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Prevalence of human polyomavirus and papillomavirus in wastewater and in stool of Egyptian patients

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ABSTRACT

Recently oncogenic viruses that are greatly associated with human cancers were detected in urban sewage and other water environments worldwide. The direct contact with contaminated water and sewage can result in serious infections associated with a wide range of diseases. Human Papillomaviruses (HPVs) and Human Polyomaviruses (HPyVs) were the most common virusesthat have been detected in urban sewage worldwide. In the present study, Ninety sewage samples were collected from Zenin wastewater treatment plant in Giza Governorate. Sixty stool samples were obtained from cancer patients. The findings showed that the prevalence of HPVs in sewage and stool samples was 24.4 % (22/90) and 28.3 % (17/60), respectively. HPV16 genotype was the most predominant genotype using sequencing. On the other hand, the prevalence of HPyV in sewage and stool samples was 78.9% (71/90) and 58.3% (35/60) respectively. JC HPyV and BK HPyV were the most common genotypes of human polyomaviruses in sewage 57.8% (41/71), 42.3% (30/71), respectively and in stool 54.3% (19/35), 45.7% (16/35), respectively. By using quantitative Real-time PCR, the number of HPyV DNA copies ranged from $5.3x10^4$ to $6.02x10^4$ GC\L in raw wastewater and $6.2x10^3$ to $6.85x10^3$ GC\L in treated effluent of wastewater treatment plant. Regarding to stool samples, their numbers range were $2.5x10^5$ and $1.24x10^7$ GC\L. These results are indicator of risk for the prevalence of HPVs and HPyVs in Egyptian environment. This is the first report in Egypt to study the human oncogenic viruses in the same study of environmental and clinical samples.

INTRODUCTION

Untreated sewage and contaminated water resources (surface water, shellfish ponds, and river water are considered a source of infection with a several human viruses and are shown to be associated with severe morbidities like, Gastroenteritis followed by respiratory diseases, neurological diseases and paralysis.

Human Papillomaviruses (HPVs) and Human Polyomaviruses (HPyVs) have been detected in urban sewage and in other water environments worldwide (Fratini *et al.,* 2014). It is well known that sewage treatment process can reduce viral contamination but cannot completely eliminate it (Okoh *et al.,* 2010).

Human Papillomaviruses (HPVs) are small, non-enveloped icosahedral capsid with circular double-stranded DNA viruses, belong to the *Papillomaviridae* family. The genome of HPV is about 8 Kb in size and contains eight open reading frames (ORFs), encode two structural proteins (L1, L2) and six nonstructural proteins (E1, E2, E4, E5, E6, E7) (Bzhalava *et al.,* 2013; Ma *et al.,* 2014). HPVs have been classified into five major genera (α, β, γ, μ, ν) according to the genetic sequence of the outer capsid protein L1 ORF. These viruses have the ability to infect squamous epithelia such as the skin and mucosa and are related with an assortment of clinical conditions ranging from common warts to intraepithelial dysplastic lesions; as a result, it is responsible for many serious diseases such as genital warts, anogenital cancers, respiratory papillomatosis, conjunctival lesions and invasive tumors (De Villiers *et al.,* 2004). Furthermore cervical cancer, HPV is causally associated with less common cancers at other sites, such as cancer of the vulva, vagina, penis, and anus, in addition to cancers of the head and neck (D'Souza and Dempsey 2011). HPVs include two types 'high-risk' and 'low-risk' according to their association with the development of cancer. The most common method of HPV infection is transmission through sexual activity; however, there is evidence that HPV can be potentially transmitted by non-sexual routes (Syrjanen, 2010). Recently, HPVs have been identified in wastewaters, and in surface waters, indicating that epitheliotropic viruses can find their way into sewage, and consequently in other water environments. The assumed source of HPV contamination is the washing of skin and mucous membranes, but recent data identified HPVs in stool specimens indicates that they may also reach wastewaters as a result of shedding in the feces and hence, can be used as a viral marker of human waste contamination (Enerly *et al.,* 2013).

