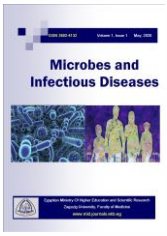
Microbes and Infectious Diseases 2024; Article-In-Press, **DOI:** 10.21608/mid.2023.227033.1579



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Quantification of adenovirus-F in the River Owena and the water's physicochemical properties

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ARTICLE INFO

Article history:
Received 3 August 2023
Received in revised form 7 December 2023
Accepted 8 December 2023

Keywords:
Adenovirus
Physicochemical
SYBR qPCR
Water quality

ABSTRACT

Background: Water quality monitoring is useful for promoting sustainable development of water resources. **Objective:** The aim of this study was to determine adenovirus concentrations in water samples from River Owena, Nigeria. **Methods:** The physicochemical characteristics of the water samples were determined immediately after collection using standard methods. A quantitative SYBR green-based real-time PCR assay for detecting human adenovirus-F (HAdV-F) was optimized using DNA extracted with a ZymoBIOMICS™ DNA Miniprep kit from filtered water samples. **Results:** Findings revealed that HAdV-F genes with end relative fluorescence units (RFUs) ranged from 7206.31 to 14248.55 and corresponding quantification cycles (Cq) ranged from 13.32 to 18.38, while the non-template control (NTC) was 31.71 in a 44-cycle reaction. Water temperature ranged from 34 to 36°C and pH 6.4 to 7.4, with total solids between 945 and 994 mg/l. **Conclusion:** Adequate treatment of the water from the river must be a priority by residents who rely on the water for domestic activities.

Introduction

Water is a basic life resource and contributes significantly to human health. In order to prevent water-borne diseases and sustain water resources development, therefore, it is important to monitor and understand water quality. Access to clean and safe drinking water is considered a basic human right, but more than 2 billion people do not have access to safe drinking water [1]. Globally, about 80% of all diseases and deaths in low-income countries are water-related as a result of polluted water [2]. Virtually all water is prone to viral contamination, including surface water such as lakes and rivers, groundwater, estuary and marine waters, and ice, as well as fish [3]. In the USA, 72% of groundwater sources have reported positive for enteric viruses [4]. Human activities, including the disposal of untreated sewage, reuse of incompletely treated effluent, use of animal waste as manure, etc., have contributed significantly to water contamination. Exposition to contaminated water may subsequently occur through numerous ways, linked to one of innumerable uses of water, such as drinking, irrigation, aquaculture, recreational activities, etc. [2,3].

Contaminated drinking water is a major cause of gastrointestinal diseases [5]. Enteric viruses are important waterborne pathogens and present in high concentrations in feces of infected people. Adenoviruses are very prevalent in water, and offered as index organisms for viral pathogens because they fit most of the criteria for such use. They are the only DNA viral pathogen in the enteric

DOI: 10.21608/MID.2023.227033.1579
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virus family and tend to outlast other enteric viruses in environmental waters due to their thermostability [6]. There are 51 serotypes of adenoviruses, of which about 30% are pathogenic in humans, most causing upper respiratory tract infections. The serotypes are classified into six species, designated A to F [7].

Human adenoviruses (HAdV) are a major cause of clinical infections including gastroenteritis, conjunctivitis and respiratory diseases, and the second most important viral pathogens of infantile gastroenteritis after rotavirus [8]. Their prevalence is high in environments where water source contamination with human feces or sewage occurs [9]. Adenoviruses are believed to have caused waterborne outbreaks in different settings, including military camps, hospitals, day care centers, schools and swimming pools [10]. Despite significant advances in water and wastewater treatment, waterborne diseases still pose a major threat to public health, especially in low-income countries where a substantial proportion of the population still rely on untreated surface waters for domestic use [11].

