Iranian Journal of Microbiology

Volume 17 Number 6 (December 2025) 1023-1032
DOI: http://doi.org/10.18502/ijm.v17i6.20371

ORIGINAL ARTICLE

Detection of the BK virus in urban wastewater inlets into the Karun River

Parvaneh Bahrami[1], Afshin Takdastan[2], Manoochehr Makvandi[3*], Sahand Jorfi[2], Mohammad Karimi Baba Ahmadi[4], Abdolkazem Neisi[2]

[1]Department of Environmental Health Engineering, Environmental Technologies Research Center, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[2]Department of Environmental Health Engineering, Environmental Technologies Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[3]Department Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[4]Department of Medical Biotechnology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Received: February 2025**, Accepted:** October 2025

ABSTRACT

Background and Objectives: The presence of the BK virus in wastewater indicates pollution, as it is shed in the urine of infected individuals. Over fifty percent of the human population remains asymptomatic for the BK virus. Reactivation of the BK virus in immunosuppressed individuals can lead to serious health issues, including cystic hemorrhagic, nephritis, and kidney graft rejection. The BK virus is associated with various cancers, such as head and neck, prostate, bladder, and colorectal cancers. The urban wastewater inlets into the Karun River cause the river contamination. This study focused on detecting the BK virus in wastewater inlet in Karun River, Ahvaz city, Iran.

Materials and Methods: Sixty raw wastewater samples were collected from diverse urban sources and concentrated using polyethylene glycol 6000 to isolate BK virus. The BK virus isolates were analyzed for genotypes and the non-coding control region (NCCR).

Results: The Nested PCR results indicated that thirty-two out of sixty samples (53.33%) were positive for the BK virus. The phylogenetic analysis revealed the dominance of BK virus genotype Ib2, followed by genotype 4. The BK virus non-coding control region (NCCR) analysis identified an archetype strain.

Conclusion: The sewage plant treatment should be implemented to remove pathogenic viruses specially BK virus and to curb the circulating of BK virus in human and environment.

Keywords: Wastewater; BK virus; Genotype; Non-coding control region (NCCR)

INTRODUCTION

The presence of BK virus (BKV) in wastewater is a significant indicator of contamination. BKV, released in the urine of infected individuals who are the carriers, enters the wastewater system and is ultimately discharged into rivers (1). Rivers constitute a primary source of potable water and can be contaminated with BK virus (2). The presence of the BK virus in drinking water leads to the establishment of the BK virus as a persistent and asymptomatic infection in various organs of the body during

*Corresponding author: Manoochehr Makvandi, Ph.D, Department Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel: +98-9166181683 Fax: +98-6133112751 Email: manoochehrmakvandi299@gmail.com

the early childhood developmental stage (3). Reactivation of latent BKV has been observed in patients with immunocompromised conditions associated with BKV infection, manifesting in ailments such as encephalitis, nephritis, hemorrhagic cystitis, retinitis, and pneumonia; this phenomenon has also been documented in individuals infected with HIV-1 (4). BK virus is characterized as a non-enveloped double-stranded DNA virus that falls under the classification of the *Polyomaviridae* family (5). The global seroprevalence rate of BK virus has been reported to be 80% (5). The dissemination of BK virus occurs via various mechanisms, encompassing the respiratory system, blood transfusions, seminal fluid, oral-fecal transmission, organ transplantation, as well as excretion through urine and feces (6). BK-PyV is classified into four genotypes, I-IV. Genotype I is the most prevalent worldwide (80%), followed by genotype IV (15%) (5, 7). Subtype II and III viruses are rarely detected. Four subgroups of subtype I have been recognized (Ia, Ib-1, Ib-2, and Ic), and six subgroups of subtype IV have been recognized (IVa-1, IVa-2, IVb-1, IVb-2, IVc-1, and IVc-2) (7). Polyomaviruses (PyVs) exhibit significant oncogenic potential. BK Polyomavirus, JC Polyomavirus, and SV40 possess viral oncoproteins, recognized as large T (Tag) and small t (tag) antigens, which are implicated in cellular transformation and oncogenic processes. (6). BK virus DNA has been identified in various malignancies, including those of the urological system, prostate, and brain cancers (8).

Molecular characterization. Two distinct variants of BKV NCCR exist: the archetype NCCR variant, found in the urine of healthy individuals, and the second, a rearranged NCCR variant, found in infected tissue samples (1). The archetypal virus is postulated to represent the transmissible variant of the virus, as it has been identified in both asymptomatic individuals and those afflicted with disease, whereas rearranged variants are predominantly obtained from patients suffering from BKV pathology (1). The BKV archetype encompasses a 376 bp linear O, P, Q, R, S boxes, where “O” denotes replication and P, Q, R, S boxes play the promoters and regulatory domains of the early viral gene region (EVGR) and the late viral gene region (LVGR) (9). The “O” box comprises 142 bp, the “P” box 68 bp, the “Q” box 39 bp, the “R” box 63 bp, and the “S” box 63 bp. The “O” box harbors the origin of DNA replication, and each box possesses a unique configuration of transcription factor binding sites (TFBS) (9). The viral genome is partitioned into three principal components: the early region, the late region, and the non-coding control region (NCCR). Alterations within the NCCR can facilitate the transition from the archetype strain to the rearranged strain, with NCCR rearrangements playing a crucial role in viral pathogenesis (10). The presence of BK virus in wastewater as an indicator of water pollution is well-established (1). The Karun river is one of the sources of the drinking water. This study aimed to detect BK virus genotypes and analyze the BK virus NCCR in wastewater from the inlet to the Karun River in Ahvaz city, Iran.

