Rapid quantitative detection of Enteric viruses in River Nile and drainage water, Egypt

Mohamed I. Azzam[1], Safaa M. Ezzat[1], K. A. El-Dougdoug[2], Badawi A. Othman[2]

[1] Central Lab. for Environmental Quality Monitoring, National Water Research Center.
[2] Microbiology Department, Faculty of Agriculture, Ain Shams University

ABSTRACT:

Contamination of surface water with enteric viruses is a major public health concern that warrants the need to develop reliable indicators for enteric viruses contamination. This work assesses the bio-diversity of coliphages to use as bio-indicators for viral water pollution. The study elucidates the occurrence of enteric viruses at various locations in Rosetta Branch of the Nile River in addition to five main drains located on its sides which selected for confirmation the eco-diversity of aquatic viral isolates. The evaluation was carried out using real time-quantitative reverse transcriptase - polymerase chain reaction (rt-qRT-PCR). Eight coliphage isolates were detected in both Rosetta Branch and drainage water samples. Transmission Electron microscopy revealed that, the isolated coliphages have an isometric head and long-contractile tail; some particles revealed a short tail with full heads, resembling those belong to the myoviridae and siphoviridae family. Restriction enzymes by EcoRI, HindIII and BamHI showed the presence of double stranded (ds) DNA as well as heterogeneity among these phages. The results showed that EcoRI produced 7, 6, 2, 9, 10, 8, 10, 7 unique fragments and HindIII produced 2, 3, 0, 1, 2, 5, 4, 3 unique fragments while BamHI produced only 4, 0, 1, 1, 0, 4, 2, 1 unique fragments, for the eight phage isolates, respectively. Out of fifteen tested sites, two only (El-Rahawy drain outlet and Sabal drain outlet) were found to be polluted with enteroviruses with rate of 3.6X104 and 3.4X104 gene copies per microliters (GC µl-1), respectively using rt-qRT-PCR.

Key words: Coliphages, Drainage water, Enteroviruses, River Nile, Rosetta branch, Restriction enzymes, Real-time-qRT-PCR.

INTRODUCTION

River Nile is the main water resource for various economic activities in Egypt. The water sector in the country is facing compound challenges in terms of both water quality and quantity due to population increase, economic development and the fixed share Egypt has of the Nile River water. The dramatic increase in pollution and environmental degradation are also decreasing water availability for various economic uses with an estimated annual water deficit of around 7 billion m3 (Dakkak, 2013).

The Rosetta receives the water of a number of agricultural drains, which are heavily polluted by industrial and domestic sewage. The drains receive large parts of the wastewater in Cairo. Domestic pollution affects water quality heavily depends on the way of disposal of this pollution. Waterborne pathogens transmit diseases to around 250 million people each year resulting in 10 to 20 million deaths around the globe. Water quality through the presence of pathogenic enteric microorganisms may affect human health. Coliform bacteria,

E.coli and coliphages are normally used as indicators of water quality (Wilkes et al., 2009; Lin and Ganesh, 2013 and Mookerjee et al., 2014).

Viruses present in the gastrointestinal tract of both symptomatic and asymptomatic individuals are excreted in the faeces in extremely high concentrations, ranging from 105-1013 viral particles per gram of stool (Espinosa et al., 2008 and Hamza et al., 2009). These particles become contaminants of urban surface waters due to the continuous discharge of domestic sewage (Donovan et al., 2008 and Sinclair et al., 2009).

Many studies confirm that bacterial indicators do not provide enough information about the occurrence of non-bacterial pathogens such as viruses in water. Therefore other indicators are advisable. Bacteriophages have been proposed worldwide as viral and fecal indicators particularly coliphages are numerous, easy, cheap to detect, morphological characteristics and sites of replication similarity to human viruses which pose a health risk, if present in water contaminated with human faeces. In addition, detection of human viruses is still a highly skilled and costly process. The resistance and incidence of coliphages against environmental factors in water environments resemble those of human viruses more closely and applicable than most other bacterial indicators commonly used (Yates 2007; Tan et al., 2008; Jurzik et al., 2010; Chandra et al., 2011; Nguyen et al., 2013 and Zahraa et al., 2014).

Several studies have confirmed out that, for monitoring purposes, phages are reliable indicators of human enteric viruses (Śliwa-Dominiak et al., 2014). Bacteriophages can be found in all the environments where bacteria grow such as River Nile and drainage water where untreated human wastes are disposed (Amenu, 2014).

Similarly, enteric viruses are found in a variety of aquatic reservoirs like sewage, ground water, drinking water, rivers and oceans. Most methods for detection and quantification of waterborne viruses from environmental samples require concentration from large volumes (100 to 1,000 L) of water (Cormier et al., 2014). Real-time-qRT-PCR allowing quantitative detection of ribonucleic acid (RNA) viruses has improved environmental virology surveys due to its sensitivity, specificity and applicability to detect low titers of viruses in complex environmental samples (Jebri et al., 2014).

Based on their morphological similarity to enteric viruses and their greater persistence and stability in water over time, the present study aims to coliphage isolates to use coliphage as reliable indicators for viral water pollution, and elucidates the occurrence of enteric viruses throughout samples from the Rosetta Branch and five main drains located on its sides.

MATERIALS AND METHODS

Sample collection

River Nile travels along Egypt for about 950 km behind the High Dam starting from downstream High Aswan Dam to upstream Delta Barrage, where it divides into two branches. The western branch is Rosetta branch (239 km in long) and the eastern branch is Demietta Branch, (about 242 km long). The Rosetta River Nile Branch has been selected for this study being the main freshwater stream in Delta region as well as the source of potable water for six governorates namely: Giza, Menofiya, Gharbiya,

Beheira, Kafr El-Sheikh and Alexandria. Rosetta branch has an average width of 180 m and depth from 2 m to 4 m. It ends at Edfina Barrage, 30 km upstream the sea, which releases excess water to the Mediterranean Sea. The area of our study extended about 120 km in Rosetta branch, starting from upstream of El-Rahawy drain to downstream of Tala drain at different representative sites as shown in Figure (1).

Samples for bacteriological and bacteriophages analyses

Water samples (1 liter clean sterilized polypropylene containers) were collected in July 2010, from Rosetta branch of River Nile as well as from five main drains namely: El-Rahawy drain, Sabal drain, El-Tahreer drain, Zawiet El-Bahr drain and Tala drain Figure (1). Sampling followed Standard Methods for Examination of Water and Wastewater (APHA, 2005).