The Owena River forms the boundary between Ondo and Osun states in Southwest Nigeria. Activities such as the introduction of domestic, agricultural, and/or industrial wastes, and of drainage and sewer systems into the river, may result in microbial contamination and degradation of its ecosystem, rendering the water a human health risk due to the possible presence of enteric pathogens [12]. The aim of this study was to determine the concentration of adenovirus-F genes in water samples from the River Owena, and the water samples' physicochemical characteristics.

Material and methods

Study area

The river Owena is about 4 km from Joseph Ayo Babalola University, Ikeji Arakeji, along the Ilesa-Akure expressway in the Oriade district, Osun State, Nigeria (latitude N7.403135 and longitude E5.014589) **(Figure 1)**. It is free flowing, freshwater body during the rainy season, but slow-moving at the onset of dry season. The study area experiences frequent rainfall between April and July, with a short break in August, but rainfall continues between September and November. The heaviest rainfall occurs in July [12]. The river was selected due to its close proximity to fecal contamination sources, and its use for both recreational activities and domestic purposes.

Sample collection

Water samples were collected weekly from the river for a period of 8 weeks from April to June, 2021. Collection took place between 08:00 and 10:00 am, and samples were taken midstream from about 20 to 30 cm below the water surface in the direction of flow, using sterile, wide-mouthed, screw-capped, 1 L plastic bottles. On each occasion, the bottle was opened under water, allowed to fill, and then capped before being removed from the water. The samples were collected in triplicates from the monitoring points on the river, labelled immediately at the sampling point and then transported in an ice-packed bag to the laboratory for analysis.

Molecular detection of adenovirus in water samples

Extraction of genomic DNA and quality assessment

Genomic DNA was extracted from the samples through silica filters using a ZymoBIOMICS™ DNA Miniprep kit (Zymo Research, Catalogue No. D4300T, USA) according to manufacturer's instruction. An aliquot of the extracted DNA was assessed for its quality and quantity using a NanoDrop™ (2000 Spectro-photometer, Thermo Scientific™, USA). The DNA products were thereafter separated on a 1.5% agarose gel and electrophoresis was carried out at 100 v for 1 hour and 30 minutes. Thereafter, DNA bands were visualized by ethidium bromide staining using 100 bp DNA ladder (New England Biolanbs) as DNA molecular weight marker. Aliquots of the purified DNA were used for polymerase chain reaction (PCR) and the remaining purified DNA stock were stored at -20°C condition.

Quantification of Adenovirus-F by real time PCR-based assay

Initially, conventional PCR assay was used to optimize and validate the real time PCR primers. A mixture containing 10 µl of 5x GoTaq colorless reaction, 3 µl of $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each of 10 µM Adeno-F forward primer (5′-3′ TTCCAGCATAATAACTCWGGCTTTG), and 10 µM Adeno-F reverse primer (5′-3′ AATTTTTTCTGWGTCAGGCTTGG) primers and 0.3 units of Taq DNA polymerase (Promega,

USA) were made up to 42 µl with sterile distilled water and 8 µl of 10µg/L DNA template. The reaction mix was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA). Amplification was carried out using PCR system thermal cycler (Applied Biosystem Inc., USA) with PCR profile of an initial denaturation at 94°C for 5 min, annealing at 55°C for 30 secs, extension at 72°C for 30 secs; all for 35 cycles and final extension at 72°C for 10 mins. The amplified fragment was run on a 2% agarose gel electrophoresis at a voltage of 100 V for 60 minutes to confirm presence or absence of expected band size of amplified target gene sequence.

A one-step SYBR green-based real-time PCR was set up containing 5 µl Luna Universal qPCR Mastermix, 0.25 µl of 10 µM Adeno-F forward primer (5′-TTCCAGCATAATAACTCWGGCTTTG-3′), 0.25 µl of 10 µM Adeno-F reverse primer (5′-AATTTTTTCTGWGTCAGGCTTGG-3′), 3.5 µl nuclease free water and 1 µL of the DNA as template to make up a 10 µl reaction mixture [13]. This was loaded into the BioRad™ Real-Time PCR Detection System (CFX96 Touch™, USA). The cycling condition includes 1 cycle of initial denaturation at 95°C for 1 minute, 45 denaturation cycles at 95°C for 15 seconds each, and extension at 60°C for 30 seconds + plate read, 1 cycle of melt curve at 62 to 95°C with 0.5°C increments [13].

