Health Risk Assessment Associated with Norovirus Incidence in Raw Wastewater in Jeddah, Saudi Arabia

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Abstract: Norovirus caused an epidemic gastroenteritis in humans. It can be transmitted by the fecal-oral and the aerosol route. Norovirus represent a most common cause of acute gastroenteritis which responsible about 42%-96% of nonbacterial gastroenteritis worldwide. Current study aims to detect a norovirus in Jeddah wastewater. A total one hundred of wastewater samples were collected from outlet of Al-Misk Lake east of Jeddah city over a period of fourteen months from January 2009 to February 2010. All samples were filtered and virus concentrated and screened for GII human. A molecular in-house detection was performed using nRT-PCR. The most conserved regions; N32, N33, N35 and N36 were used for primers design. Of 19 positive samples were signaled a band of 338bp. In conclusion, this study revealed that the norovirus was frequently present in Jeddah wastewater, which should be alert to do not use this water in land irrigation.

Key words: wastewater, norovirus, detection, nRT-PCR.

INTRODUCTION

Water virology started around half a century ago, with scientists attempting to detect poliovirus in water samples. Since that time, other enteric viruses responsible for gastroenteritis such as: rotaviruses (RVs), Adenoviruses (AVs), Hepatitis A viruses (HAVs) and Noroviruses (NVs) have replaced enteroviruses as the main target for detection in the water environment (Bosch, 1998 and Redwan, et al. 2008). NV is recognized as the second most common cause of infectious nonbacterial gastroenteritis among persons of all ages (Kamel et al., 2009 and Widdowson et al., 2005). NVs have frequently been associated with gastroenteritis in the elderly were between 70 and 90 years of age, especially those living in institutional settings such as nursing homes (Hedlund et al., 2000 and Dedman et al., 1998). Also, NVs are shed into stool samples of children ©10 years of age with diarrhea (Soares et al., 2007). Infections with NVs may lead to death in immunocompromised persons (Widdowson et al., 2005).

Rivers water has been frequently and inevitably contaminated with human enteric viruses via the discharge of untreated domestic and industrial wastewater. The presence of enteric viruses in aquatic environments is an emerging issue because the viral infection can be caused by ingestion even at low concentrations (Keswick et al., 1985, Lee et al. 2011). Norovirus is a cause of epidemic acute gastroenteritis. This virus belongs to a single-stranded RNA virus which has been classified as belonging to the family Caliciviridae. It has been reported that cases of norovirus outbreaks unexpectedly increased in Europe and the United States during the winter season of 2002–2008 (Kamel et al., 2009). NVs in the environmental condition are stable and can survive under power of hydrogen (pH, 3-10) for prolong time at low temperatures. Viruses have been detected from water such as: seawater, freshwater, wastewater, food and soil (Fong and Lipp, 2005 and Green et al., 1998).

Since no cell culture technique is as yet adequate for detection of human noroviruses, and beyond, the classical viral detection methods, the DNA based method, namely reverse transcription-nested PCR (RT nested PCR), is used for sensitive detection. Recently, RT-PCR has been successfully used for enteric virus detection in shellfish and water samples (Suñén and Sobsey, 1999). This method has various types; Nested RT-PCR (nRT-PCR) is one of highly specific and sensitive assay to use in noroviruses detection (Tong et al., 2011), which will harness in current study.

The current study aims to determine the incidence of NVs in wastewater of Jeddah city and to depict the seasonal variation of the NVs load.

MATERIALS AND METHODS

Wastewater Samples Collection:

A total of one hundred wastewater samples, 2 Liters each, were collected in plastic gallons from the outlet (Fig. 1) of the wastewater lake (previously called Al-Misk Lake) at the east of Jeddah city, Saudi Arabia. Two samples were collected weekly in two different days from different outlet. Samples were collected over a period of fourteen months starting in January 2009 to February 2010.
Concentrating And Re-Concentrating Viruses From Wastewater Samples:

Viruses were recovered from the collected wastewater samples using modified Adsorption-Elution technique according to Katzenelson et al., 1976. Each wastewater sample was first clarified to remove large particles and then passed through two layers of filter membranes. The first was the Whatman filter paper; the second was 0.45 μm nitrocellulose membrane. The adsorbed viral particles were eluted from nitrocellulose membrane by adding 70 ml of 3% B.E. (3% Beef Extract) pH 9.5. The 70 ml eluate was re-concentrated by organic flocculation method as follows. The pH of the eluate was adjusted to pH 3.5 with HCl, then mixed at room temperature for 30 min. The product was centrifuged by the ultracentrifugation system at 1680xg for 15 min at 4°C. The pellet was suspended in 4 ml of (0.45N) Na2HPO4 7.5 pH, then the samples were concentrated followed by storage at -20°C until use.