MATERIALS AND METHODS

The Karun River is situated in the southwestern region of Iran, it extends approximately 850 km, originating from Zard-Kuh Bakhtiari in the Zagros Mountains and flowing into the international waters of the Persian Gulf. The river traverses the Ahvaz metropolitan zone and bifurcates the city into western and eastern segments. The quality and quantity of the river’s water are experiencing a significant decline, primarily attributable to population growth, and the release of wastewater to the river. In this investigation, 60 raw wastewater samples were collected from different locations where untreated wastewater is directly discharged into the Karun River from March to August 2023. The Personal Protective Equipment (PPE), Goggles, Protective face mask or splash-proof face shield, Liquid-repellent coveralls, Waterproof gloves, Rubber boots were provided for the worker, since experts handling raw sewage may be exposed to infectious or oncogenic viruses.

Concentration of wastewater samples. Various techniques such as Polyethylene glycol (PEG), ultracentrifugation, electronegative membrane filtration, and ultrafiltration have been utilized for virus concentration in different water environments (11). The World Health Organization (WHO) has endorsed the PEG-based separation technique (12). The PEG method is favored for its efficiency, cost-effectiveness, reliability, and non-destructiveness to viruses (11), thus the Amdiouni method using PEG was chosen for this study (13).

The concentration methodology was executed in accordance with the procedures delineated by Amdiouni (13). A total of 500 ml of wastewater sample was collected, put on the ice, and sent to the virology laboratory. Then the sample underwent clarification via centrifugation for a duration of 30 minutes at a gravitational force of 1000 g, at 4ºC, after which the pellet was reconstituted in 10 ml of the supernatant. The residual supernatant was retained for further analysis. Chloroform was introduced to the resuspended sample to achieve a concentration of 10%, followed by thorough mixing, and the resultant mixture was subjected to a secondary centrifugation for 5 minutes at 1000 g. The first and second supernatants were amalgamated, and the total volume was quantified. The consolidated supernatants were augmented with NaCl and polyethylene glycol (PEG) to reach final concentrations of 2.2% (w⁄w) NaCl (Sigma) and 7% (w⁄w) PEG 6000 (Fluka, Steinheim, Germany). The mixture was agitated at a temperature of 4°C overnight and subsequently centrifuged for 2 hours at 2000 g at the same temperature. The supernatant was discarded, and the pellet was reconstituted in phosphate buffer at a dilution of 1 ⁄ 100 of the initial volume and kept at -20ºC until DNA extraction.

DNA extraction. The deoxyribonucleic acid (DNA) was isolated from concentrated samples utilizing a viral nucleic acid extraction kit (High Pure Viral Nucleic Acid Kit, Roche, Germany) in accordance with the guidelines prescribed by the manufacturer. The concentration of the isolated viral DNA was assessed employing a Nano-Drop spectrophotometer produced by Thermo Fisher Scientific, United States. The ultimate concentration of the purified DNA derived from the 60 raw wastewater specimens exhibited a range between 10 and 75 ng/µl. The purified DNA samples were preserved at -20ºC to enable future analytical assessments.

BK virus genotyping. Nested Polymerase Chain Reaction (PCR) was utilized for identification of the BK virus VP1 region, employing an outer primer pair VP1-7/VP1-2R to amplify a fragment measuring 579 bp, subsequently followed by the application of the inner primer pair 327-1/2 for amplification of a 327 bp sequence (14) (Table 1). The genomic segment from 1630 to 1937 (327 bp) of BK viruses is characterized as hypervariable and serves as a critical determinant for the classification of BK virus genotypes (14). The PCR mixture encompassed 10 µL of a 2X master mix (Amplicon, Denmark), 1 µL (10 pmol/µL) of each primer (Table 1), 500 ng of the DNA template, and distilled water adjusted to a final volume of 25 µL. In each PCR assay, both negative and positive controls (the extracted DNA from the urine sample of positive for BK virus DNA) were incorporated, utilizing the following thermal cycling protocol: an initial denaturation at 94°C for 5 minutes, succeeded by 35 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 60 seconds, culminating in a final extension at 72°C for 10 minutes. The second amplification round was conducted utilizing 1 µL of the product obtained from the initial amplification, alongside 1 µL (10 pmol/µL) of each primer, and 10 µL of a 2x PCR master mix, with the volume adjusted to 25 µL using distilled water. The thermal cycling parameters implemented were as follows: an initial denaturation at 94°C for 5 minutes, followed by 32 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and an extension phase at 72°C for 60 seconds. The resultant PCR product, measuring 327 base pairs, yielded a positive result (14).