Samples for enteric viruses analyses

Water samples were collected according to Kfir et al., (1995), water sample (40 liters in clean container) was collected from each site. For each container, 10ml per of 2M magnesium chloride was added to increase the stability of the viruses in the samples during transportation while containers were put in an ice box to the laboratory for analyses to take place within 8 hours.

Isolation and identification of bacterial isolates

E.coli detection was done according to (Pettibone, 1992), using multiple tube fermentation technique. Lauryl tryptose broth with 4-methylumbellifery-ß-D-glucuronide (MUG) medium was used in test tubes containing inverted fermentation vials. The tubes were inoculated with appropriate sample volume and incubated at 44.5oC/24 hrs. Tubes showing gas production with growth is considered a positive indication for presence of E.coli. The Analytical Profile Index (API) 20E (Enterobacteriaceae) strips obtained from Biomerieux, France were used as biochemical system for identification of E.coli isolates. The API 20E strip consists of 20 micro-tubes containing dehydrated substrates. These tests were inoculated with a bacterial suspension that reconstitutes the media. The strips were incubated for 18 - 24 hrs at 37oC. The reactions were read according to the reading table and the identification was obtained by referring to the API (Juang and Morgan, 2001). E.coli strain B American Type Culture Collection (ATCC) (reference strain) was obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbial Resources Center.

Bacterial viruses assay

Coliphages were qualitatively and quantitatively assayed by the spot test and the over layer agar techniques (plaque assay technique) according to method of Othman (1997).

Purification of coliphages

Dextran sulfate-polyethylene glycol system was used for phage purification according to method of (Watanabe et al., 1970).

Transmission electron microscope (TEM)

The isolated coliphages were examined as described by Accolas and Spillmann (1979). The viruses were stained with a 2% (W/V) solution of phosphotungstic acid (PTA), pH 6.5 and examined by TEM (Model Jeol JEM-100CX II) operated at 60 KV at Electron Microscope Unit, at Assiut University, Assiut, Egypt.

Enteric viruses assay

Virus concentration

Enteric viruses were concentrated by ultrafiltration of 40 liters of each water sample using a hollow fiber cartridge (Amersham BioScience, VFP-50-E-9A model, NRC). The porosity of the ultrafiltration cartridge was 50 KDa and 8400 cm2 surface area in which almost all viruses will be trapped (Kfir et al., 1995). After finishing filtration of the whole sample, the adsorbed viruses on the filter were re-concentrated as described by Katzenelson et al., (1976).

Coliphages DNA isolation and Restriction enzyme digestion

Purified phage particles (108 PFU/ml) were treated with 1µg of DNase I and RNase A (Bangalore Genei,Bangalore, India) at 37oC for 30 min. To the mixture, Proteinase K (Bangalore Genei, Bangalore, India) and Sodium dodecyl sulfate (SDS) were added at a final concentration of 0.05 mg/ml and 0.5% respectively and incubated at 56oC. After 1 hrs incubation, an equal volume of phenol: chloroform was added to remove proteinaceous material. The extraction was repeated thrice with phenol-chloroform-isoamyl alcohol (25:24:1). The nucleic acid was precipitated with chilled ethanol and suspended in 20 µl of TE buffer (10 mM Tris-HCl, pH 7.0, 1.0 mM EDTA, pH 7.0) according to Sambrook et al., (1989). Restriction enzyme digestion of isolated phage DNA was carried out following the instructions provided by suppliers. Three types of restriction endonucleases EcoRI, HindIII and BamHI were added to purified coliphage DNA. The restriction digests were separated on 0.8% agarose gel in 1× TAE buffer (40 mM tris-acetate and 1 mM EDTA, pH 8.0) (Sigma) containing 0.5 µg/ml of ethidium bromide at 100 V for 2-3 hrs.

Molecular detection of enteric viruses

Extraction of viral RNA was performed in a biosafety level 2 laboratories Dept. of Microbiology, National Water Research Center "NWRC" at El-Kanater El-Khyria, Qalubia Governorate, Egypt with enhanced containment practices. Ribonucleic acid was extracted using QIAmp Viral RNA Mini Kit® (QIAamp, Qiagen®, Hilden) according to the instructions of the manufacturer. The extracted viral RNA was stored at -80°C for later analysis and confirmed spectrophotometerically at A260/A280 (UV-2401 PC UV-Vis spectrophotometer, Virology Lab. NWRC). The RNA yield was determined by the following equation:-

$$\text{RNA concentration (µg/µl)} = \frac{\text{Absorbance reading at 260nm X (Dilution factor)}}{\text{Extinction coefficient}}$$

Primer synthesis

Oligonucleotide primers and TaqMan® pr for enteoviruses

For enteroviruses (EntF, EntR and EntP)* were selected based on the alignment of all 2.028 enteroviruses sequences available in GenBank was designed according to **Tan *et al.*, (2008)**: EntF: 5' GAG AGT TCT ATA GGG GAC AGT -3', EntR: 5' AGC TGT GCT ATG TGA ATT AGG AA -3', Probe: 5' 6FAM-ACTT ACC CAG GCC CTG CCA GCT CC- TAMRA. Primers and probe sequences were modified to allow detection of all enteroviruses, the primer sets were stored at – 20°C. EntF: 5' GAR AGT TCY ATA GGR GAY AGY -3', EntR: 5' AGC TGT GCT RTG YGA RTT RAG RA -3', Probe:

5' 6FAM-ATT GGR GCD TCR TCA AAT GCT AGT GA-TAMRA

Real-time-qRT-PCR for enteroviruses detection

RNA concentrations present in collected water samples were estimated using the standard curves, according to the end point dilution of the positive control **(Rutjes *et al.*, 2005)**. Quantitative reverse transcription-PCR was performed using an MX3000P detection system (Stratagene, La Jolla, CA, USA). Place tubes in a thermal cycler and run with a setting of 1 cycle at 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec, and 60°C for 1 min.