Detection Of HNV Gene:

Extraction Of Hnvs RNA From Concentrated Sample:

Viral RNA in the concentrated wastewater samples were extracted using the QIAamp® Viral RNA Mini Kit, according to the manufacturer’s instructions. The resulting RNA concentrate was stored at -80°C until used.

Detection Of HNV By Nrt-PCR:

NRT-PCR amplification reactions were performed using the QIAGEN® One step RT-PCR Kit; a method was done according to Schreier et al. (2000). The reaction mixture 1 was prepared. The reaction was carried out with an initial RT step at 42°C for 1 h, followed by PCR activation at 95°C for 15 min for a hot star and initial denaturation step. 35 cycles of amplification, 94°C for 30 s for denaturation, 42°C for 30 s for annealing, 72°C for 45 s for extension and a final extension at 72°C for 5 min for first round. PCR products were then prepared to the second round PCR that carried out with the same above program. A positive and a negative control were included in every run.

Visualization Of Nrt-PCR Product:

The final nRT-PCR product (338 bp fragment of the VP60 gene) were separated by 2% agarose gel electrophoresis at 70 voltage, stained with ethedium bromide (0.5 µg/ml) for 30-45min. The resolved bands were visualized under ultra violet-transilluminator light (Schreier et al., 2000) on the gel documentation system and bands were analyzed in comparison to the 100 bp DNA ladder and positive control (Norovirus NV-GII was kindly gifted of Dr. Marina Höhne, Robert Koch Institute, Berlin, Germany), which was previously identified cited in GeneBank under accession # AB039777).

Results:

Screening Of Hnv Genome From Wastewater Samples:

A total of 100 wastewater samples were collected during 28 January 2009 to 25 February 2010 from two outlets of Al-Misk Lake. All samples were investigated for the presence of NV using molecular technique. Amplification of HNV genome was carried out using nRT-PCR and specific primers. nRT-PCR product (338bp DNA fragment) was analysis in agarose electrophoresis. Results were confirmed by using reference sample of NV-GII as positive control, which is proceeded through extraction using QIA gene kit and in parallel with the DNA ladder included in each nRT-PCR. NV was detected in 19 samples (1, 10, 23, 24, 42, 48, 56, 57, 58, 65, 72, 73, 79, 80, 82, 87, 88, 90 and 98) as depicted in Fig. (2a and 2b).
Fig. 2 (a&b): Agarose gel (2%) showing the positive samples of the nRT-PCR products with 338 bp fragment length on the gel electrophoresis. Ladder 1Kbp ladder, PC (Positive control, stool sample contained NV GII as positive control, which is proceeded through extraction using QIA gene kit and in parallel with the DNA ladder included in each nRT-PCR). 19 Positive samples (1, 10, 23, 24, 42, 48, 56, 57, 58, 65, 72, 73, 79, 80, 82, 87, 88, 90 and 98).

Seasonality Of NV Infection:

The result of this study revealed that the norovirus is present in the wastewater of Al-Misk Lake. Accordingly, the date collected for the positive samples are distributed as follows; four positive samples were collected in January, one sample was collected in February, one sample was collected in March, two samples collected in April, one sample was collected in June, one sample was collected in July, three samples were collected in August, one samples was collected in September, two samples were collected in October, one sample collected in November, two samples were collected in December. These data demonstrate that the NV could be distributed in winter and summer months as well throughout the year off 2009 (Fig. 3).

Fig. 3: Positive samples of Norovirus distributed in wastewater collected over January 2009 to February 2010, each point represent a triplicate experiment.
Discussion:

Enteric viruses may be present naturally in aquatic environments and can be transported in the environment through groundwater, seawater, aerosols emitted from sewage treatment plants, insufficiently treated water and private wells that receive treated or untreated wastewater either directly or indirectly (Fong and Lipp, 2005). Since NV is mainly waterborne viruses and the infectious dose has been reported to be low (Jothikumar et al., 2005), methods proposed for detection of viruses from water generally imply two steps: a viral concentration procedure by membrane filtration, followed by a secondary concentration step, and a detection step using RT-PCR, especially for NVs that unable to multiply in cell cultures (Gassilloud et al., 2007).

A good concentration method should fulfill several requirements: it should be technically simple, fast, provide high virus recoveries, be adequate for a wide range of enteric viruses, provide a small volume of concentrate, and be inexpensive (Bosch et al., 2008), but with a large volume of water the second step becomes necessary (Katzenelson et al., 1976). The ideal method for virus concentration from water should be capable of processing large volumes of a variety of waters in the least possible time, sensitive enough to concentrate most types of viruses known to be present in water and wastewater, easy to perform and economical to use, and able to detect viral aggregates and viruses adsorbed to suspended solids (Gerba and Goyal, 1982).