Polyomaviruses are non-enveloped viruses with a circular double-strand DNA genome, belong to the *Polyomaviridae* family. The average of HPyV genome was approximately 5Kb in size. In 1971 BK and JC genotypes of human polyomaviruses were discovered, BK was isolated from the urine of a renal transplant patient (Gardner *et al.,* 1971) while JC was isolated from the brain tissue of a patient with Hodgkin's lymphoma who developed progressive multifocal leukoencephalopathy (PML) (Padgett *et al.,* 1971). Both of JC and BK are known to endure in many different sites such as the central nervous system, peripheral blood cells, lymphoid organs in addition to the urinary and genital tract (Goudsmit *et al.,* 1982; Dorries *et al.,* 1994). HPyVs were detected in the gastrointestinal tract and many studies indicated the presence of HPyVs in stool specimen from both hospitalized patients and healthy individuals (Vanchiere *et al.,* 2005; Vanchiere *et al.,* 2009). However, the mechanism of HPyVs transmission, persistent, and reactivation is still unknown. Primary infection with BK virus occurs in early childhood whereas primary infection with JC virus occurs in late childhood, both viruses persist in renal tissue after primary infection, and probably also in B-lymphocytes (Dolei *et al.,* 2000).

Recent studies have demonstrated presence of HPVs and HPyVs among immunocompromised cancer patients (Bruni *et al.,* 2017; Loutfy *et al.,* 2017). However, to the extent of our knowledge, in Egypt, there is no sufficient environmental data available concerning the seasonal variation of HPVs and HPyVs in water.

HPVs and HPyVs are not classic waterborne pathogens. Their presence in water environments is a relatively recent discovery and they are thus considered as emerging or potentially emerging waterborne pathogens Therefore, the present study was aimed to invistigatethe presence of HPVs and HPyVs in wastewater (Activated sludge, raw wastewater, and treated effluent) and also in stool specimens from hospitalized patients with clinical signs of diarrhea of unknown etiology and investigating their seasonal distribution.

In Egypt, there are a few reports about the incidence of papilloma-viruses and polyomaviruses in environmental or clinical samples (Bruni et al., 2017; Loutfy et al., 2017). However, to the extent of our knowledge, there is no sufficient data available concerning the seasonal variation of HPVs and HPyVs. The objective of this study is to investigate the presence of HPVs and HPyVs in wastewater and fecal samples.

MATERIALS AND METHODS

Wastewater and activated sludge samples

A total of 60 sewage samples (30 of raw sewage and 30 of treated sewage) and 30 activated sludge samples were collected from Zenin wastewater treatment plant (WWTP) in Giza governorate from October, 2014 to October, 2017. The capacity of this wastewater treatment plant was 330,000 m^3 per day, activated sludge was obtained from secondary treatment step. Finally treated wastewater was discharged into Nahia effluent system then to River Nile.

Clinical specimens

During the same period as mentioned above a total of 60 stool specimens were collected from hospitalized children, Giza governorate. Samples were collected in clean containers and transferred to the laboratory within 3 hours after collection for analysis.

Concentration of collected wastewater samples

Wastewater samples (inlet and outlet) were concentrated according to (USEPA, 2001). Briefly, 2.5 mL/1L sample of 1M magnesium chloride (Merck-Schuchardt, Germany) was added to increase the stability of the viruses in the samples during transportation (APHA, 2005). Wastewater samples were adjusted to pH 3.5 by 1N HCl for detection of viruses. The samples were concentrated by filtration on Nitrocellulose membrane filter, (0.2µm pore size, and 142mm diameter) on a stainless steel filter holder. The viruses were adsorbed on the membrane filter and were subsequently eluted in 100ml of 3% beef extract-0.05M glycine solution, pH 9.5. All samples were re-concentrated using an organic flocculation method (Katzenelson *et al.,* 1976). Concentrated Samples were stored at -20˚C until used.

Concentration of sludge samples

One hundred g of sludge were dissolved in 300ml of 10% beef extract, 1.34%$Na_2HPO_4.7H_2O$ and 12% citric acid. The mixture was stirred for 30 min at pH 7 and centrifuged at 4500 rpm for 15 min, the supernatant was adjusted to pH 3.5, stirred for 30min, centrifuged at 4500 rpm for 15min,finally the obtained sediment was dissolved in 0.15M Na_2HPO_4, stored at -80˚C until analyzed (USEPA,1984).