Physicochemical characteristics of water samples

Physicochemical parameters like pH and temperature were determined on the sampling site with a Hanna multi-meter instrument (HI 98107) and mobile thermometer (model HI98107) respectively. Electrical conductivity (EC) (µs/cm), turbidity (NTU), salinity (mg/L), hardness (mg/L), total solids (TS) (mg/L), total suspended solids (TSS) (mg/L), total dissolved solids (TDS) (mg/L), dissolved oxygen (DO) (mg/L), biological oxygen demand (BOD) (mg/L) and phosphate content (mg/L), were determined at the Department of Marine Science, Federal University of Technology, Akure, Ondo State, Nigeria [14,15].

Determination of the salinity of water samples

The salinity of water samples was determined by adding five drops of phenolphthalein indicator solution to 50 ml of the water sample and neutralized with 0.1 N sulphuric acids to the colorless side of phenolphthalein. About 1 ml of potassium chromate indicator solution was added before titration with standard silver nitrate solution to a pinkish-yellow endpoint. A reagent blank titration was carried out in parallel to the sample titration [15].

Chloride quality was calculated as follows:

Chloride, mg/l = [(A-B) (N) (35.45) / V] x 100

A = Silver nitrate solution, in ml for sample titration.

B = Silver nitrate solution, used for blank titration in milliliters.

N = Normality of the silver nitrate solution.

V = Sample volume in milliliters.

Measurement of turbidity of water samples

This was determined using a standardized Hanna H198703 turbidimeter. The samples were poured into the measuring bottle and the surface of the bottle was wiped with silicon oil. The bottle was then inserted into the turbid meter and the reading was obtained [14, 15].

Measurement of total dissolved solids in water samples

A portion of water sample was filtered out and 10ml of the filtrate was measured into a pre-weighed evaporating dish which was then dried in an oven at a temperature of 103 to 105 °C for two and half hours. The dish was transferred into a desiccators and allowed to cool at room temperature and was weighed. The total solid was represented by the increase in the weight of the evaporating dish. Total dissolved solids content of the water was calculated [14].

Total dissolved solids (mg/l) = [(W2-W1) mg x 1000] / ml of filtrate used.

Where, W1 = Initial weight of evaporating dish

W2 = Final weight of the dish (evaporating dish + residue).

Measurement of dissolved oxygen in water samples

About 300 ml BOD bottles were filled with the samples respectively, 2 ml of manganese sulphate and 2ml of alkali-iodide-azide solution added by inserting a pipette just below the surface of the liquid. The bottles were stoppered to avoid the introduction of air and were mixed by inverting several times. The bottles were left to stand for few minutes. The presence of oxygen was indicated by the formation of a brownish –orange precipitate. Two millimeters (2 ml) of tetraoxosulphate (vi) acid

were added to the samples. It was mixed again by inverting to dissolve the precipitate. Two hundred and one milliliters of the sample were then measured into a clean 250 ml conical flask and titrated against sodium thiosulphate Solution ($Na_2S_2O.5H2O$) using the starch indicator until the solution turned colorless [14,15].

Calculation

DO (mg/L) = [16000 x M x V] / [V2/V1 (V1-2)]

M = Molarity of thiosulpahte used.

V = volume of thiosulphate used for titration.

V1 =Volume of bottle with stopper.

V2 = Volume of aliquot taken for titration.

Measurement of electrical conductivity of water samples

Conductivity was determined using a conductivity meter. The probe was dipped into the container of the water samples until a stable reading was obtained and recorded (Oakton instrument, model lCON510series) [15].