Adsorption-elution of viruses with an electropositive filter is one of the most commonly used techniques and is the method for recovery of enteric viruses from water. Under ambient conditions, enteric viruses are negatively charged and will adsorb to a positively charged membrane under acidic conditions. In addition, as a second concentration method an organic flocculation method commonly used 3% beef extract with high pH (pH 9.5) is the most widely used to elute absorbed viruses from filters and gives a high viral recovery. In organic flocculation, buffered beef extract is used to precipitate viruses from concentrated samples by lowering pH of a protein solution to 3.5 (Fong and Lipp, 2005; Lakhe and Paunikar, 2002 and Katzenelson et al., 1976).

Based on NV genetic divergence in regions of the RdRp and VP60 (Hardy, 2005 and Boga et al., 2004), the primers for NV were chosen to amplify sequence of a 338 bp which represent? RdRp and the major capsid protein (VP60) (Boga et al., 2004) coding gene corresponding to nucleotides 4226-4707 for first round using NV 32 and NV 36 primers and 338 bp fragment in the position 4280-4617 for the second round using NV 35 and NV 33 primers in the same region Norwalk virus genome strain (accession number X86557) (Schmid et al., 2004; Oh et al., 2003 and Schreier et al., 2000).

Today, RT-PCR technique has become the standard for diagnosis of NV infection worldwide; among the problems with traditional RT-PCR has been the inability to enumerate viruses (Jothikumar et al., 2005). Recently, most reported conventional RT-PCR assays have been modified, because it is unable to detect all NVs, to increase specificity, sensitivity and efficiency (Fong and Lipp, 2005 and O'Neill et al., 2002).

NRT-PCR assays, with the use of an internal primer or primer set, have been successfully employed for amplification of low levels of virus found in naturally contaminated food and water (O'Neill et al., 2001; Oh et al., 2003 and Schreier et al., 2000). This method is one of highly specific and sensitive assays. I used this technique in this study, to detect NV from wastewater samples.

In this study, NV was screened in wastewater collected from the outlet of the wastewater Lake of Jeddah city. HNV was detected in 19 out of 100 (19%) wastewater samples by using nRT-PCR. Our results were concordant with Pusch et al. (2005) who recorded 44%, 53%, 15% and 18% of positive NV samples in German mining lake wastewater samples by using nRT-PCR technique. Moreover, nRT-PCR was the essential technique for detection NVs from stool samples in several studies, O'Neill et al. (2002) has been detected NVs in 17 (16.8%) of 101 stool samples. Also, Oh et al., 2003 results revealed 70 (32%) of 217 stool samples were positive. In addition, NVs was detected in 26 (50%) of 52 stool samples (Schmid et al., 2004) and detected in 16 (18.8%) of 85 stool samples (Medici et al., 2005). Zahorsky (1929) suggested the first described of NVs characterized by the winter vomiting disease. Also, a review of 12 studies from 1978 to 1998 around the world showed that NVs was seen during winter months in 10 surveys (Mounts et al., 2000), which clearly demonstrate that the NVs infection distribution is not restricted on a specific time and/or environmental factors (Steyera et al., 2011). While, Lopman et al. (2003) b in his study conducted that NVs infection exclusively have wintertime seasonality. Also, in local studies in Saudi Arabia, during the winter season of the years 2004 and 2005 a study at Al-Qassim shown that 13 (9.2%) of 142 stool samples were belonged to NV (Meqdam and Thwiny, 2007). On the other hand, current results are agreed with previous study (Tayeb et al., 2008), which estimated of 9 (3.5%) out 253 stool samples were positive for NVs.

Conclusions:

HNVs are one of the most common etiologic agents causing acute nonbacterial gastroenteritis worldwide. This study showed that NV presents in 19% of wastewater samples collected from Wastewater Lake in Jeddah city and throughout the years 2009- 2010. In addition, HNV could appear in winter and summer months throughout the year off 2009.
Recommendations:

Our study highly recommends treating wastewater using sufficient methods to avoid underground water sources contamination. Also, community education about NV infection has been recommended, especially at nursing homes to protect ourselves from infections. Consumer education is useful to reduce consumption of illegally harvested, and to increase awareness of the hazards associated with eating raw foods. Fresh fruits and vegetables may also be contaminated with NVs during production or processing prior to distribution. Contaminated irrigation water or wash water can transfer NVs to fresh products, and surrogate viruses have been shown to attach and persist on fruit and vegetable surfaces. Finally, Ministry of Health has to enroll the NVs diagnosis in parallel to the other enteric viruses detection, because there is no vaccine available until now, and the virus was detected especially in acute gastroenteritis infections of all age groups. However, substantial outbreaks continually occur in developed as well as developing countries. The produce-related illnesses cost United States up to $39 billion annually (Lee et al., 2011, Steyera et al 2011).

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REFERENCES