Concentration of the collected stool specimens

Approximately 0.2 g of stool specimens was weighed, diluted by 1ml PBS (20% wt/v), and vortex for 30sec. Samples were clarified by centrifugation at 7000 rpm at 4°C for 10min, and then the supernatant was taken and archived at -08°C until use.

Extraction of viral nucleic acid

Viral nucleic acids were extracted from 300 µl of concentrated samples using DNA extraction kit (Patho Gene-spinTM, Korea) according to the instructions of the manufacture. Total nucleic acids were eluted in 60µl elution buffer and stored in -20°C. Standard precautions were followed to avoid contamination.

Spike experiment and inhibition test

The effect of sewage sample's inhibitors on PCR was analyzed by using human adenovirus type 2 as an internal control. In the current study inhibitors were monitored by spiking our samples using adenovirus type 2 ($2x10^9$ GC/mL). Serial dilutions of adenovirus (stock) was prepared from 10^{-1} to 10^{-6}. Viral DNA was extracted using patho Gene-spin TM cat. No. 17154 from the viral stock. The PCR was performed as previously described by Puig *et al.,* (1994). Briefly, 100 µl from adenovirus (stock) was inoculated in 900 µl of raw wastewater sample and then incubated for two hours at room temperature to determine the presence of PCR inhibitors in our samples. Serial dilution was prepared of the inoculated sample from 10^{-1} to10^{-9}. DNA was extracted and PCR was performed, similarly spiked experiment was done for activated sludge sample as before. After, Inoculated samples were stored at -20^{o}C for two weeks then DNA was extracted from inoculated samples, and serial dilution of the inoculated samples from 10^{-1} to10^{-9} was performed, DNA was extracted and PCR was performed again.

Detection of Human Polyomaviruses using qualitative PCR assays

Molecular detection of HPyVs was performed using qualitative semi-nested version of PCR assay to identify the HPyVs strains. The PCR amplification was performed using a set of primers that target an intergenic T region according to previously published protocol (Arthur *et al.,* 1989, Loutfy *et al.,* 2017)

Real Time PCR for Quantification of Human polyomaviruses

Quantitation of HPyVs viral load in stool, activated sludge, raw wastewater and treated effluent samples was performed using real-time detection system (Applied Biosystems StepOne TM Real-Time PCR system Thermal Cycling Block, Singapore) according to previously published protocols (Arthur *et al.,* 1989, Loutfy *et al*., 2017) using sets of primers as shown in Table (1).

Detection of Human Papillomaviruses using nested PCR assay

DNA was extracted from activated sludge, raw wastewater and treated effluent samples of Zenine wastewater treatment plant, and stool specimens. All samples subjected to nested PCR assay according to previous protocol (Manos *et al.,* 1989). In the 1st PCR , MY09 F1 , MY11_R1 primers targeted the L1 region were used, but in the 2nd PCR assay GP5+/GP6+ were used as shown in Table (1).

Table 1: The group of primers used in nested and semi-nested PCR

Virus	Name	Primer	Orientation	PCR Product size	Reference
HPVs	MY09	5'GCA CAG GGA CAT AAC AAT GG3'	Sense	455bp	(Manos *et al.,* 1989)
	MY11	5' CGT CCA AAA GGA AAC TGA TC 3'	Antisens		
	GP5+	5'TTTGTACTGTGGTAGATACTAC3'	Sense		(de Roda *et al.,* 1995)
	GP6+	5'GAAAAATAAACTGTAAATCATATTC3	Antisense	151bp	
HPyVs	F1	5'AAG TCT TTA GGG TCT TCTAC 3'	Sense	179bp	(Arthur *et al.,* 1989)
	R1	5' GTG CCA ACC TAT GGA ACA GA 3'	Antisense		
	BK	5' GAG TCC TGG TGGAGT TCC3'	Antisense	71bp	(Loutfy *et al.,* 2017)
	JC	5'TGA TGAA AA CAC AGG ATCC3'	Antisense	149bp	
HAdV	Ad40(hexon)	5'GCCCGCAGTGGTCTTACATGCACATC3'	Sense	300bp	(Puig *et al.,* 1994)
	Ad41(hexone)	5'CAGCACGCCGCGGATGTCAAAGT 3'	Antisense		