Measurement of biological oxygen demand of water samples

Initial dissolved oxygen was determined by filling 300 ml of BOD bottles with the diluted samples previously prepared and the initial dissolved oxygen (DO) was determined. After incubation for 5 days, the final dissolved oxygen (DO) was determined following the earlier stated procedure [15].

BOD (mg/L) = [DO1-DO0] / B

Where, DO0 = initial dissolved oxygen (immediately after preparation).

DO1 = final dissolved oxygen (after 5days of incubation).

B = Fraction of sample used.

Statistical analysis

Pearson's correlation analysis was carried out to determine whether there were positive correlations between the concentration of adenovirus-F genes and physicochemical properties of the water samples.

Results

Concentration and quality of extracted DNA

The DNA concentrations obtained ranged from 14.50 to 75.20 µg/L, with most of the samples showing a good quality nucleic acid **(Table 1)**.

Qualitative detection of adenovirus

Good quality DNA was obtained in the water samples. Agarose gel electrophoresis showed varied band intensity in positive amplified samples, indicating the presence of adenovirus-F genes in six samples – numbers 1, 2, 5, 6, 7 and 8 – at about 244 base pairs (bp). Sample numbers 3 and 4 were negative **(Figure 2)**.

Quantitative detection of adenovirus

The SYBR green-based real-time PCR assay used to detect and quantify adenovirus-F showed the presence of the virus target in six samples – 1, 2, 5, 6, 7 and 8 – with high end relative fluorescence unit (RFU) values. Again, samples 3 and 4 had very low end RFUs, indicating the absence of adenovirus-F genes. The highest end RFU (14248.55) was detected in sample 2 with Cq 13.32. The lowest end RFU (7206.31) was detected in sample 7 with Cq 18.38. The non-template control (NTC) was 31.71 in a 44 cycles reaction **(Table 2)**.

Physicochemical properties of the water

The temperature of the river water ranged from 34 to 36°C, the pH from 6.4 to 7.4. TS concentration was between 945 to 994 mg/L, with TSS ranging from 569.0 to 910.0 mg/L and TDS from 73.0 to 403.0 mg/L. EC was between 145.0 to 807.0 µS/cm, while salinity ranged from 50.0 to 350.0 mg/L, and the DO from 6.1 to 8.7 mg/L. The Pearson's correlation coefficient showed that was no relationship between the concentration of adenovirus-F genes and physicochemical properties of the water samples **(Table 3)**.

Table 1. Quantity and quality of nucleic acid extracted from water samples

Samples	Nucleic acid concentration (µg/L)	A260/A280	A260/A230
1	17.70	0.62	0.59
2	14.50	1.90	1.65
3	52.60	0.90	0.45
4	17.50	1.76	2.12
5	18.20	1.75	2.16
6	75.20	1.53	0.96
7	15.00	0.23	0.54
8	20.40	1.87	2.08

Table 2. Adenovirus-F quantification parameters

Well	Sample	Cq Mean	End RFU
B02	1	18.03	12226.92
D02	2	12.32	14248.55
F01	3	20.71	22.71
F02	4	31.69	12.04
D03	5	15.78	12216.74
E01	6	18.00	10227.97
E02	7	18.38	7206.31
E04	8	17.53	12190.55
H03	NTC	31.71	7.58

Table 3. Physicochemical properties of the water samples from River Owena and influence on the concentration of adenovirus-F genes

Parameter	Minimum value	Maximum value	End RFU HAdV-F genes (*r*)
Temperature (°C)	34.0	36.0	0.42
pH	6.4	7.4	-0.27
EC (µS/cm)	145.0	807.0	-0.27
TS (mg/L)	945.0	994.0	0.19
TSS (mg/L)	569.0	910.0	0.29
TDS (mg/L)	73.0	403.0	-0.27
Salinity (mg/L)	50.0	350.0	-0.35
DO (mg/L)	6.1	8.7	-0.19
BOD (mg/L)	2.5	6.7	0.27
Turbidity (NTU)	16.3	128.0	0.19

