African Journal of Microbiology Research Vol. 5(18), pp. 2733-2736, 16 September, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.541
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Molecular characterization of norovirus from acute gastroenteritis patients in Malaysia

H. Zuridah[1,2]*, Sufiyan Hadi M[1], L. K. Teh[2] and A. H. Zed Zakari[1]

[1]Faculty of Health Sciences, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.
[2]Pharmacogenomic Centre (PROMISE), Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.

Accepted 30 July, 2011

Rotavirus and recently norovirus have been described as important and most common cause of acute gastroenteritis in children. The mode of transmission is fecal-oral. Diagnosis of both of these viruses can be made by rapid antigen detection of the viruses in stool specimens and strains can be further characterized by enzyme immunoassay or reverse transcriptase polymerase chain reaction. Stool specimens collected from various hospitals in Malaysia were examined for norovirus by both immunochromatography and reverse-transcription polymerase chain reaction techniques. Rotavirus antigen was screened by a commercially available latex agglutination test kit. Altogether, 168 stool samples were collected for both norovirus and rotavirus screening. Out of these, 77/168 (45.8%) were examined for rotavirus with 17/77 (22%) rotavirus antigen positive. Due to very small amount of fecal materials obtained in some cases, only 151/168 (89.8%) were sufficient for norovirus and 14/151 (9.3%) were positive for norovirus genogroup II (GII). This study highlights that rotavirus remains the main agent for acute gastroenteritis and identification for emerging norovirus among the children is becoming important for proper patient management.

Key words: Norovirus, rotavirus, immunochromatography, latex agglutination, RT-PCR, phylogenetic analysis.

INTRODUCTION

Noroviruses (NoV) are the worldwide major cause of viral gastroenteritis in human (Marshall et al., 2003). They belong to the *Norovirus* genus of the *Caliciviridae* family, which also includes the *Sapovirus* genus. NoV which is also called small round structured viruses (SRSVs) are non-enveloped, single-stranded positive sense RNA viruses (Frankhauser et al., 2002). The primary route of NoV transmission is through the fecal-oral route (Alice et al., 2004). Primary infection results from either the consumption of focal contaminated food or water (Rabanaeu et al., 2003). Currently, at least five NoV genogroups (GI, GII, GIII, GIV and GV) are being reported with GI, GII and GIII commonly found in humans and GII the most predominant genogroup causing worldwide acute gastroenteritis infecting persons of all age groups (Frankhauser et al., 2002; Alice et al., 2004; Kirkwood and Bishop, 2001). Outbreaks of gastroenteritis in USA showed that GII strains were the predominant type accounting to 73% in all outbreaks (Frankhauser et al., 2002), 94% of positive specimens in children hospitalized with acute gastroenteritis in Australia (Kirkwood and Bishop, 2001) and 64% in Thailand (Guntapong et al., 2004).

Rotavirus (RV) forms the genus Rotavirus of the *Reoviridae* family which are often associated with children under the age of 5 years, (Parashar et al., 2006), whilst NoV is common in the adults and older children (Kirkwood and Bishop, 2001). However, in Malaysia, the aetiology and frequency of viral gastroenteritis due to NoV is still poorly investigated and understood unlike the vast studies already reported on rotavirus (Zuridah et al., 2010; Yap et al., 1998; Rasool et al., 1993). The aim of our study was to detect for both RV and NoV and further characterization of NoV for epidemiological purposes.

MATERIALS AND METHODS

Stool samples were obtained from 8 different hospitals in Malaysia. These samples were tested for RV antigen using the Rotalex kit

*Corresponding author. E-mail: drzuridah@salam.uitm.edu.my. Tel: +603-32584421. Fax: +603-32584599.

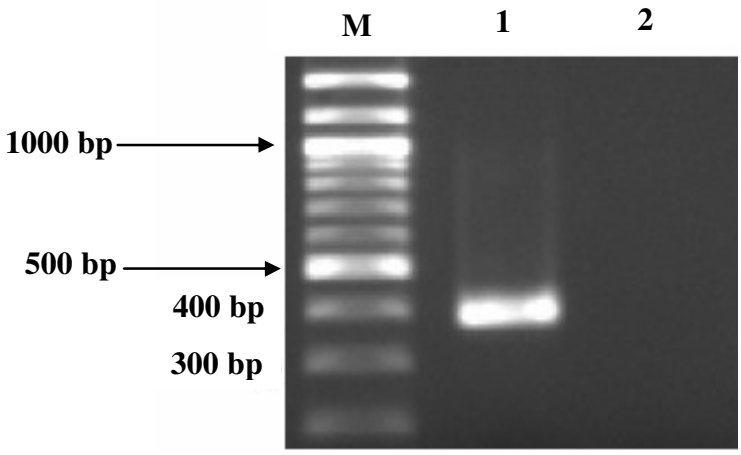

Figure 1. Detection of NoV GII gene from stool samples. M: marker (100 bp ladder); amplicons of NoV GII positive (Lane 1); negative control (Lane 2); PCR product for NoV GII: 379 bp.