Gel electrophoresis. Gel electrophoresis was performed utilizing a 2% agarose gel in conjunction with a 100-bp DNA ladder to facilitate the separation and detection of PCR products within a timeframe of 20 minutes. Positive samples were subsequently subjected to Sanger sequencing for further analysis. The duplicate PCR assays were repeated for the positive tests.

Sequencing and phylogenic analyses of VP1. For the purposes of verification, the sequencing outcomes pertaining to the partial "VP1" region of the BK virus genome from the isolated samples were systematically aligned utilizing the NCBI BK virus database (https://blast.ncbi.nlm.nih.gov). The isolated VP1 BK virus sequences were further aligned with the reference sequence of the VP1 BK virus through the application of SnapGene software (version 3.2.1). To ascertain the genotyping of the BK virus, a phylogenetic tree was constructed employing the Maximum Likelihood method for each isolated partial VP1 region of the BK virus genome, adhering to the Kimura 2-parameter distance model with 1000 boot-

Table 1. Primer sets used for BKV detection

Primers	Sequence	Fragment size	Reference
VP1-7	5'-ATCAAAGAACTGCTCCTCAAT-3'	579 bp	(14)
VP1-2R	5'-GCACTCCCTGCATTTCCAAGGG-3'		
327-1	5'-CAAGTGCCAAAACTACTAAT-3'	327 bp	(14)
327-2	5'-TGCATGAAGGTTAAGCATGC-3'		
NCCR Outer- F	5'- GAGCTCCATGGATTCTTC -3'		(15)
NCCR Outer-R	5'- CCAGTCCAGGTTTTACCA -3'		
NCCR Inner-F	5'- CCCTGTTAAGAACTTTATCCATTT -3'	375bp	(15)
NCCR Inner-R	5'- AACTTTCACTGAAGCTTGTCGT -3'		

strap replicates; the scale bar utilized for this analysis was configured at 0.01. The MEGA software version 6 was utilized to execute these methodologies. The sequences corresponding to the partial VP1 region of the BK virus, along with their respective accession numbers, were randomly selected from five wastewater samples and aligned with VP1 sequences representing various BK virus genotypes isolated from diverse geographical locations worldwide.

BK virus NCCR assessment. Nested Polymerase Chain Reaction (PCR) was utilized for identification of the BK virus NCCR region. The PCR mixture encompassed 10 µL of a 2X master mix (Amplicon, Denmark), 1 µL (10 pmol/µL) of each primer (table 1), 500 ng of the DNA template, and distilled water adjusted to a final volume of 25 µL. In each PCR assay, both negative and positive controls were incorporated, utilizing the following thermal cycling protocol: an initial denaturation at 94°C for 5 minutes, succeeded by 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 53°C for 45 seconds, and extension at 72°C for 45 seconds, culminating in a final extension at 72°C for 10 minutes. The second amplification round was conducted utilizing 1 µL of the product obtained from the initial amplification, alongside 1 µL (10 pmol/µL) of each primer, and 10 µL of a 2x PCR master mix, with the volume adjusted to 25 µL using distilled water. The thermal cycling parameters implemented were as follows: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and an extension phase at 72°C for 45 seconds, culminating in a final extension at 72°C for 10 minutes. The resultant PCR product, measuring 375 bp, yielded a positive result (15).

Gel electrophoresis. Gel electrophoresis was performed utilizing a 2% agarose gel in conjunction with a 100-bp DNA ladder to facilitate the separation and detection of PCR products within a timeframe of 20 minutes. Positive samples were subsequently subjected to Sanger sequencing for further analysis. The duplicate PCR assays were repeated for the positive tests. The sequences corresponding to the NCCR region of the BK virus, along with their respective accession numbers, were randomly selected from five wastewater samples and aligned with a consensus sequence of the NCCR region of the BK virus archetype.

RESULTS

The identification of BK DNA was substantiated in 32 out of 60 (53.33%) wastewater specimens. A subset of 10 samples was randomly selected, comprising 5 samples that tested positive VP1 BK virus with accession numbers OR723817 - OR723821, and 5 samples that tested positive NCCR BK virus with accession numbers OR933745-OR933749, all of which were recorded in GenBank. The VP1 BK virus isolates OR723817 - OR723821 from Ahvaz, Iran, exhibited a phylogenetic clustering with the BK virus genotype 1b2 (HE650868) isolate from Kuwait, alongside (OR113380, OR113382, and OR113383) isolates derived from the colon specimens of patients in Ahvaz, Iran. Conversely, the BKV isolates obtained from Ahvaz, specifically OR723818 and OR723820, clustered with genotype IV (JX195569) isolated in Spain, as well as with HE650847 isolated in Kuwait, and OQ129438 and OR509396 isolated in Iran. The phylogenetic tree results are illustrated in Fig. 1.

