative	42	84	46	92	48	96	48	96	
	Total	50	100	50	100	50	100	50	100	
PCR Results	Positive	13	26	8	16	5	10	3	6	0.031*
	Negative	37	74	42	84	45	90	47	94	
	Total	50	100	50	100	50	100	50	100	

Discussion

In spite of the recommendation of the who for the necessity of surveillance system for periodic detection and typing of Enteroviruses in sewage and all water sources that may be employed for providing treated water for human consumption consistent with the drinking - water quality standards [10]. During July and August in Iraq the temperature reached 50, and Iraq suffers for more than three years of water scarce seasons, and not obtaining water from the upstream

countries for the Tigris and Euphrates rivers, and for the role of some surrounding countries in building dams and diverting some of the tributaries that Fred the rivers as in the Diyala river [9]. Adenoviruses are one of the causative agents of Gastroenteritis, responsible for a large proportion of Gastroenteritis in children, as they are the second cause of Gastroenteritis after rotavirus, and children between the ages of (6) months to two years are the most vulnerable to infection with adenoviruses [7].

The results of the current study found that the detection rate of adenoviruses in sewage water plants (SWPs) and draining canals (DCs) was 18 (36%) by Immunochromatographic Assay (ICA) using polymerase chain reaction (PCR) the detection rate of Adenoviruses in SWPs and DCs was 29 (58%) this high detection rate undoubtedly reflects the high prevalence of these viruses in the Diyala province community. Unfortunately, several studies approved the adenoviruses. The clinical specimens and responsibility of these viruses for outbreak of gastroenteritis particularly among children less than 5 years old [10, 11] in addition the asymptomatically infected children and adults. who were continuously shed Enteroviruses in feces and contaminate water and food [12] since these viruses can cause persistent infections lasting for longer time and acting as permanent reservoirs for these viruses in the community [13] furthermore the Enteroviruses had certain characteristics enabling them to withstand a wide range of pH and temperature charge. Permitting flourishment of these viruses in the Environment with their potential risks on public health [14, 15]. using PCR technique the detection rate of the adenoviruses in sewage water plants and draining canals was 29 (58%) and using ultracentrifugation to increase the viral nucleic acid (NAs) concentration, Furthermore, the detection rate of adenoviruses in sewage water plants. (SWPs) and draining canals (DCs) by using Immunochromatographic Assay (ICA) was 18 (36%). The convergent results obtained by ICA and PCR technique may endorse each other as the ICA technique has sensitivity and the PCR technique has characterize by high sensitivity and specify [16, 17] therefore, it seems that both techniques are essential for detection of Adenovirus in sewage and drinking water [18] Except the study of Abbas et al (2011). in which the presence of Enteroviruses in drinking water was indicated by coli phages, this is the only study. were found dealing with the detection of EVs or adenovirus in sewage water plants or canals. However, the current detection rate was

close with studies conducted in regional countries [19]. In Nigeria Enteroviruses were detected in 34.6% [7] from total of 100 samples of sewage water samples using the PCR technique using the RT - PCR and Nested – PCR on water samples collected from raw sewage and Al-Zarqa River, Jordan the Enteroviruses were detected in 2-8% including the norovirus, astrovirus and human adenovirus 40/41[8]. In a study conducted in India using PCR technique on sewage water samples, researcher found that (40.4%) of sewage water samples were positive for enteroviruses including adenoviruses, and that there was a close correlation between virus samples isolated from stool and samples isolated from sewage water [20]. Considering Diyala province as an example of the general situation in Iraq, actually several predisposing factors were collaborated leading to this tacky status. first of these is the general deterioration of infrastructure particularly those related to water treatment plants which results, in lowering drinking water standards in Iraq [21, 22]. Several shortages of water supply due to drought, global climate change and regional Geopolitical disturbance are collectively leading to general water crisis in tray Iraq generally and the Baquba city in Particular [23]. Additionally, the neglected health programs plus the reduction in public health commitments which paralyze important part of humanitarian demands, largely due to forced immigration and population displacements as a result of the effects of armed conflicts [24].

All these events plus the absence of national or local water surveillance and vigilance systems lead to impairment of sewage water and drinking water treatment plants yields unsafe drinking water over the country thus it can be concluded that there is a huge contamination rate of adenoviruses in sewage and draining canals water. The results found that the detection rate of adenoviruses was higher in districts compared with sub-districts. perhaps the reason for this is the population density. The districts compared to the sub-districts and the large use of water and the disposal of that water through the canals to sewage plants, or into the revers that pass through districts, which the pollution of those rivers. [25].

The results also found that the detection rate of adenoviruses was higher in Cold months compared to that of hot months, the results also found that the detection rate of adenoviruses was higher in the January month compared to that of years months. This is admittedly due to the fact that during or cold months presence the rains which dispersed waste directly or

indirectly to rivers, sewage water plants and draining canals, this leads to increase in viral the rivers or draining canals [26] Concentration in Consequently the adenoviruses were shredded of in sewage water in a large number 105-1000 viral particles. per gram of feces [27] additionally, the ability of Enteroviruses to survive over a wide range of pH high concentrations of Sodium Chloride (NaCl) and large change in the temperature: these abilities allow EVs to flourish in a water environment, their natural reservoir and in winter months and early spring in temperate regions [28].

Conclusion

It can be concluded that there was a high contamination rate of sewage and draining canals water by adenoviruses in Diyala province, recommending a periodic. surveillance system including epidemiological and molecular studies.

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