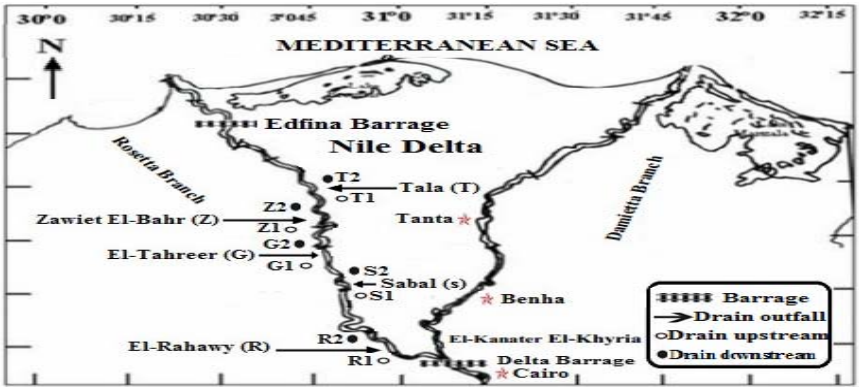


Figure (1): Map of sampling locations (Drains and Rosetta branch).

Result calculation using standard curve quantification

After amplification, given that both the standards and experimental samples are amplifying efficiently, the threshold line (C_T) for each standard dilution and unknown samples can be determined and plotted against the initial template quantity. Sample C_T values can be used to estimate template quantity by comparing them to the standard curve. Data from a standard curve run can be viewed in multiple formats including: standard curve, initial template quantity, and plate sample values. In the standard curve view, as seen in **Figure (2)**, the efficiency and linearity will automatically be displayed by the software according to the following equation: $\mathbf{Xn = X_0\ (1+E)_a}$ Where, $\mathbf{X_n}$: amplified target amount (target quantity at cycle n). $\mathbf{X_0}$: starting quantity. **E**: efficiency of amplification. **n**: number of cycles.

Table (1): Standard curve dilution of enterovirus.

Standard curve	Gene copies (GC) per microliters
Tube 1 (Positive control)	$2x10^5$ GC μl^{-1}
Tube 2	$2x10^4$ GC μl^{-1}
Tube 3	$2x10^3$ GC μl^{-1}
Tube 4	$2x10^2$ GC μl^{-1}
Tube 5	20 GC μl^{-1}
Tube 6	2 GC μl^{-1}

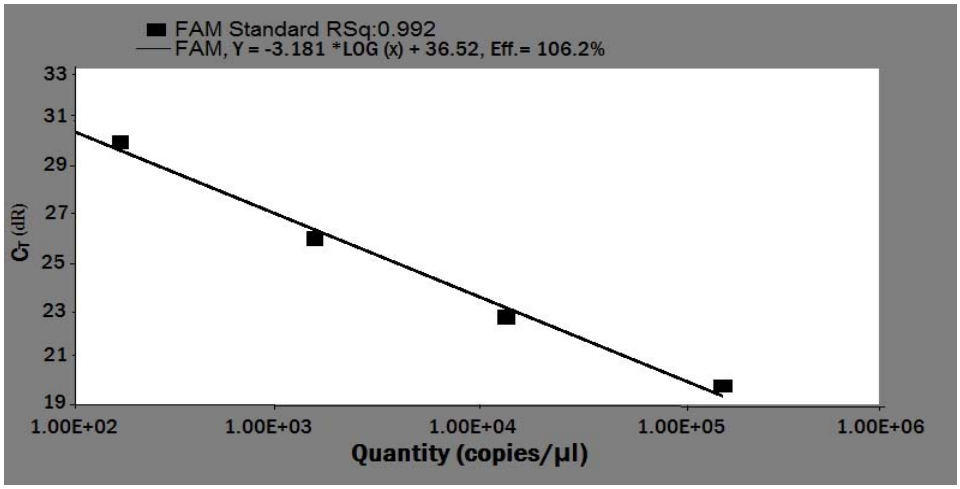


Figure (2): A photographe of standard curve of enterovirus by real time-qRT-PCR.
(y) = -3.51 (x) + 37.67 R2 = 0.995 y = C_T x=Log (quantity).

The rt-qRT-PCR reaction was carried out using Power SYBR® Green master Mix (Applied biosystem, Cambridge, UK), a reporter dye is used. SYBR green has the capability to bind to double stranded DNA upon amplification and emit fluorescence. Such fluorescence could be quantitively detected by fluorescence detector in the smart cycler (Cepheid, Sunnyvale, USA). Data are produced as sigmoidal-shaped amplification profile TaqMan when using a linear scale, in which the fluorescence is plotted against the number of cycles. The threshold cycle (C_T) serves as a tool for calculation of the starting template amount in each sample. This is the cycle in which there is the first detectable increase in fluorescence. Determination of C_T values is carried out slightly differently on Applied Biosystems and Light Cycler systems.

RESULTS

Qualitative and quantitative assay of coliphages

Results demonstrated in Table (2), showed that coliphages were detected in collected water samples from both Rosetta Branch and the 5 drains, according to *E.coli* isolates using spot test technique. The presence of bacteriophages specific for *E.coli* isolates (strain 1, 3 & B) were confirmed by spot test and plaque assay test as shown in Figure (3). The recorded concentrations were $30x10^2$, $36x10^2$ and $37x10^2$ pfu ml^{-1} (Drains) and $34x10^2$, $49x10^2$ pfu ml^{-1} (Rosetta branch). On the contrary no coliphages were detected specific for *E.coli* strain 2 as an indicator host.

As shown in Table (3), using single plaque isolation technique recorded eight single isolates of coliphages were picked up from the plates of *E.coli* strain 1, *E.coli* strain 3 and *E.coli* strain B. All isolated phages formed circular, clear plaques whose diameters ranged between 2mm to 7mm. These phage isolates specific for *E.coli* were named C1, C2, C3 (*E.coli* strain 1), C4, C5, C6 (*E.coli* strain 3) and C7, C8 (*E.coli* strain B). Dextran sulfate-polyethylene glycol two phase systems were used to obtain a purified and concentrated phage preparation. About twenty five milliliters of turbid phase were collected from the separating funnel after precipitation with PEG and centrifuged at low speed (3000rpm/20min). About 2ml of intermediated phase (Cake) were collected in 1ml eppendorf tube.

Virulence of the eight phage isolates (C1 to C8) examined by spot test showed that phages C1 to C5 had lysosensibility of *E.coli* strain 1, while phage C6 had lysosensibility of *E.coli* strain 3 and both of C7 and C8 phages had lysosensibility of *E.coli* strain B. But all isolated phages failed to lyse *E.coli* strain 2 as shown in Table (4).