The results of five BK virus NCCR isolates from

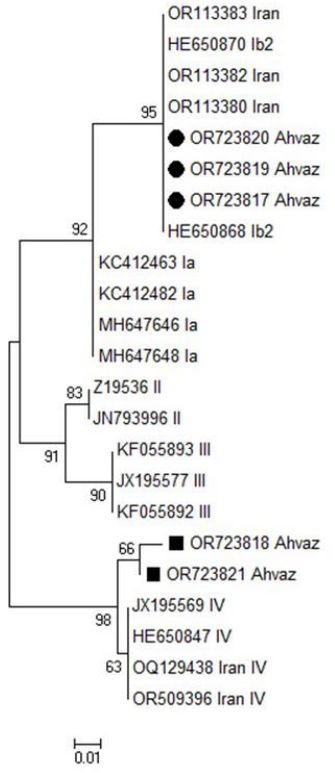

Fig. 1. The phylogenetic tree was constructed utilizing the Maximum Likelihood Method for VP1 sequences derived from BK virus genome isolates obtained from wastewater samples. The BK Virus isolates from Ahvaz (OR723817, OR723819, OR723820) were found to cluster with the BK genotype 1b2, alongside the BK virus genotype 1b2 (HE650868) isolate from Kuwait, as well as the OR113380, OR113382, and OR113383 isolates from Ahvaz, Iran. Conversely, the BKV isolates from Ahvaz (OR723818, OR723821) were observed to cluster with genotype IV (JX195569), which was isolated in Spain, along with HE650847 isolated in Kuwait, and OQ129438 and OR509396 isolated in Iran. The Tamura-Nei model was utilized for the Maximum Likelihood method, incorporating 1000 bootstrap replicates for enhanced statistical reliability. The scale bar was established at 0.01.

wastewater samples revealed that all five NCCR isolates show the archetype with few nucleotides substitution in some blocks of NCCR, when compared with consensus BK virus archetype (AY628236) isolate from urine of a healthy control individual in USA (Fig. 2). The results of BK virus NCCR alignment exhibit 100% homology with the consensus BK virus archetype (AY628236) for wastewater samples isolates OR933745, OR933747, and OR933748, while wastewater samples isolate OR933746 and isolate OR933749 show 99.57% and 97.85% homology with the BK virus archetype strain (AY628236), respectively. Of the 5 isolates archetype variants, the sequences and transcription factors of the three BK virus NCCR boxes, P, Q, R, S, (OR933745, OR933747 and OR933748) found to be 100% homology with the consensus BK virus archetype (AY628236). The nucleotide substitution at position C6G was observed in Q box of BKV NCCR isolate (OR933746). The R box of the isolate OR933749 demonstrates three nucleotide substitutions: G9A, A11C, and G46T. The first two substitutions were not found in the transcription factors, whereas G46T was observed in transcription factor NFI-3. Moreover, the S box of isolate OR933749 reveals two nucleotide substitutions: G19A, observed in transcription factor p53, and G25A, which was not found in the transcription factor region (Fig. 2).

DISCUSSION

The surveillance of wastewater has emerged as an essential approach for tracking the propagation of viruses within populations, as well as for anticipating potential outbreaks of viral illnesses.

The monitoring of wastewater is especially crucial for viruses that cause subclinical infections (16), considering the examination of JCV and BKV in wastewater may serve as a valuable method for detecting human-derived fecal contamination (1).

The global seroprevalence rate of BK virus has been reported to be 80% (9). BKPyV can be classified into four predominant genotypes (BKPyV I, II, III, and IV) predicated upon neutralization assays and the distinct sequences within the principal BKPyV capsid gene VP1 (14). Nonetheless, contemporary genotyping methodologies predominantly utilize VP1- and LTAG-sequences to delineate BKPyV subgroups Ia, Ib1, Ib2, Ic, II, III, IVa1, IVa2, IVb1, IVb2, IVc1, and IVc2 (11).

The prevalence of BKV DNA in the urine samples of healthy individuals were reported in different regions of the world. the frequency of BKV DNA in urine samples was in Ahvaz city, Iran (41.8%) (17), Brasilia (12.5%) (18), Tunisia (6%) (19). BKV has been mainly interlinked renal failure with BKV-associated nephropathy (BKVAN) in kidney-transplant recipients (8) and hemorrhagic cystitis (HC) (4) in hematopoietic stem cell transplant recipients (HSCTRs) (20, 21).