Sequencing of purified PCR products

PCR products were purified using MEGA quick spinTM Total fragment DNA purification kit iNtRON (Lot.NO.15163048 Company Name) according to manufacturer instructions. The nucleotide sequences were determined directly with an ABI Prism® 310 Genetic Analyzer (Applied Biosystems) Clinilab Company, Egypt. Briefly, Cycle sequencing was performed on 7µl of the purified products with an ABI Prism Big dye termination cycle sequencing ready reaction kit (applied biosystem) using the same primers as described in PCR section. The DNA was

sequenced with an ABI Prism (model: 310) automated DNA sequence. CLUSTAL multiple sequence alignment was carried out by MUSCLE (3.8**).**

RESULTS

Physicochemical Characteristics of raw and treated Wastewater

The performance of the integrated system was monitored monthly for two years from (October 2015 to October 2017). The raw wastewater was analyzed. The physico-chemical analysis covered: Total chemical oxygen demand (COD_{tot}), soluble chemical oxygen demand (COD_{sol}), Biochemical oxygen demand (BOD_{tot}), soluble biological oxygen demand (BOD_{sol}), total Kjeldahl nitrogen (TKN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), Total suspended solids (TSS), oil and grease and total phosphorus (TP) see Table (2).

Table 2: Physicochemical analysis for Zenine wastewater treatment plant

Parameter	Units	Influent	Effluent
pH		7.5±0.3	7.7±0.3
COD_{tot}	mg/l	354±78	148±47.8
COD_{sol}	mg/l	168±51	72.9±23.8
BOD_{tot}	mg/l	207±53	80.3±28.3
TSS	mg/l	185±44	71.5±26.1
TKN	mg/l	48±9.5	40.5±10
Ammonia	mg/l	24±4.2	19.8±4
Nitrite	mg/l	0.09±0.2	0.15±0.3
Nitrate	mg/l	0.22±0.3	0.5±0.5
Phosphorous	mg/l	1.9±0.7	1.1±0.5

Evaluation of inhibitors by using spiked experiment

Aiming to obtain high sensitivity of PCR product in the collected samples, PCR detection protocol was first optimized for wastewater DNA extracts to overcome common inhibitors. In that respect, it was observed that the amplification efficiency was reduced over time in stored samples. To assess the effect of storage time on the PCR amplification of target oncogenic viruses, the control Adenovirus was used to spike the collected samples and monitor the amplification efficiency for 45 days. Results showed that the amplification was not affected after two hours of Adenovirus spiking (Fig. 1), while after two weeks the effect on inhibitors began to appear (Figures 2 and 3)

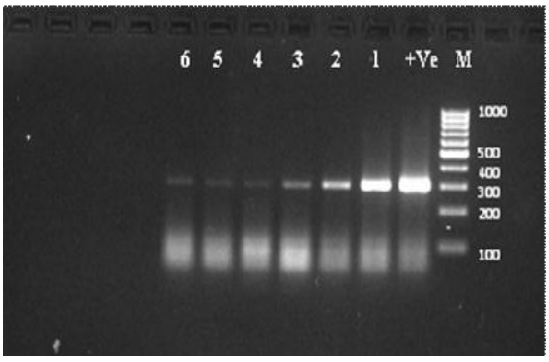

Fig. 1: PCR inhibition test after two hours from inoculation. Amplification product of the 300 bp from Hexone region. M was DNA ladder 100 bp, followed by control positive. Lanes from 1 to 6 represented serial dilution from 10^{-1} to 10^{-6} of inoculated samples.