(Orion Diagnostica, Finland). For NoV, antigens were screened by immunochromatography (IC) test (The RIDA® QUICK Norovirus, R-Biopharm, Germany) and ELISA (The RIDASCREEN® Norovirus, R-Biopharm, Germany). The IC test is based on the principle that it uses specific monoclonal antibodies directed against NoV capsid protein. Results were interpreted visually after 15 min.

The ELISA is based on monoclonal antibodies bound on the surface of the microwell strips to capture the antigen in stool specimens and samples were assayed accordingly (R-Biopharm, 2006).

For RNA extraction, NoV ssRNA was treated with Ultraspec^{TM}-II RNA isolation system (BioTecx/USA). The eluted ssRNA were then transferred to new clean sterile tubes and frozen until further use (BioTecx/USA). Next, the extracted ssRNA was used as the template for reverse transcription (RT) (Qiagen, Germany protocol). Two microlitre RNA template was added to a 20 µl reaction mixture containing a final concentration of 10X reverse transcription buffer, 2.0 µl of 5 mM dNTPs mixture, 2.0 µl of 10 µM random primers, 1 µl Sensiscript RT, 2 µl of RNase-free water and 10 units/µl RNase inhibitor (Fermentas) and incubated at 37°C for 60 min using the random primers (Qiagen, 2004).

Further amplification was performed using primers NV_OF2 and NV_OR (Bull et al., 2005). Five microlitre of cDNA was added to 20 µl of a PCR mixture containing 2.0 µl of 10X PCR buffer (Fermentas), 1.2 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM dNTPs mix (Promega), 0.24 µl of 50 µM each primer, and 0.4 µl of 2.5 U *Taq* polymerase (Fermentas). Pre-denaturation was carried out at 95°C for 5 min followed by 40 cycles of PCR (95°C for 30 s, 55°C for another 30 s, and 72°C for another final 60 s). Electrophoresis of the amplicons was carried out in 1% agarose gel with 1X TBE buffer with a 100 bp ladder (Fermentas) included.

For phylogenetic studies, all the 14 positive amplicons coded as C7, C4, C11, C12, C14, D1, D3, D6, D7, E1, E2, E8, and E9 were sent to an independent laboratory. The nucleotide sequences obtained were then analyzed using the public database (BLAST algorithm) and an alignment was made with the ClustalW algorithm.

RESULTS

Of the 168 specimens, 77 samples (45.8%) that were screened for RV antigen were from patients less than 5 years old by latex agglutination test. For NoV, not all the 168 samples were screened for NoV. Only 151 samples were screened, of which 14/151 (9.3%) samples were positive.

The amplicons with 379 bp size were confirmed to be NoV genogroup II (GII) (Figure 1). The sequences were compared against the GenBank database using the BLAST programme. Phylogenetic analysis showed that the NoV analyzed was grouped into 2 distinct genotypes GII/4 and GII/3 (Figure 2). GII/4 was the most predominant genotype, accounting for 71.4% (10 of 14), and followed by 29% (4/14) GII/3. All the samples were more than 95% similar to other strains found in Asian countries, especially in Japan. NoV GII gene of patients C7, C4, C11, C12, C14, D1, D3, D6, D7, E1 and E2 were clustered in the same branch with those in Japan (GII/4Nagano). Meanwhile, samples from patients E8 and E9 were clustered in the same branch with strains in Argentina (GII/3Arg320/95/AR).

DISCUSSION

This study describes the incidence and genetic diversity of NoV in diarrheal patients in Malaysia. The incidence found in this study (9.3%) which confirms that infection rates in our study population are comparable to Thailand, Taiwan and Vietnam (14, 8.2, and 5.4%, respectively) (Hansman et al., 2004b; Chen et al., 2007; Hansman et al., 2004a).