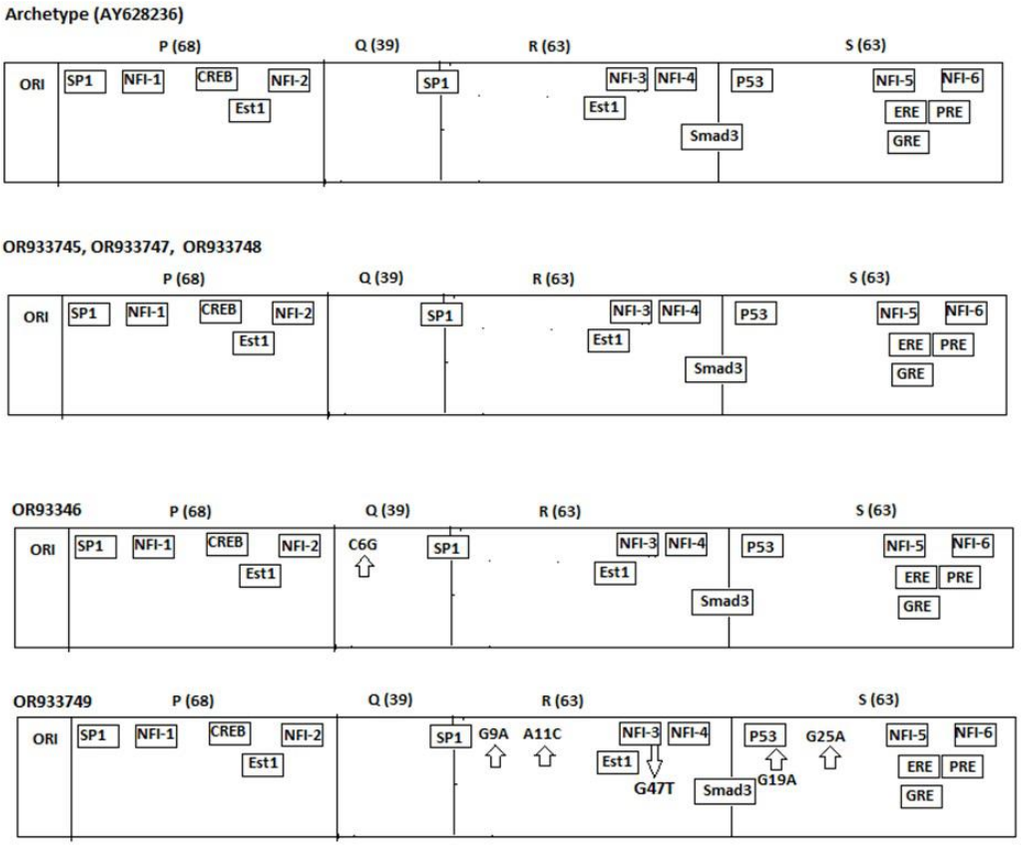

Fig. 2. Illustrates that isolates OR933745, OR933747, and OR933748 exhibited 100% homology with the consensus BK virus archetype (AY628236) isolated in the USA. The Q box of isolate OR933746 presents a nucleotide substitution at position C6G; this mutation was not observed in the transcription factor region of the Q box. The R box of isolate OR933749 demonstrates three nucleotide substitutions: G9A, A11C, and G46T. The G46T substitution was observed in transcription factor NFI-3, while the others were not found in the transcription factors. The S box of isolate OR933749 reveals two nucleotide substitutions: G19A, which was observed in transcription factor p53, and G25A, which was not found in the transcription factor regions of the S box.

BKV has been correlated with kidney and bladder cancer (8), head and neck cancers (22), BK virus association in HIV-associated salivary gland disease (HIVSGD) (9), and colorectal cancer (23).

In the absence of effective treatments, management focuses on adjusting immunosuppressive therapies during viral reactivation or graft injury. Early detection of viral reactivation is vital to reduce the risk of allograft rejection, especially soon after kidney transplantation. Current screening relies on quantifying plasma BKPyV DNA via PCR. The International Consensus 2024 guidelines advocate for monthly BKPyV DNA screening in kidney transplant recipients for nine months, followed by every three months screenings until the second year (or third year for pediatric cases) (24).

In the present investigation, a high rate of BK virus DNA was identified in 53.33% of urban wastewater samples. The relative prevalence of BK virus across various wastewater samples reinforces its potential for transmission through the fecal-oral route (6). BK virus has been identified in urban wastewater across diverse geographical regions, including Egypt (Ahmed et al. 2019) (25), and Pakistan (Ijaz et al. 2023) (26).

It is remarkable that fecal matter and wastewater encompass a multitude of pharmaceuticals, industrial discharge, heavy metals, reagents, complex polysaccharides, lipids, proteins, metal ions, and RNases, which can inhibit PCR amplification by several mechanisms. These mechanisms include the inhibition of DNA polymerase activity, degradation or sequestration of target nucleic acids, fluorescent signaling, and chelation of essential metal ions necessary

for amplification (27, 28). We diluted each positive BKV sample (e.g., 1:10) to confirm that inhibition does not mask true positives (29, 30).

The phylogenetic tree analyses revealed that the BK virus isolated from Ahvaz corresponds to the sequences, specifically OR723817, OR723819, and OR723820 were found to cluster with the BK virus genotype 1b2 isolate from Kuwait (HE650868), and the OR113380, OR113382, and OR113383 isolates from Ahvaz, Iran. Conversely, the BKV isolates from Ahvaz (OR723818, OR723821) were observed to cluster with genotype IV (JX195569), which was isolated in Spain, along with HE650847 isolated in Kuwait, and OQ129438 and OR509396 isolated in Iran. Within this geographical context, the BK genotype 1b2 was identified as the predominant strain, followed by genotype IV. It is noteworthy that both BK genotypes 1b2 and IV were identified in urine and tumor tissues of patients diagnosed with colorectal cancer within this region (23).