Partially purified coliphages examined by TEM revealed that the phage particles have isometric heads and long-contractile tails. Some particles appeared containing short tail with full heads as shown in Figure (4). The bacteriophage resembles those of the Myoviridae and siphoviridae families of International Committee on Taxonomy of Viruses (ICTV) (Othman, 1997 and Azzam *et al.*, 2009).

Restriction digestion of bacteriophage DNA

DNA of all the selected *E.coli* phages were isolated and electrophoresed on 0.8% agarose gel. Molecular weight of all the isolated *E.coli* phages DNA was found to be ranging from 20-25kb. For molecular characterization of phages, the isolated phage DNA samples were subjected to digestion with three types of restriction enzymes *Eco*RI, *Hind*III and *Bam*HI. The results revealed that all the coliphages were sensitive to *Eco*RI, *Hind*III and *Bam*HI and exhibited different banding patterns confirming that all the coliphages were genetically different and harbored dsDNA as genetic material as shown in Figure (5). However, in lane 3, two extra bands were observed when C3 coliphage DNA digested with restriction enzyme *Eco*RI and *Hind*III was analysed on agarose gel. Restriction enzyme of the eight isolated coliphages (C1 to C8) by *Eco*RI, *Hind*III and BamHI showed the presence of double stranded (ds) DNA as well as heterogeneity among these phages. The results showed that *Eco*RI produced 7, 6, 2, 9, 10, 8, 10, 7 unique fragments, and *Hind*III produced 2, 3, 0, 1, 2, 5, 4, 3 unique fragments while *Bam*HI produced only 4, 0, 1, 1, 0, 4, 2, 1 unique fragments, for eight phage isolates respectively as shown in Figures 6, 7 and 8 and Table (5).

Qualitative and quantitative assay of enteroviruses

When procedure was used to investigate the water quality of 15 samples collected from both of drains and Rosetta branch water samples, RNA extraction was applied using mini spin column reagent followed by real-time-qRT-PCR and tested for enteroviruses. To assess the quality of the isolated RNA by mini spin column protocol, the isolated RNA had an A260/280 ratio of 1.7 (Extension coefficient = 0.3178 μM^{-1} cm^{-1}). The RNA concentration was 50µg/200µg concentrated samples. The result showed that only two sites were positive for enteroviruses among all tested sites, namely El-Rahawy drain outlet (R) and Tala drain outlet (T) recording $3.6X10^4$ and $3.4X10^4$ GC μl^{-1}, respectively. The real-time-qRT-PCR results are depicated in Figure (9) for two positive samples.

Table (2): Qualitative and quantitative assay of coliphages collected from drains and River Nile water samples.

E.coli isolates	Spot test		Plaque assay test pfu ml^{-1}	
	Drains	Rosetta branch	Drains	Rosetta branch
***E.coli* strain 1**	*+	+	$36x10^2$	$49x10^2$
***E.coli* strain 2**	**-	-	-	-
***E.coli* strain 3**	+	+	$30\ x10^2$	$34x10^2$
***E.coli* strain B ***(ATCC)**	+	-	$37x10^3$	-

* +: lysis. ** - : No Lysis. *** ATCC: American type culture collection (Reference strain).

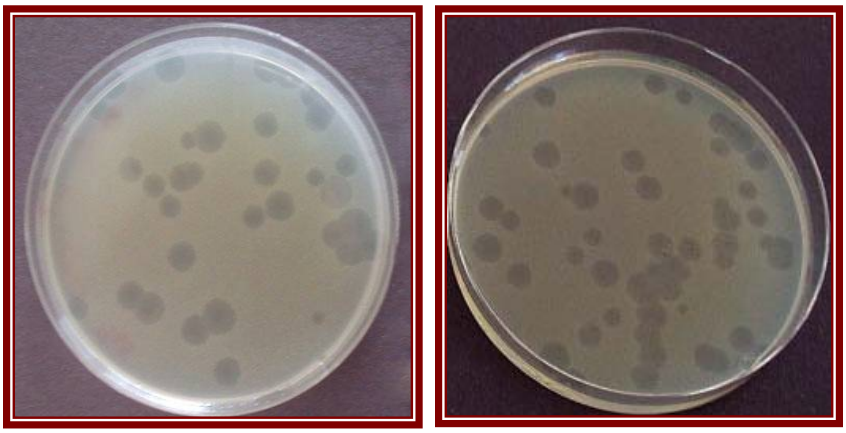


Plate (3): Plaque assay technique illustrates the presence of phages specific for *E.coli strains* 1 and B.

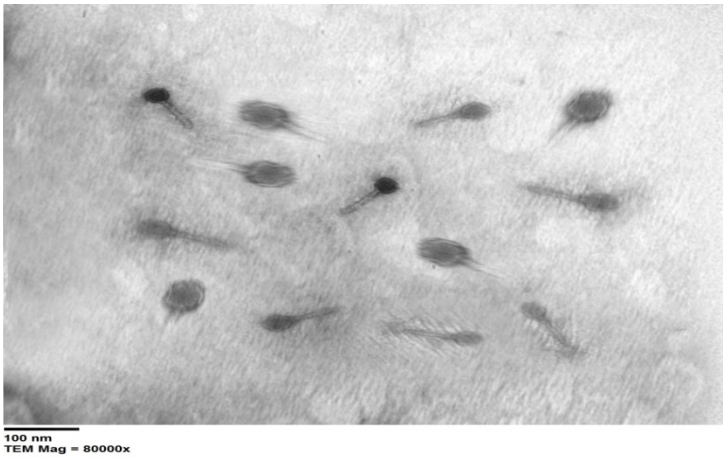


Plate (4): Electron micrograph illustrates phages specific for *E.coli* showing phage with isometric head and long contractile tail and short tail with full heads.

Table (3): Incidence of *E.coli* phage isolates recovery from collected water samples.

Phage isolates code	Bacterial host	Diameter	Plaque assay test pfu ml^{-1}	Sites
C1*	*E.coli* strain 1	2 mm	$24x10^2$	Rosetta branch
C2	*E.coli* strain 1	4 mm	$16x10^2$	Rosetta branch
C3	*E.coli* strain 1	1 mm	$15x10^2$	Rosetta branch
C4	*E.coli* strain 3	3 mm	$10x10^2$	Rosetta branch
C5	*E.coli* strain 3	5 mm	$46x10^2$	Rosetta branch
C6	*E.coli* strain 3	3 mm	$22x10^2$	Drains
C7	*E.coli* strain B	4 mm	$58x10^2$	Drains
C8	*E.coli* strain B	1 mm	$33x10^2$	Drains

* The letter C refers to coliform bacteria *E.coli* and numbers 1 to 8 are the isolated phage numbers.