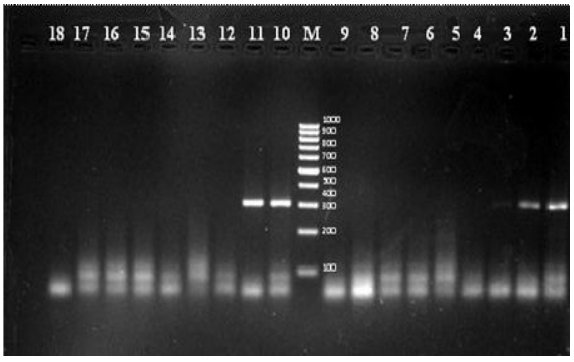

Fig. 2:PCR inhibition testing after two weeks from inoculation. Samples from 1 to 9 represented serial dilution from 10^{-1} to 10^{-9} of inoculated raw wastewater sample respectively; M was DNA ladder 100bp samples from 10 to 18 represented serial .dilution from 10-1 to 10-9 of inoculated activated sludge sample respectively

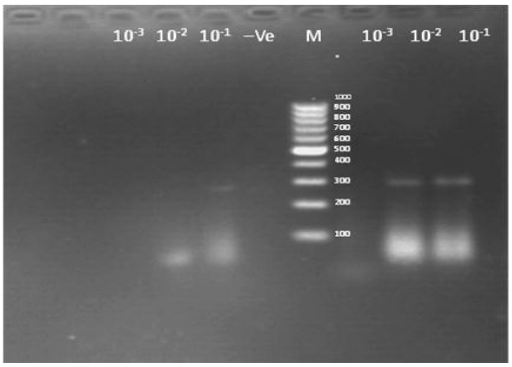

Fig. 3: PCR inhibition test after 45 days from inoculation. Lanes 1, 2, 3 represented serial dilution from 10^{-1} to 10^{-3} of inoculated raw wastewater sample respectively; M was DNA ladder 100bp while samples from 10 to 18 represented serial dilution from 10^{-1} to 10^{-9} of inoculated activated sludge sample, respectively.

Human Polyomaviruses (HPyVs) in Zenin wastewater treatment plant

HPyVs were detected in 78.9% (71/90) of samples collected from Zenin wastewater treatment plant during the current study. For activated sludge, 100% (30/30) of HPyVs DNA were detected. JCV was detected in 60% (18/30), while BKV was detected in 40% (12/30) of all positive activated sludge samples. For raw wastewater, 83.3% (25/30) of HPyVs DNA were detected. JCV was detected in 56% (14/25), while BKV was detected in 44% (11/25) of all positive raw wastewater samples. For treated effluent, 53.3% (16/30) of HPyVs DNA were detected. JCV was detected in 56.3% (9/16), while BKV was detected in 43.8% (7/16) of all positive treated effluent samples as shown in (Table 3).

Table 3: Detection of Human polyomavirus (HPyV) in Zenin (WWTP).

HPyVs	**Type of sample**		
	Raw wastewater samples	**Treated effleunt samples**	**Activatd sludge samples**
Percentage of positive HPyV	(25/30) 83.3%	(16/30) 53.3%	(30/30) 100%
BKV	(11/25) 44%	(7/16) 43.8%	(12/30) 40%
JCV	(14/25) 56%	(9/16) 56.3%	(18/30) 60%

Frequency of Human polyomavirus (HPyVs) in clinical stool specimens

HPyVs DNA was detected in 58.3% (35/60) of stool samples of cancer patients and patients with gastroenteritis. Of 35 positive polyomavirus we found 45.7% (16/35) JC, and 54.3% (19/35) of them were BKV. HPyVs DNA was higher in females than males as shown in Fig (4).

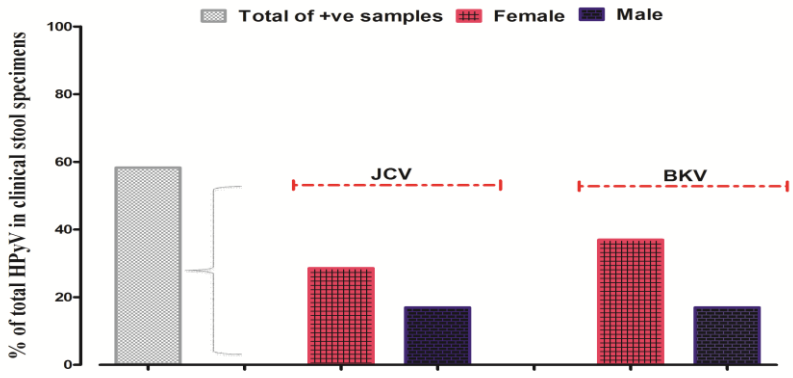

Fig. 4: Presence of Human HPVs in clinical stool specimens according to gender.