Key: n = 8, r = Pearson's correlation coefficient

Figure 1. River Owena and the Ilesa-Akure expressway, in the study

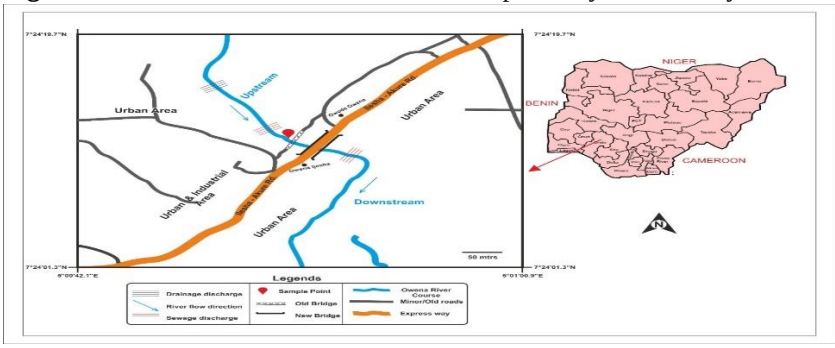
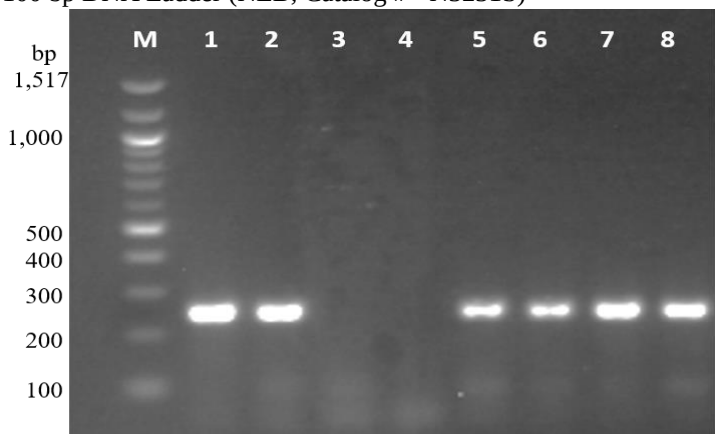


Figure 2. Agarose gel (2%) showing PCR amplified target sequence genes of DNA extracts using Adenovirus-F forward and reverse primers at about 244 base pair. The numbers at the top are those of the 8 test samples, M-100 bp DNA Ladder (NEB, Catalog # - N3231S)



Discussion

Adenoviruses are a major cause of symptoms such as diarrhea, vomiting, abdominal pain and febrile-related ailments in children [16]. Such symptoms normally last for only about 10 days or so before disappearing, although the infections can be fatal in neonates and immune-compromised individuals. They can cause symptomatic infections in, among other things, the respiratory system (pharyngitis, acute respiratory disease and pneumonia), eye (conjunctivitis), gastrointestinal tract (gastroenteritis), central nervous system (meningoencephalitis) and genitalia (urethritis and cervicitis) [17]. Gastroenteritis in children, for example, has been linked to HAdV types 40 and 41 [16]. HAdV serotypes 40 and 41 belong to HAdV subgenus F and have been recognized as the second most important etiological agent of viral gastro-enteritis in children and in contrast to other adenoviruses, do not shed in respiratory secretions, but in feces thus, their transmission is limited to the oral-fecal route. They have also been associated with several outbreaks associated with drinking water [18]. Infected patients excrete them in high concentrations, up to 1011 viral particles per gram of feces [10]. The risk posed by adenovirus- F serotypes 40 and 41, as leading causes of childhood diarrhea, led the USEPA to bring in the Information Collection Rule in 1996, which required water utilities serving more than 100,000 households to monitor their source water for viruses and recommended a range of 10-4 to 10-6 as the acceptable risk limit for enteropathogens [19]. This recommended range should be adopted by the National Environmental Standards and Regulations Enforcement Agency (NESREA) responsible for providing quality control, quality standards and requirements of surface waters in Nigeria. In a recent study, both human and animal HAdV were found in water environments, suggesting that water, including drinking water, could be a significant route of AdV transmission in both developed and developing economies [20].