Table (4): Lysosensibility of different *E.coli* strains to phage isolates.

Phage isolates code	*E.coli* strains			*E.coli* strains (ATCC)
	1	**2**	**3**	**B**
C1	++++	–	–	–
C2	+++	–	–	–
C3	+++	–	–	–
C4	++	–	–	–
C5	++++	–	–	–
C6	–	–	++++	–
C7	–	–	–	++++
C8	–	–	–	+++

++++ = Strong lysis; +++ = lysis; ++ = moderate lysis and + = weak lysis. – = resistance (failed lysis).

Table (5): Number of fragments for coliphage isolates using different types of restriction enzymes.

Type of restriction enzyme	No. of fragments for DNA coliphage isolates							
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**
***Eco*RI (15 - 45kb)**	7	6	2	9	10	8	10	7
***Hind*III (5 - 14kb)**	2	3	0	1	2	5	4	3
***Bam*HI (0.5 – 4.3kb)**	4	0	1	1	0	4	2	1

* The letter C refers to coliform bacteria *E.coli* and numbers 1 to 8 are the isolated phage numbers.

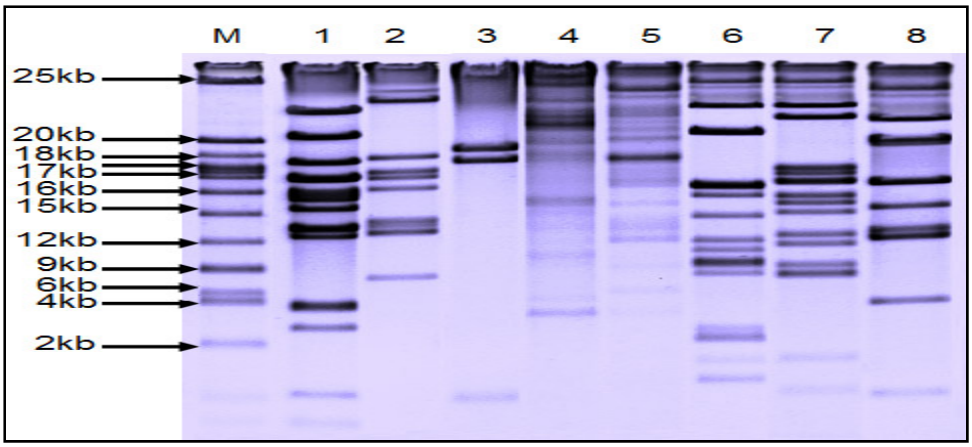


Figure (5): The restriction endonuclease digestion patterns of DNA isolated from eight isolated *E,coli* phages. Lane M, Marker; Lane 1, C1 DNA; Lane 2, C2 DNA; Lane 3, C3 DNA; Lane 4, C4 DNA; Lane 5, C5 DNA; Lane 6, C6 DNA; Lane 7, C7 DNA and Lane 8, C8 DNA digested with both of *Eco*RI, *Hind*III and *Bam*HI.

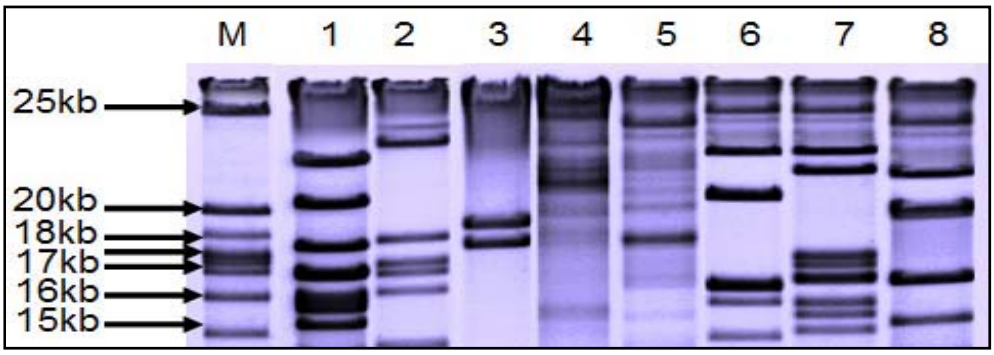


Figure (6): The restriction endonuclease digestion patterns of DNA isolated from eight isolated *E,coli*
phages digested with *Eco*RI.

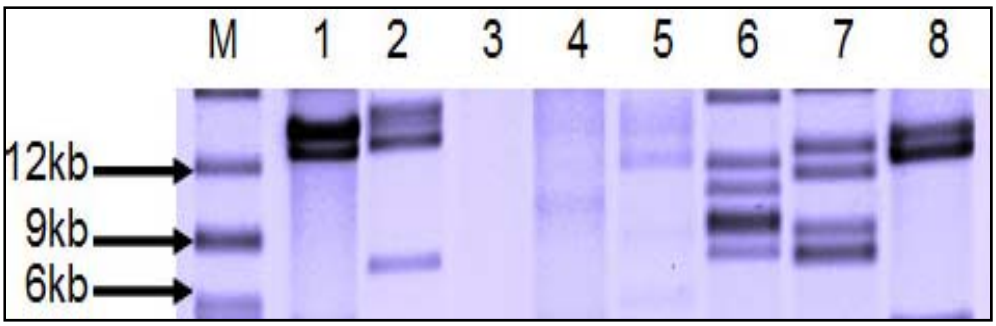
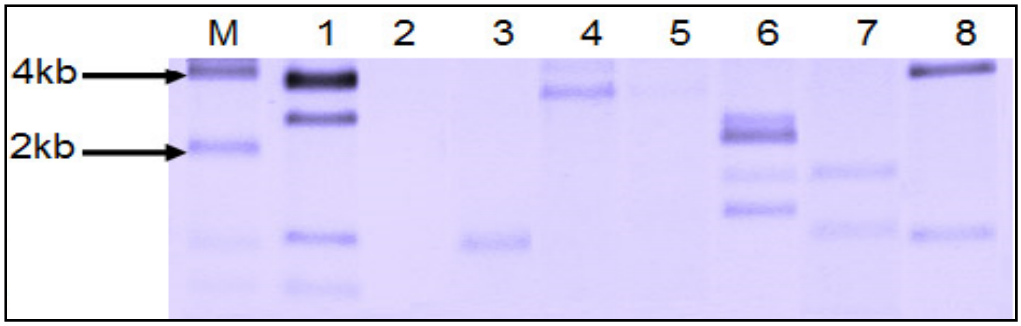


Figure (7): The restriction endonuclease digestion patterns of DNA isolated from eight isolated *E,coli* phages digested with *Hind*III.