Quantitative detection of HPyVs by Real time PCR

The median viral load of HPyVs was $3.5x10^3$ to $4.02x10^4$ GC/L.in activated sludge samples. But it was $6.02x10^4$ to $5.3x10^4$ GC/L and $6.85x10^3$ to $6.2x10^3$ in raw wastewater and in treated effluent samples, respectively. In stool specimens, its range was found to be between $2.5x10^5$ and $1.24x10^7$ GC/L. The highest load of the stool specimens and raw wastewater samples was in winter season, but the lowest viral load was for treated effluent samples in summer season.

Presence of HPVs in wastewater using nested PCR assay

Presence of HPVs in Zenin wastewater treatment plant was 12.2 % (11/90) in total collected samples using MY09/MY11 whereas, it was 24.4 % (22/90) using GP5+/GP6+ of the same samples. The detection rate was (8/30) 26.6% in activated sludge, while it was (14/60) 23.3% in collected wastewater samples divided as 33.3% and 13.3% in raw wastewater and treated effluent, respectively as shown in Table (4).

Table 4: Detection of Human Papillomavirus (HPVs) in Zenin (WWTP)

No. of samples	Percentage of positive HPVs (nested PCR)			
	Raw Wastewater samples	Treated effluent Samples	Total	Activated sludge samples
90	(10/30) 33.3%	(4/30) 13.3%	(14/60) 23.3%	(8/30) 26.6%

Seasonal distribution of HPyVs and HPVs in sludge and wastewater treatment plant

HPyVs were present all over the year, the highest distribution was in winter 38%, (27/71) and the lowest one was in summer 17% (12/71) as shown in figure (5). While HPVs were detected in 45.6% (10/22) of the collected wastewater samples in winter whereas; it was detected in 36.4% (8/22) and 19% (4/22) during autumn and spring respectively. HPV was not detected in summer season as shown in Fig (5).

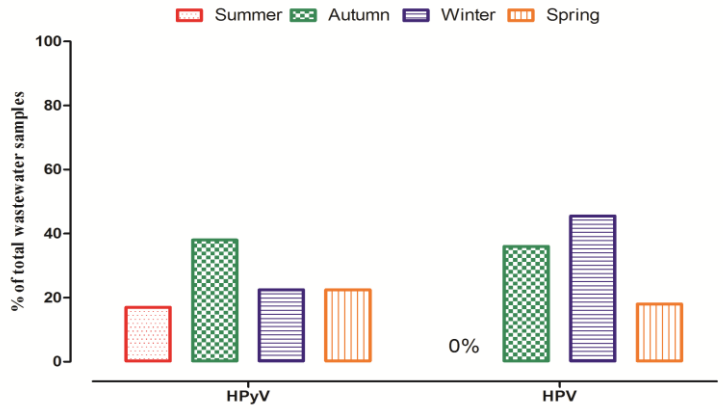

Fig. 5: Seasonal distribution of HPyVs and HPVs DNA in Zenine wastewater samples

Presence of HPVs in clinical stool specimens

HPV DNA was detected in 28.3% (17/60) hospitalized patients suffering from cancer and gastroenteritis. The percentage of HPV DNA was higher in females 64.7% (11/17) than in males 35.3 % (6/17) as shown in Fig (6).

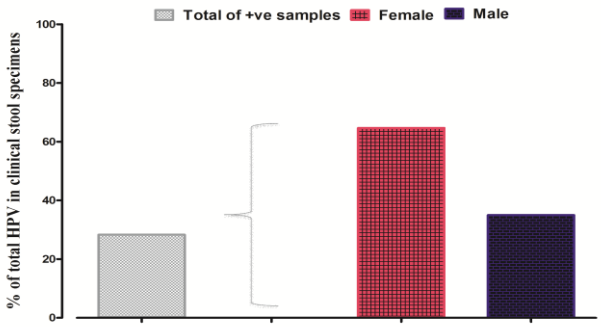

Fig. 6: Presence of Human HPV in clinical stool samples according to sex

Sequencing of Papillomaviruses (HPVs) PCR products

The PCR products (150bp) was purified from wastewater and stool samples of hospitalized patient and subjected to sequencing. Nucleotide blast analysis of the consensus sequence revealed partial homology with previously published HPV sequences in NCBI Gen bank database, with the highest homology annotated with the sequence of: MH057740.1 type 16 isolate HPJ16-6 with nucleotide identity 98%. The phylogenetic tree was constructed by alignment of our obtained partial sequence with 21 highly similar HPV nucleotide sequences published in Gen Bank Fig. (7).