The predominant rotavirus infection (22%) was in concordance with previous investigations on the viral etiology of pediatric gastroenteritis (Zuridah et al., 2010; Chen et al., 2007; Zuridah et al., 2009; Nguyen et al., 2007). However, due to sampling volumes, no mixed infections were investigated in this study. This should be pursued in future because many studies have reported

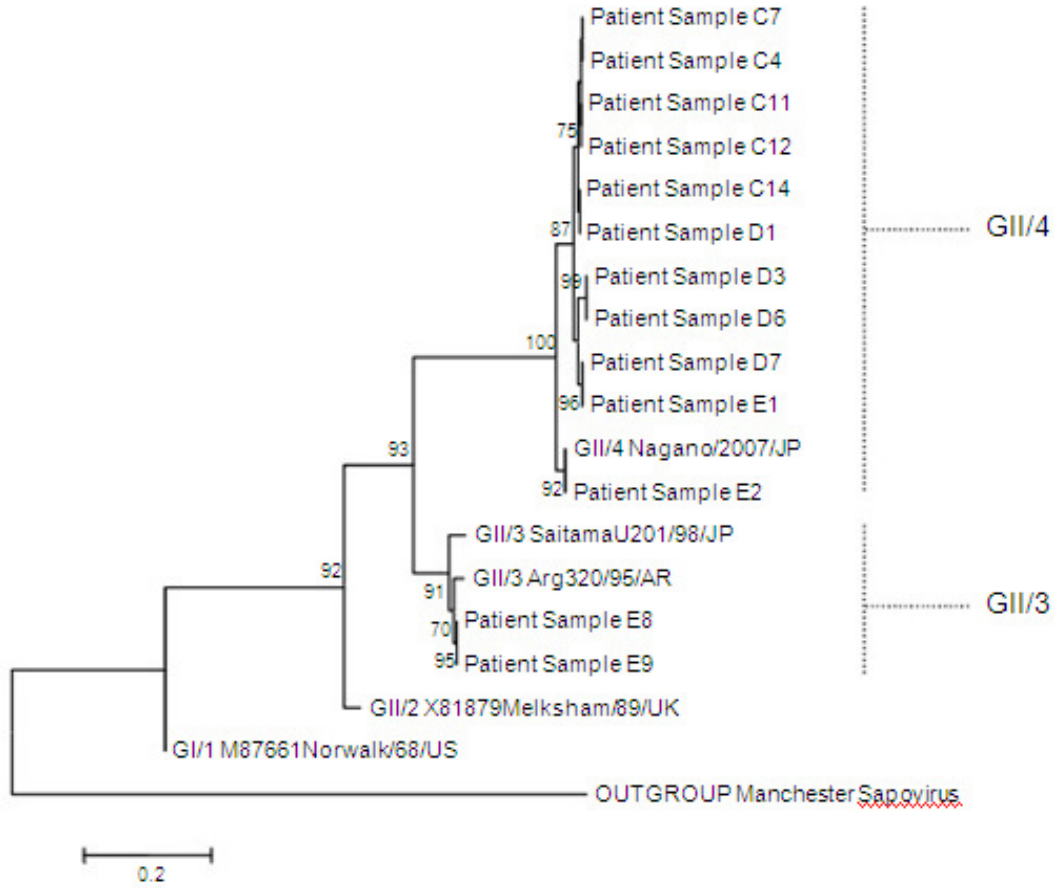

Figure 2. Phylogenetic analysis of NoV local strains (labeled as C7, C4, C11, C12, C14, D1, D3, D6, D7, E1, E2, E8 and E9) and other global strains GII-4 Nagano/2007/JP from Japan, GII-3Saitama from Japan, GII-3Arg from Argentina, GII-2X81879 Melksham from UK, GI/1M87661Norwalk from USA. OUTGROUP Manchester Sapovirus is used as an outgroup control.

that children are vulnerable to several viruses at the same time (Yap et al., 1998). Besides norovirus and rotavirus, adenovirus and astrovirus were also detected in small percentage (Tran et al., 2010).

To our knowledge, this is the first preliminary report on NoV infection in the hospitalized patients in Malaysia. Genetic diversity was observed, encompassing 2 genotypes, namely GII/3 and GII/4 with GII/4 the most prevalent in concordance to other reports worldwide (Nguyen et al., 2007; Bucardo et al., 2008).

However, in Japan, a change in distribution of predominant NoV genotypes, GII/4 to GII/3 in 2003 to 2004 and GII/3 to again GII/4 in 2004 to 2005 was observed in a surveillance conducted over a three year period (Phan et al., 2006a; Phan et al., 2006b). Therefore, continuous monitoring of NoV genetic property in Malaysian population is important for updating and comparing global trends of NoV infection. Great caution is needed in interpreting this result; moreover the prevalence strain cannot be concluded as final due to the small sample size analyzed thus this study warrants further investigation. In conclusion, this study concurs with other reports which indicate that NoV is another important enteric virus that can cause gastroenteritis.

ACKNOWLEDGEMENT

This study was supported by the Excellence Fund UiTM 600-RMI/ST/DANA5/3/Dst (90/2009). Sincere gratitude to RMI, UiTM for permission to publish this work.