Figure(8): The restriction endonuclease digestion patterns of DNA isolated from eight isolated *E,coli* phages digested with *Bam*HI.

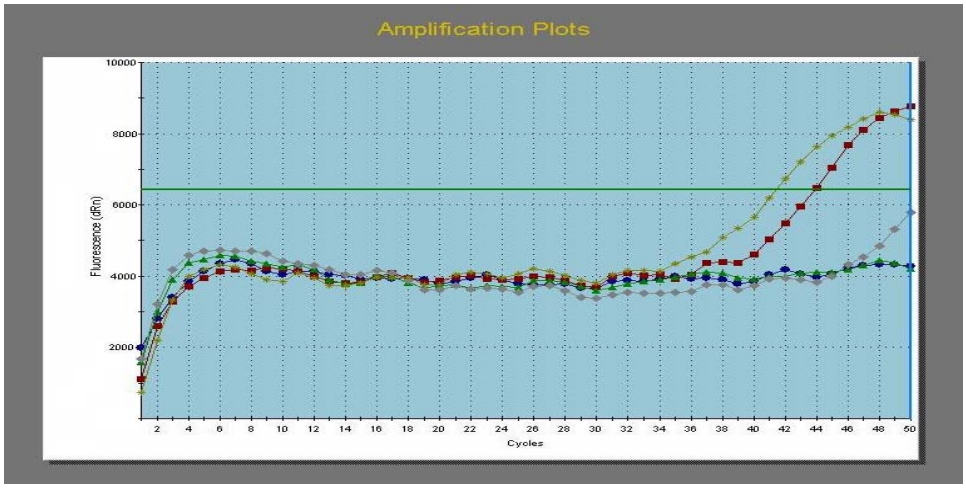


Figure (9): Amplification profiles of enteric viruses obtained by real-time-qRT-PCR with specific primers pairs in drainage water samples.

DISCUSSION

Coliphages were recently proposed by the United States Environmental Protection Agency (US EPA) as surrogates for water contamination because of their similar survival rate to human viruses, while genotyping of coliphages has been successfully used for pollution source identification. Enteric viruses' occurrence was related to the coliphages and the adaptation to a broad host-range may enable the proliferation of coliphages in the aquatic environment. Bacteriophages are increasingly employed in various environmental applications as indicator organisms of human pathogenic viruses because they possess all the elements of true viruses and permit easy, fast and inexpensive isolation.

Coliphages have been shown to be complementary or equivalent to other indicators, therefore it is highly advisable to include them as faecal pollution indicators and also as index of enteric virus for water quality monitoring programs.

In the present investigation, eight phages specific for *E.coli* strains 1, 3 and B (coliphage) were isolated from drainage and River Nile water with concentrations ranged between 10^2 to 10^3 pfu ml^{-1}, after enrichment and designed as C1 to C8 phages. Several authors have also isolated *E.coli* phages from

River Nile and drainage water (Azzam *et al.,* 2010 and Dhevagi and Anusuya, 2013). The isolated phages specific for *E.coli* (C1 to C8 phages) produced different morphological plaques as circular, clear plaque about 2mm and 7mm in diameter. Coliphages (C1 to C8 phages) particles have an isometric head with a long contractile tail and a short tail; these results are in agreement with (Othman, 1997; Azzam, 2010; Lee and Sobsey, 2011 and Enan *et al.,* 2013).

According with the results, coliphages are useful as index or model organism of the presence of EV, due to the significant relationship showed. These agree with results reported for coliphages and enterovirus where there is also highlighted the similarities in physical particle characteristics, as well as resistance to wastewater treatment; which support the idea of using coliphages as enteric viruses index, and also as a process indicator.

Molecular weight of all the isolated *E.coli* phages DNA was found to be ranging from 20-25kb. Obire *et al.,* (2010) found that DNA size of the coliphages genome isolated from creek were ranged from 26,000 to 42,000 base pairs while the DNA size of the coliphage genome isolated from domestic sewage ranged from 26,000 to 53,000 base pairs.

The fact that the phage isolates closely related by RFLP differ in their host ranges may reflect an intensive selection for new host range variants. The abilities of phages to adapt to a new host presumably involve point mutations as well as recombination events of a larger scale. Also, the effect of formation of small and unstable fractions of virus particles of some phages that have extended ranges of host adsorption, known as "nascent phage quality" may contribute to phage host range variability (Alla *et al.,* 2007).

Differences between phages were confirmed by comparison between them using restriction fragment number. The eight phages are genetically different and therefore are distinct. Our results are in agreement with Oliveira *et al.,* (2008) they isolated and characterized bacteriophages from sewage poultry. They found that 70.5% of the tested *E. coli* strains were sensitive to a combination of three of the five isolated phages, that seemed to be virulent and taxonomically belong to the *Caudovirales* order. Two of them like T4-like phages (*Myoviridae*) and the third is a T1-like phage and belongs to *Syphoviridae* family and all of them are genetically different. In our study, the number of coliphages detected has different concentration, morphology particle character, plaque diameter and genome according to ecosystem based on nature of polluted sites, which elucidates the biodiversity of detected coliphages.

Contamination of surface water with enteric viruses is a concern for public health, especially if this surface water is used for drinking purposes (Mans *et al.,* 2013). Fortunately, results of this study indicate the occurrence of enteroviruses in El-Rahawy drain outlet (R) and Tala drain outlet (T) but not in Rosetta branch. This may be due to heavy fecal contamination of drainage water before feeding the Rosetta branch. Azzam (2010); Adeniji and Faleve (2014) and Hegazy *et al.,* (2014) mentioned that enteroviruses were detected in raw water, tap water and wastewater in Egypt.