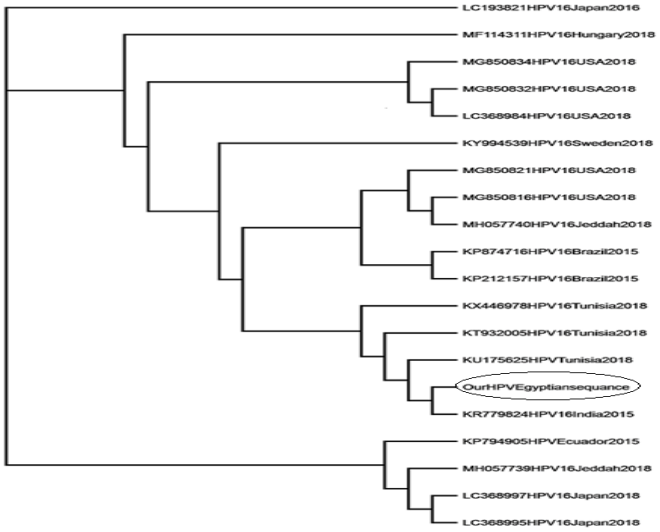

Fig. 7: A Neighbor-joining Phylogenetic Tree for all reported sequences of HPV with studied partial sequence of positive HPV sample 2018. Data showed that the obtained sequence is closely related to sequences with Accession No(s). (MH057740.1 HPV16), (KX446978.2 HPV16) and (KT932005.2 HPV16)

DISCUSSION

In the current study, presence and seasonal distribution of HPVs and HPyVs were investigated in sewage sludge, influent, effluent of wastewater samples; In addition, their removal during the municipal treatment over a period of thirty months was investigated as well. We observed that treatment of wastewater was not efficient enough to remove these viruses as they were detected in sludge, inlet, and outlet

samples. This results were not far from that published data which reported the stability and highly resistant strains of HPyV to environmental conditions in different water resources and shedded the light on the possible use of these viruses as indicator for fecal contamination in water sources (Hundesa *et al.,* 2006; Hamza *et al.,* 2009).

Comparing to many studies that reported a high level of dissemination of HPyVs among a panel of different human-associated microbes in different water and wastewater samples (McQuaig *et al.,* 2009; Fumian *et al.,* 2010; Fumian *et al.,* 2013; Hewitt *et al.,* 2013; Hamza *et al.,* 2014; Kitajima *et al.,* 2014); our results revealed that the presence of HPyV was 79% (71/90) in wastewater samples. These findings were slightly higher than Hamza *et al.,* (2018) who reported that HPyV was detected in 72% (48/66), of Egyptian wastewater as which might be attributed to the larger volume of our collected samples. Similarly, there was a marginal difference in HPyVs detection rate obtained in our study with that reported by Bo fill-Mas *et al.* (2013) that can be justified by several factors including different geographical area, enrichment process, and sensitivity of PCR assay.

Quantitatively, the median viral load of HPyVs in wastewater samples used in this study ranged from $6.02x10^4$ to $5.3x10^4$ GC/L in raw wastewater samples decreased tenfold in treated effleunt samples to be $6.85x10^3$ to $6.2x10^3$ GC/L. These results were lower than previously reported by Rusinol and his colleagues. (Rusinol *et al.,* 2015) in Spain, who detected HPyV in raw and treated wastewater as $7.5x10^5$ GC/L and $1.6x10^5$ GC/L, respectively. In another study from Egypt by Hamza *et al.,* (2018); HPyVs viral load was detected as 10^5 GC/L in the influent's samples. Regarding to our results HPyVs viral load in the treated effluent was one to two magnitudes lower than the raw water and this reflect deficiency of wastewater treatment plant in removing all viral loads during municipal treatment process.