Staggemeier et al. [21] stated that water's role in HAdV epidemiology and the potential health risks constituted by the virus in water environments can't be over emphasized. In this study, adenovirus-F genes were detected in six of the eight water samples from River Owena, the load detected suggesting fecal contamination. This may be attributed to intense human activity from the pressure of urbanization leading to sewage, industrial and agricultural discharges to the river, which is in agreement with many studies reporting the presence of adenovirus in surface waters [22]. The SYBR green-based real-time PCR assay detected and quantified adenovirus-F genes with high end RFUs between 7206.31 and 14248.55. The qPCR adenovirus assay's outcome in this study also aligns with the results of comparison of conventional PCR and qPCR for HAdV detection in water and sediment samples, in which high virus loads were reported [21].

The temperature found in this study was between 34 and 36°C, higher than the 26.3 to 30.4°C reported by **Hart and Zabbey** [23] in the Lower Niger delta. **Okoye and Okoye** [24] observed that high water temperatures enhance the growth of microorganisms and hence taste and corrosion challenges.

The pH range recorded in this study – 6.4 to 7.4 – is similar to the 6.68 and 7.03 reported by

Abowei and George [25] for the pH in Okpoka Creek, Niger Delta. The characteristics of the creek that include continuous input of freshwater into the estuarine tidal water may be responsible for the similar pH range with water from River Owena. The EC, at between 145 and 807 µS/cm, is consistently below the 1000 µS/cm maximum recommended by WHO [1]. The wide range may arise from extensive evaporation from the river when the temperature is high, which is also thought to have contributed to the relatively high TDS concentrations reported in some samples. The range recommended by WHO for TDS in potable water is 500 to 1000 mg/l [1]. TDS includes all of the dissociated electrolytes that make up salinity, as well as dissolved non-ionic solutes e.g., organic matter. DO refers to the concentration of free oxygen dissolved in water. DO, an important environmental parameter, favors the growth of micro-organisms that can affect the aquatic environment adversely [26]. The DO concentration in the River Owena puts it into the "very clean" class. The BOD concentrations observed in the water from the river may be as a result of high runoff from farmland close to the river and/or the discharge of raw sewage into the water body. **Kolawole et al.** [27] reported that the high BOD concentrations in the River Asa, in Nigeria, may be derived from the presence of organic and inorganic pollutants.

Turbidity is another key parameter in water quality analysis. The results from this study showed turbidity levels above the maximum 5 NTU recommended by WHO [28]. The elevated values of turbidity, TS (total solids) and TDS reported in this study could have been caused by the large volume of storm water and associated suspended materials in the river (TS and TSS are the portions of fine particulate matter that remain in suspension in water). Seasonal distribution has been showed to insignificantly influenced HAdV prevalence among sampling areas in the study area [29]. The frequency and extent of adenovirus disease outbreaks appears to be on an increase, thus understanding their emergence and transmission routes are therefore essential [30].

Conclusion

The SYBR green-based real-time PCR assay is efficient for the detection and quantification of adenovirus-F nucleic acid in water, employing an economical approach of primer optimization using conventional PCR assay. This has a great economic value to reduce cost of detection and suggest platform for development of rapid diagnostics toolkit for on-site detection of adenovirus. The study has shown the presence of adenovirus-F in the River Owena, at least over the sampling period and suggest adequate treatment of the water from the river before domestic use.

Funding

None received.

Competing interest

Authors declare no competition of interest.

Acknowledgements

The authors are grateful to the Department of Microbiology, School of Life Sciences, The Federal University of Technology, Akure, Ondo State, Nigeria for providing the equipment and laboratory used for the study. The Technical assistance of Inqaba Biotech West Africa laboratory for the molecular studies is appreciated.

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