The findings of the BK virus NCCR alignment reveal that the wastewater sample isolates OR933745, OR933747, and OR933748 demonstrate complete homology (100%) with the consensus BK virus archetype (AY628236) isolated in USA, whereas the wastewater sample isolates OR933746 and OR933749 exhibit homologies of 99.57% and 97.85% with the BK virus archetype strain (AY628236), respectively. The nucleotide substitution at position C6G was observed in Q box of BKV NCCR isolate (OR933746). The R box of isolate OR933749 exhibits three nucleotide substitutions: G9A, A11C, and G46T. While G9A and A11C were not found in transcription factors, G46T was observed in transcription factor NFI-3. The S box of isolate OR933749 reveals two nucleotide substitutions: G19A, found in transcription factor p53, and G25A, which was not found in the transcription factor region. The transcription factor binding sites (TFBSs) affect viral replication; a mutation in a Sp1 site that can hinder binding and modify viral gene expression, enabling the viral strain to adopt features of a BKV strain with a rearranged NCCR (31, 32). The O block harbors the origin of replication, yet various TFBSs, including Sp1, NF1, NF-kB, and Ets-1, are distributed throughout the NCCR and regulate BKV gene transcription (31, 32). Six Nuclear Factor I (NFI) binding sites (NFI-1 to NFI-6), acting as viral enhancers, occur in sequences flanking the late side of the origin. Their mutation in BKV NCCRs boxes reduced BKV DNA (33).

NFI-3 has been associated with the regulation of early-late transcription (33). The role of BK virus p53 within the NCCR's S box is influenced by interactions with other transcription factors, like Sp-1 and NF-1, which may modify its transactivation or repression capabilities in infected cells (34). Numerous studies have indicated that nucleotide substitutions or rearrangements within the BK virus NCCR box result in enhanced replication of the BK virus genome and play a pivotal role in the pathogenesis associated with BK virus (9). The activation of BK virus (BKV) is typically associated with the rearrangement of the Non-Coding Control Region (NCCR), wherein the deletion or insertion of Transcription Factor Binding Sites (TFBS) modulates the expression of Early Viral Gene Regulatory (EVGR) and Late Viral Gene Regulatory (LVGR) elements (33, 34).

Cloning of 10 rr-NCCRs demonstrated various duplications or deletions that enhanced gene expression, replication capacity, and cytopathology of recombinant BKV in vitro (35). The BKV NCCRs from throat wash samples of individuals with HIV-associated systemic disease (HIVSGD) displayed a specific block arrangement termed “OPQPQQS” in immunosuppressed patients (9). The expression of Large T Ag (TAg) was found to inactivate P53 and retinoblastoma, contributing to oncogenesis (8). Additionally, BKV encodes two miRNAs, 5p-miRNA and 3p-miRNA, that are perfectly complementary to the mRNA of Large T Ag (Tag). BKPyV-infected bladder cancer cells exhibited enhanced proliferation alongside increased expression of miR-B1–3p and −5p (36).

The BK virus and JC virus are categorized as potentially carcinogenic infectious agents for humans (8). Indeed, the classified BKPyV and JCPyV as 2B are regarded as potential oncogenic viruses (37). Consequently, the eradication of BK virus should be prioritized as a primary consideration by environmental authorities in the context of wastewater treatment facilities.

Various methodologies, including skimmed milk flocculation, polyethylene glycol precipitation (PEG), glass wool filtration, ultrafiltration, virus adsorption, and elution have been documented for the concentration of viruses (11, 12). We implemented techniques for viral concentration utilizing PEG, which is straightforward, economically viable, and effective, as previously reported (13).

CONCLUSION

The results of the present investigation demonstrated that 32 (53.33%) wastewater specimens tested positive for the BK virus. The phylogenetic assessment reveals a predominance of BK virus genotype Ib2, followed by genotype 4. These findings pertaining to the BK virus non-coding control region (NCCR) indicated that three BK virus isolates were identified as archetype strains, whereas the Q box of isolate OR933746 exhibits a nucleotide substitution at position C6G; concurrently, the R box of isolate OR933749 displays multiple nucleotide substitutions at positions G9A, A11C, and G46T, while the S box of the same isolate OR933749 shows nucleotide substitutions at positions G19A and G25A, which cannot significantly contribute to the pathogenesis of the BK virus. Consequently, the eradication of BK virus should be prioritized as a primary consideration by environmental authorities in the context of wastewater treatment facilities.

Membrane technology is recognized for its efficacy in eliminating emerging viruses and antimicrobial-resistant genes from wastewater. Microfiltration is widely utilized in commercial membrane filtration to remove protozoa and bacteria (38). Sequential UV-chlorine disinfection enhances virus inactivation synergistically (39). Ultraviolet C radiation efficiently eradicates microorganisms within the 200–280 nm range. The UV disinfection mechanism involves the formation of pyrimidine dimers, which obstruct RNA and DNA replication. Chlorine disinfection operates by disrupting enzyme structures essential for bacterial and viral survival. It is notably effective in deactivating both bacteria and viruses (40). It is notable, the sewage plant treatment should be implemented to remove pathogenic infectious agents especially BK virus, and to restrain the circulation of BK virus in humans and the environment.