REFERENCES

Alice CT, Thornton MD, Karen SJ, Malkanthie IM (2004). Noroviruses: Agents in outbreaks of acute gastroenteritis. Emerg. Nursing Assoc., 2(1): 1-9.

Bucardo F, Nordgren J, Carlsson B, Paniagua M, Lindgren PE, Espinoza F, Svensson L (2008). Pediatric norovirus diarrhea in Nicaragua. J. Clin. Microbiol., 46:2573-2580.

Bull RA, Tu ETV, McIver CJ, Rawlinson WD, White PA (2005). Emergence of a new norovirus genotype II variant associated with global outbreaks of gastroenteritis. J. Clin. Microbiol., 44(2): 327-333.

Chen SY, Chang YC, Lee YS (2007). Molecular epidemiology and clinical manifestations of viral gastroenteritis in hospitalized pediatric patients in Northern Taiwan. J. Clin. Microbiol., 45: 2054-2057.

Fankhauser RL, Noel JS, Monroe SS, Humphrey CD, Bresee JS, Parashar UD, Ando T, Glass RI (2002). Epidemiologic and molecular trends of “Norwalk like Viruses” associated with outbreaks of gastroenteritis in the United States. J. Inf. Dis., 186: 1-7.

Guntapong R, Hausman GS, Oka T, Ogawa S, Kageyama T, Pongsuwana Y, Kotayama L (2004). Norovirus and sapovirus in Thailand. J. Inf. Dis., 57: 276-278.

Hansman GS, Doan LT, Kguyen TA (2004a). Detection of norovirus and sapovirus infection among children with gastroenteritis in Ho Chi Minh City, Vietnam. Arch. Virol., 149: 1673-1688.

Hansman GS, Katayama K, Maneekarn N (2004b). Genetic diversity of norovirus and sapovirus in hospitalized infants with sporadic cases of acute gastroenteritis in Chiang Mai, Thailand. J. Clin. Microbiol., 42: 1305-1307.

Kirkwood CD, Bishop RF (2001). Molecular detection of human calivirus in young children hospitalized with acute gastroenteritis in Melbourne, Australia, during 1999. J. Clin. Microbiol., 39(7): 2722-2724.

Marshall JA, Hellard HE, Sinclair MI, Fairley CK, Cox BJ,Cotton MG (2003). Incidence and characteristic of endemic Norwalk-like virus-associated gastroenteritis. J. Med. Virol., 69: 568-578.

Nguyen TA, Yagyu F, Okame M, Phan TG (2007). Diversity of viruses associated with acute gastroenteritis in children hospitalized with diarrhea in Ho Chi Minh City, Vietnam. J. Med. Virol., 79: 582-590.

Parashar UD, Gibson CJ, Bresee JS, Glass RI (2006). Rotavirus and severe childhood diarrhea. J. Emerg. Infect. Dis., 12: 304-306.

Phan TG, Kuroiwa T, Kaneshi K (2006a). Changing distribution of norovirus genotypes and genetic analysis of recombinant GIIb among infants and children with diarrhea in Japan. J. Med. Virol., 78: 971-978.

Phan TG, Takanashi S, Kaneshi K (2006b). Detection and genetic characterization of norovirus strains circulating among infants and children with acute gastroenteritis in Japan during 2004-2005. Clin. Lab., 52: 519-525.

Qiagen, Sensiscript® reverse transcription handbook. Qiagen; 2004.

Rabenaeu HF, Sturmer M, Buxbaum S, Walczok A, Preiser W, Doerr HW (2003). Laboratory diagnosis of norovirus: which method is the best? Intervirol., 46: 232-238.

Rasool NBG, Green KY, Kapikian AZ (1993). Serotype analysis of rotaviruses from different locations in Malaysia. J. Clin. Microbiol., 31(7): 1815-1819.

Tran A, Talmud D, Lejeune B (2010). Prevalence of rotavirus, adenovirus, norovirus and astrovirus infections and co-infections among hospitalized children in northern France. J. Clin. Microbiol. doi:10.1128/J. Clin. Microbiol., 02181-09:1-11.

Yap KL, Lim YH, Tan SC (1998). Rotavirus electropherotypes from the Kuala Lumpur Hospital: a re-examination after an interval of seven years. Malays. J. Pathol., 20(1): 25-30.

Zuridah H, Kirkwood CD, Bishop RF, Bogdanovic-Sakran N, Yap KL (2009). Molecular characterization and epidemiology of rotavirus isolates obtained from children with diarrhea in Malaysia. Med. J. Malaysia, 64:193-196.

Zuridah H, Kirkwood CD, Bogdanovic-Sakran N, Bishop RF, Yap KL (2010). Circulating human group A rotavirus genotypes in Malaysia. J. Med. Virol., 82: 707-711.