Additionally, seasonal distribution of HPyVs was observed all over the year in wastewater environmental samples, this indicated that HPyVs was highly resistant to the most environmental conditions with the highest percentage in winter (45.5%). Also, Fumian *et al.* (2010) observed a seasonal variation during summer months taking into consideration that the study performed in a geographic region belongs to the southern hemisphere. These data might support the hypothesis of using HPyVs as indicator of viral contamination of human origin (Pina *et al.,* 1998; Hundes *et al.,* 2006).

The percentage of JC in raw wastewater was 56% (14/24) this result was lower than (Fumian *et al.,* 2010) in Rio De Janeiro, Brazil, where JC was detected by PCR in 96% of raw sewage samples. Also another report from Spain, (Bofill-Mas *et al.,* 2000) detected JC in 98% of the 52 sewage samples collected from disparate geographical areas around the world. Such discrepancy in results might be justified by using different sets of primers to amplify VP1 region. During this study, a spiked experiment was conducted to determine the potential effect of PCR inhibitory substances in our collected wastewater samples through spiking the collected samples with the control human adenovirus type 2 over 45 days. We found that the effect of PCR inhibitors began to appear after two weeks of inoculation, which reflects the necessity of immediate extraction of nucleic acid to avoid false negative results.

Based on our findings, the overall prevalence of HPyV in sewage and stool samples was 79% and 58.3%, respectively. These outcomes in both sewage and stool samples are similar to the findings of the previously reports conducted in Egypt or worldwide, which demonstrated that this virus is highly abundant in sewage as well as stool samples. Moreover, the virus may reach wastewaters as a result of shedding in feces which support its probability to be transmitted via fecal-oral route (Hamza *et*

al., 2018; Iaconelli *et al.,* 2015; La Rosa *et al.,* 2015; Kitajima *et al.,* 2014; Vanchiere *et al.,* 2009; Vanchiere *et al.,* 2005). Indeed, 83.3%, 53.3% and 100% of raw wastewater, treated effluent and activated sludge samples were positive for HPyV, respectively. These data demonstrated that the virus was able to stay stable in waters and resistant to disinfectants. These results are consistent with the previously published studies of La Rosa *et al.,* (2015); Hamza *et al.,* (2009); Fernandez-Cassi *et al.,* (2016) which reported there is no significant reduction in viral concentration before and after treatment processes.

For viral quantitative study, the difference between viral load in sewage samples before (10^4 GC\L) and after (10^3 GC\L) treatment was only one log, while the viral load of HPyV in stool samples (10^7 GC\L) was one to two folds more than the inlet effluent. These concentrations for wastewater samples are relatively similar to the findings of the previously reports conducted in sewage (Hamza *et al.,* 2014; Rusinol *et al.,* 2015; Hamza *et al.,* 2018) and conducted also in stool (Vanchiere *et al.,* 2009).

No seasonal peak was observed for circulating of polyomaviruses in sewage or fecal samples. This outcome is agreeing with the previously studies of Pina *et al.,* (1998) and Hundes *et al.,* (2006) which suggested that the polyomaviruses might be used as an indicator for fecal viral contamination.

On the other hand, the present study showed that human papillomaviruses were identified in 24.4% of sewage samples and 28.3% of fecal samples with the presence only one mucosal genotype HPV-16 which is mainly isolated from anal, colon and cervical cancers. These findings are relatively similar with the data reported in La Rosa *et al.,* (2013), Di Bonito *et al.,* (2015), Yu, *et al.,* (2015), Loutfy *et al.,* (2017), and Hamza *et al.,* (2018). Therefore, the present study recommends that the fecal samples are the main source of human papillomaviruses in sewage samples and this is agreeing with report of Di Bonito *et al.,* (2015) which identified HPVs in fecal samples. For seasonal distribution, we found that the papillomaviruses were detected throughout the year except hot months. This finding is consistent with Albert, (2007) that reported the high temperatures have effect on virus's survival in water, since lower temperatures are the key for longer virus survival. Eventually, the presence of HPyV and HPV in stool and sewage samples might be considered as possible link for their transmission by fecal-oral route.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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