ACKNOWLEDGEMENTS

We acknowledge the financial support and approval of this study by Environmental Technologies Research Center, Medical Basic Sciences Research Institute, Department of Environmental Health Engineering Ahvaz Jundishapur University of Medical Sciences Ahvaz, Iran with the registration number ETRC-0216 and ethics code number (IR.AJUMS.REC.1402.353).

REFERENCES

1. Shaheen MNF, Ahmed N, Rady Badr K, Elmahdy EM. Detection and quantification of adenovirus, polyomavirus, and papillomavirus in urban sewage. *J Water Health* 2024; 22: 401-413.
2. Rashed MK, El-Senousy WM, Sayed ETAE, AlKhazindar M. Infectious pepper mild mottle virus and human adenoviruses as viral indices in sewage and water samples. *Food Environ Virol* 2022; 14: 246-257.
3. Ambalathingal GR, Francis RS, Smyth MJ, Smith C, Khanna R. BK polyomavirus: clinical aspects, immune regulation, and emerging therapies. *Clin Microbiol Rev* 2017; 30: 503-528.
4. Alcendor DJ. BK polyomavirus virus glomerular tropism: implications for virus reactivation from latency and amplification during immunosuppression. *J Clin Med* 2019; 8: 1477.
5. Kotla SK, Kadambi PV, Hendricks AR, Rojas R. BK polyomavirus-pathogen, paradigm and puzzle. *Nephrol Dial Transplant* 2021; 36: 587-593.
6. Krajewski W, Kamińska D, Poterek A, Małkiewicz B, Kłak J, Zdrojowy R, et al. Pathogenicity of BK virus on the urinary system. *Cent European J Urol* 2020; 73: 94-103.
7. Morel V, Martin E, François C, Helle F, Faucher J, Mourez T, et al. A simple and reliable strategy for BK virus subtyping and subgrouping. *J Clin Microbiol* 2017; 55: 1177-1185.
8. Zhou X, Zhu C, Li H. BK polyomavirus: latency, reactivation, diseases and tumorigenesis. *Front Cell Infect Microbiol* 2023; 13: 1263983.
9. Burger-Calderon R, Ramsey KJ, Dolittle-Hall JM, Seaman WT, Jeffers-Francis LK, Tesfu D, et al. Distinct BK polyomavirus non-coding control region (NCCR) variants in oral fluids of HIV- associated Salivary Gland Disease patients. *Virology* 2016; 493: 255-266.
10. Sahragard I, Yaghobi R, Mohammadi A, Afshari A, Pakfetrat M, Karimi MH, et al. Impact of BK Polyomavirus NCCR variations in post kidney transplant outcomes. *Gene* 2024; 913: 148376.
11. Lu D, Huang Z, Luo J, Zhang X, Sha S. Primary concentration–the critical step in implementing the wastewater-based epidemiology for the COVID-19 pandemic: a mini-review. *Sci Total Environ* 2020; 747: 141245.
12. WHO. In: Guidelines for Environmental Surveillance of Poliovirus Circulation. Biologicals DoVa, editor.