Currently the standard methods in this study for the detection of enteroviruses in environmental water samples involve real-time-qRT-PCR able to identify all human viruses (Hryniszyn *et al.,* 2013). Real-time-qRT-PCR is a rapidly and sensitive method for the routine monitoring of enteroviruses in water samples because it is faster and simpler (qualitative and quantitative) than conventional RT-PCR and cell culture methods, also this protocol allows the

results to be obtained within few hours, especially when we used these primers sets together with the rt-qRT-PCR systems (Jothikumar *et al.,* 2009 and Chongmiao *et al.,* 2010; Hamza *et al.,* 2011; Okoh and Chigor, 2012; Xiao *et al.,* 2012; Okoh and Sibanda, 2013 and Jebri *et al.,* 2014).

In this study, direct concentration of human viruses through an ultrafiltration process-based adsorption, elution, re-concentration, RNA purification and rt-qRT-PCR amplification demonstrated a peak and cut of threshold (C_T) through in the detection of enteroviruses. This study confirms the probable occurrence of enteric viral genomes in drainage water compared to Rosetta branch, suggesting that drains may represent a source of environmental contamination with potentially infectious human viruses. Of all tested sites, coliphages and enteroviruses had the highest concentrations in drains outlets, confirming the relevance of evaluating these viruses as possible indicators of viral contamination of water.

Enterovirus, *E.coli* and coliphages considered in this study were present in water to be used for irrigation. The abundant presence of indicator bacteria and enteric viruses in irrigation water proves a continuous raw residual water supply to the aquatic system.

Conclusions and Recommendations

- Analysis to identify the presence of coliphages as reliable indicators of faecal contamination is recommended. These should be considered as complementary to bacterial indicators, and to reflect the general survival conditions of enteric viruses.
- The fact that coliphages are tolerant to wastewater treatment makes them reliable indicators for the evaluation of recycled water to be used for irrigation and recreational purposes.
- The proposed strategy of using viral and bacterial indicators for water quality assessment is of relatively low cost and could be widely use to confirm water quality norms for drinking and irrigation. In addition to regular water quality parameters measurements. Periodical monitoring for viral pollution must be carried out for River Nile. For this to be effective, it should be coupled to proper lesigtalation for law enforcement.

REFERENCES

Accolas, J.P. and Spillmann, H. (1979). The morphology of six bacteriophages of *Streptococcus thermophilus*. *J. Appl. Bacteriol.* 47: 135-144.

Adeniji, J.A. and Faleve, T.O. (2014). Isolation and Identification of Enteroviruses from Sewage and Sewage-Contaminated Water in Lagos, Nigeria. *Food Environ. Virol.* 6(2):75-86.

Alla, G.; Eugene, K.; Alina, I.; Anatoly, M. and Andrey, L. (2007). The Diversity of Coliphages and Coliforms in Horse Feces Reveals a Complex Pattern of Ecological Interactions. *Appl. Environ. Microbiol.* 73(19):5975-5981.

Amenu, D. (2014). Isolation of Bacteriophages Specific to *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. *Bio-Genetic J.* 2(1):32-36.

American Public Health Association "APHA" (2005). Standard Methods for the Examination of Water and Wastewater (21st ed.), Washington, D. C.

Azzam, M.I.; Hakim, A.S.; Othman, B.A. and El-Dougdoug, K.A. (2010). Virological evaluation of drinking water

in Egypt. *Egyptian J. Virol.*, SP. Issue, 435-452.

Azzam, M.I.; Hakim, A.S.; Othman, B.A. and El-Dougdoug, K.A. (2009). Evaluation of microbial typing as indicators for the efficiency of water treatment stations in El-Menofia Rayah, Egypt. *Egyptian J. Virol.* 6:435-452.

Chandra, M.; Thakur, S.; Narang, D. and Saxena, H. M. (2011). Isolation of a bacteriophage against Salmonella Dublin and determination of its physical resistance under varied in vitro conditions. *African Journal of Microbiology Research*. 5(15):2044-2047.

Chongmiao, Z.; Xiaochang, W.; Yongjum, L. and Dangcong, P. (2010). Simultaneous detection of enteroviruses from surface waters by real-time RT-PCR with universal primers. *J. Environ. Sci.* 22(8):1261-1266.

Cormier, J.; Gutierrez, M.; Goodridge, L. and Janes, M. (2014). Concentration of enteric virus indicator from seawater using granular activated carbon. *J. Virol. Methods.* 196:212-8.

Dakkak, A. (2013). "Egypt's Water Crisis-A Recipe for Disaster". Middle East and North Africa. Available at: www.ecomena.org. [Quoted, 23.10.2013].

Dhevagi, P. and Anusuya, S. (2013). A novel approach for pathogen reduction in wastewater treatment. *J. Environ. Health Sci. and Eng.* 11:12.

Donovan, E.; Unice, K.; Roberts, J.D.; Harris, M. and Finley, B. (2008). Risk of gastrointestinal disease associated with exposure to pathogens in the water of the lower Passaic River. *Appl. Environ. Microbiol.* 74: 994-1003.

Enan, G.; Shaaban, K. A.; Askora, A. and Maher, M. (2013). Coliphages Lysing *Escherichia coli* Bacteria: Their Morphology and Their Response to Some Physical Factors. Research Journal of Applied Sciences. 8(10):516-522.

Espinosa, A.C.; Mazari-Hiriart, M.; Espinosa, R.; Maruri-Avidal, L.; Méndes, E. and Arias, C.F. (2008). Infectivity and genome persistence of rotavirus and astrovirus in groundwater and surface water. *Wat. Res.* 42: 2618-2628.

Hamza, I.A.; Jurzik, L.; Stang, A.; Sure, K.; Überla, K. and Wilhelm, M. (2009). Detection of human viruses in rivers of a densely-populated area in Germany using a virus adsorption elution method optimized for PCR analyses. *Wat. Res.* 43: 2657-2668.

Hamza, I. A; Jurzik, L.; Überla, K. and Wilhelm, M. (2011). Methods to detect infectious human enteric viruses in environmental water samples. *International Journal of Hygiene and Environmental Health.* 214 (6):424–436.

Hegazy, A. M.; El-Salakawy, A. H.; Shaban, M.M.; Yehia, M.M. and Abu Salam, M.S. (2014). Eco-friendly management of enteroviruses in wastewater. *Wat. Sci.* 27 (54):19-29.

Hryniszyn, A.; Skonieczna, M. and Wiszniowski, J. (2013). "Methods for Detection of Viruses in Water and Wastewater," *Advances in Microbiology*. 3 (5): 442-449.