World Health Organization Switzerland 2003.
13. Amdiouni H, Faouzi A, Fariat N, Hassar M, Soukri A, Nourlil J. Nourlil. Detection and molecular identification of human adenoviruses and enteroviruses in wastewater from Morocco. *Lett Appl Microbiol* 2012; 54: 359-366.
14. Anzivino E, Bellizzi A, Mitterhofer AP, Tinti F, Barile M, Colosimo MT, et al. Early monitoring of the human polyomavirus BK replication and sequencing analysis in a cohort of adult kidney transplant patients treated with basiliximab. *Virol J* 2011; 8: 407.
15. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, et al. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 2009; 199: 837-846.
16. Carmo Dos Santos M, Cerqueira Silva AC, Dos Reis Teixeira C, Pinheiro Macedo Prazeres F, Fernandes Dos Santos R, de Araújo Rolo C, et al. Wastewater surveillance for viral pathogens: a tool for public health. *Heliyon* 2024; 10(13): e33873.
17. Kaydani GA, Makvandi M, Samarbafzadeh A, Shahbazian H, Hamidi Fard M. Prevalence and distribution of BK virus subtypes in renal transplant recipients referred to Golestan Hospital in Ahvaz, Iran. *Jundishapur J Microbiol* 2015; 8(3): e16738.
18. Machado DM, Fink MC, Pannuti CS, Succi RC, Machado AA, Carmo FB, et al. Human polyomaviruses JC and BK in the urine of Brazilian children and adolescents vertically infected by HIV. *Mem Inst Oswaldo Cruz* 2011; 106: 931-935.
19. Boukoum H, Nahdi I, Sahtout W, Skiri H, Segondy M, Aouni M. BK and JC virus infections in healthy patients compared to kidney transplant recipients in Tunisia. *Microb Pathog* 2016; 97: 204-208.
20. Laskin BL, Denburg MR, Furth SL, Moatz T, Altrich M, Kleiboeker S, et al. The natural history of BK polyomavirus and the host immune response after stem cell transplantation. *Clin Infect Dis* 2020; 71: 3044-3054.
21. Peras M, Bilić E, Mareković I. Recent Insights into the Pathogenesis, Diagnostics, and Treatment of BK Virus Infections in Children After Hematopoietic Stem Cell Transplantation. *Pathogens* 2025; 14: 236.
22. Mody MD, Rocco JW, Yom SS, Haddad RI, Saba NF. Head and neck cancer. *Lancet* 2021; 398: 2289-2299.
23. Javadi M, Kaydani G, Pirmoradi R, Talaiezadeh A, Navand AH, Ahmadi MKB, et al. Frequency of BK virus genotypes in patients with colorectal cancer. *Iran J Microbiol* 2025; 17: 137-143.
24. Nourie N, Boueri C, Tran Minh H, Divard G, Lefaucheur C, Salmona M, et al. BK Polyomavirus Infection in Kidney Transplantation: A Comprehensive Review of Current Challenges and Future Directions. *Int J Mol Sci* 2024; 25: 12801.
25. Ahmed NI, Elmahdy EM, Allayh AK, Mohamed El-CB, Loutfy SA, Barakat A, et al. Prevalence of human polyomavirus and papillomavirus in wastewater and in stool of Egyptian patients. *Egypt J Aquat Biol Fish* 2019; 23: 29-41.
26. Ijaz R, Shahzad N, Farhan Ul Haque M. Detection of BK and JC polyomaviruses in sewage water of the urban areas of Lahore, Pakistan. *Biologia (Bratisl)* 2023; 1-8.
27. Kumblathan T, Liu Y, Uppal GK, Hrudey SE, Li XF. Wastewater-Based Epidemiology for Community Monitoring of SARS-CoV-2: Progress and Challenges. *ACS Environ Au* 2021; 1: 18-31.
28. Sims N, Kasprzyk-Hordern B. Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. *Environ Int* 2020; 139: 105689.
29. Yan L, Toohey-Kurth KL, Crossley BM, Bai J, Glaser AL, Tallmadge RL, et al. Inhibition monitoring in veterinary molecular testing. *J Vet Diagn Invest* 2020; 32: 758-766.
30. Schrader C, Schielke A, Ellerbroek L, Johne R. PCR inhibitors - occurrence, properties and removal. *J Appl Microbiol* 2012; 113: 1014-1026.
31. Bethge T, Ajuh E, Hirsch HH. Imperfect symmetry of Sp1 and core promoter sequences regulates early and late virus gene expression of the bidirectional BK polyomavirus noncoding control region. *J Virol* 2016; 90: 10083-10101.
32. Walder TA, Odegard EA, Meeds HL, Kleiboeker SB, Ziady A, Sabulski A, et al. Single-nucleotide polymorphisms within the BK polyomavirus non-coding control region are genotype-associated. *Microbiol Spectr* 2025; 13(8): e0049425.
33. Liang B, Tikhanovich I, Nasheuer HP, Folk WR. Stimulation of BK virus DNA replication by NFI family transcription factors. *J Virol* 2012; 86: 3264-3275.
34. Bethge T, Hachemi HA, Manzetti J, Gosert R, Schaffner W, Hirsch HH. Sp1 sites in the noncoding control region of BK polyomavirus are key regulators of bidirectional viral early and late gene expression. *J Virol* 2015; 89: 3396-3411.
35. Gosert R, Rinaldo CH, Funk GA, Egli A, Ramos E, Drachenberg CB, et al. Polyomavirus BK with rearranged noncoding control region emerge in vivo in renal transplant patients and increase viral replication and cytopathology. *J Exp Med* 2008; 205: 841-852.
36. Li YJ, Chen YH, Wang JW, Wu HH, Hsu HH, Ho DR, et al. Suppression of cisplatin induced ATF3 expression and apoptosis by BK polyomavirus and its encoded microRNA in bladder cancer cells. *Biomed Pharmacother* 2025; 186: 118032.
37. Galati L, Chiantore MV, Marinaro M, Di Bonito P. Hu-

man oncogenic viruses: characteristics and prevention Strategies—Lessons learned from human papillomaviruses. *Viruses* 2024; 16: 416.

38. Wang R, Xiao M, Furusho H, Kodera N, Hoek EM, Honda R. Enhancing Adsorptive Removal of Viruses and Extracellular Antimicrobial Resistance Genes in Microfiltration: Impacts of Membrane Materials and Solution Chemistry. *ACS ES T Water* 2025; 5: 5079-5087.
39. Guerrero-Latorre L, Gonzales-Gustavson E, Hundesa A, Sommer R, Rosina G. UV disinfection and flocculation-chlorination sachets to reduce hepatitis E virus in drinking water. *Int J Hyg Environ Health* 2016; 219: 405-411.
40. Shi Q, Chen Z, Liu H, Lu Y, Li K, Shi Y, et al. Efficient synergistic disinfection by ozone, ultraviolet irradiation and chlorine in secondary effluents. *Sci Total Environ* 2021; 758: 143641.