Jebri, S.; Hmaied, F.; Lucena, F.; Saavedra, M.; Yahya, M. and Hamdi, M. (2014). A comparison of two extraction methods for the detection of Enteroviruses in raw sludge. *J. Virol. Methods.* 200:1-5.

Juang, D.F. and Morgan, J.M. (2001). The applicability of the API 20 E and API rapid NET systems for the identification of bacteria from activated sludge. Elect. *J. Biotech.* 4(1); 0717-3458.

Jurzik, L.; Hamzaa, I.A.; Puchert, W.; Überla, K. and Wilhelm, M. (2010). Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. *Int. J. Hyg. Environ. Health.* 213:210–216.

Jothikumar, N.; Kang, G. and Hill, V.R. (2009). Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. *J. Virol. Methods.* 155:126–131.

Katzenelson, E.; Fattal, B. and Hostovsky, T. (1976). Organic flocculation: an efficient second-step concentration method for the detection for viruses in tap water. *Appl. Environ. Microbial*. 59: 1213-1219.

Kfir, R.; Hilner, C.; du Preez, M. and Bateman, B. (1995). Studies evaluating the applicability of utilizing the same concentration techniques for the detection of protozoan parasites and viruses in water. *Wat. Sci. Techol.* 31: 417-423.

Lee, S.H. and Sobsey, M.D. (2011). Survival of prototype strains of somatic coliphage families in environmental waters and when exposed to UV low-pressure monochromatic radiation or heat. Wat. Res. 45(12):3723-3734.

Lin, J. and Ganesh, A. (2013). Water quality indicators: bacteria, coliphages, enteric viruses. *International Journal of Environmental Health Research.* 23(6): 484-506.

Mans, J.; Netshikweta, R.; Magwalivha, M.; Van Z.W. and taylor M.B. (2013). Diverse norovirus genotypes identified in sewage-polluted river water in South *Africa. Epidemiol. Infect.* 141 (2): 303-313.

Mookerjee, S.; Batabyal, P.; Halder, M. and Palit ,A. (2014). Specificity of coliphages in evaluating marker efficacy: A new insight for water quality indicators. *J. Virol. Methods.* 208:115-8.

Nguyen, X. H.; Fang, T.; Qinqin, B.; Chanjuan, S.; Thanh, T. V. and Chengping, L. (2013). Isolation and characterization of bacteriophages against enterotoxigenic *E.coli* strains causing swine diseases. *African J. of Microbiology Research.* 7:1-7.

Obire, O.; Lockhart, M. and Putheti, R. (2010). Plaque Assay and Nucleic Acid Analysis of Lytic Coliphages from Creek Water and Domestic Sewage. *Academic Journal.* 2 (7): 335

Othman, B.A. (1997). Isolation of lambdoid bacteriophage β_4 EC from sewage polluted drinking water. Proceedings of the 9th Conference of Microbiology, Cairo, pp: 25-27.

Okoh, A. I. and Chigor, V. N. (2012). Quantitative RT-PCR Detection of Hepatitis A Virus, Rotaviruses and Enteroviruses in the Buffalo River and

Source Water Dams in the Eastern Cape Province of South Africa. *Int. J. Environ. Res.* 9(11):4017-4032.

Okoh, A. L. and Sibanda, T. (2013). Real-time PCR quantitative assessment of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River located in the Eastern Cape Province, South Africa. *Water Sci.* 39 (2): 295-304.

Oliveira, A.; Sillankorva, S.; Quinta, R.; Henriqoes, A.; Sereno, R. and Azeredo, J. (2008). Isolation and characterization of bacteriophages for avian pathogenic *E.coli* strains. *J. of App. Microbiol.* 106:1919-1927.

Pettibone, G.W. (1992). The use of Lauryl tryptose broth containing 4-methyl lumbelliferyl-beta-D-glucuronide"MUG" to enumerate *E.coli* from fresh water sediment. *Letters in applied microbiology,* 15 (5):190-192.

Rutjes, S.A.; Italiaander, R.; Berg, H.L.; Lodder, W.J. and Husman, M.R. (2005). Isolation and detection of enteroviruses RNA from large-volume water samples by using the NucliSens mini MAG system and real-time-RT-PCR nucleic acid sequence-based amplification. *National Institute for Public Health and the Environment, American Society for Microbiology. Appl. Environ. Microbiol.,* 71(7): 3734-3740.

Sambrook, J.; Fritschi, E.F. and Maniatis, T. (1989). Molecular cloning: a laboratorymanual, Cold Spring Harbor Laboratory Press, New York.

Sinclair, R.S.; Jones E.L. and Gerba, C.P. (2009). Viruses in recreational water-borne disease outbreaks: a review. *J. Appl. Microbiol.* 107:1769-1780.

Śliwa-Dominiak, J.; Tokarz-Deptuła, B. and Deptuła, W. (2014). Occurrence of F-Specific RNA Coliphages and microbial indicators in municipal lake water. Pol. *J. Environ. Stud.* 23(2):467-473.

Tan, E.L.; Yong, L.L.; Quak, S.H; Yeo, W.C. and Chow, V.T. (2008). Rapid detection of enterovirus 71 by real-time TaqMan RT-PCR. *J. Clin. Virol.* 42: 203–206.

Watanabe, K; Takesue, S.; Jin-Nai, K. and Yoshikawa, T. (1970). Bacteriophage Active Against the Lactic acid Beverage. Producing Bacterium *Lactobacillus casei*. *Appl. Microbiol.* 20:409-415.

Wilkes, G.; Edge, T.; Gannon, V.; Jokinen, C.; Lyautey, E.; Medeirose, D.; Neumann, N.; Ruecker, N.; Topp, E. and Lapen, D.R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface water within an agricultural landscape. *Wat. Res.,* 43:2209-2223.

Xiao, Y.Y.; Xing, M.; Yong, L. Z.; Wen, Q.X.; Xia, N.H.; Yu, G.C. and Kang, D. G. (2012). Real-Time PCR Detection of Enteric Viruses in Source Water and Treated Drinking Water in Wuhan, China. *Current Microbiology J.* 65 (3):244-253.

Yates, M. V. (2007). Classical indicators in the 21st century – far and beyond the coliform. *Wat. Environ. Res.* 79:279–286.

Zahraa, J. J.; Amin, S. B. and Akeel, H. A. (2014). Bacteriolytic Activity of Coliphages on Diarrhea Associated *E. coli*. *Journal of Natural Sciences Research.* 4(6):69-75.