Abstract

Acute gastroenteritis is a leading cause of child mortality and morbidity worldwide making it an important public health problem. Several aetiological agents such as bacteria, parasites and viruses are known to cause the disease however; ~70% of the cases are attributed to viruses. Among enteric viruses, rotavirus (RV), caliciviruses (norovirus-NoV and sapovirus-SaV), adenovirus (AdV) and human astrovirus (HAstV) account for maximum disease burden of acute gastroenteritis. Recently, several novel picornaviruses like aichivirus, enterovirus, cosavirus, saffoldvirus and salivirus and other viruses such as human bocavirus (HBoV), coronavirus, picobirnavirus, pestivirus and torovirus have been detected in diarrhoeal stools of acute gastroenteritis patients, suggesting their possible aetiological involvement in the disease. The epidemiologies of viral gastroenteritis with respect to important causative agents like RV, NoV, AdV and HAstV have been well established in India. However, other enteric viruses associated with the disease have not been focused upon.

SaV, which has been recognized as an important aetiological agent in sporadic cases and outbreaks of acute gastroenteritis worldwide but has not received much attention in the Indian context. After initial reports of SaV detection in gastroenteritis patients from northern, eastern and southern parts of India between 2007 and 2009, none of the Indian research groups have attempted detailed studies on SaV. Till date, there is no report available from western India. Currently, there is no report available on the presence of HBoV in acute gastroenteritis from India. In case of salivirus, there is a single report documenting its detection from Vellore, southern India. This lone report does not address the genetic diversity in the salivirus strains detected in India. Also, there is no report on detection of these three viruses in outbreaks of acute gastroenteritis from India so far.

The present five year retrospective study was undertaken to determine the prevalence of SaV, HBoV and salivirus in sporadic cases and outbreaks of acute gastroenteritis from western India. A total of 778 faecal samples collected from children (age < 5 years), hospitalized with acute gastroenteritis to three local hospitals in Pune between 2007 and 2011, were examined for
the presence of these three viruses. Faecal samples (n=315) from two outbreaks that occurred in Solapur, western India, in 2010 (n=268) and 2011 (n=47) were also analysed for the presence of these viruses. Additionally, asymptomatic control samples (n=207) collected from children (age < 5 years) hospitalized for non-gastrointestinal ailments were also examined for emerging HBoV and salivirus. Since HBoV is known to cause respiratory infections, all control samples showing history or presence of respiratory illness (n=46) were omitted from the study. All the study samples from the study subjects were previously analysed for presence of important enteric viruses such as RV, NoV, AdV, HAstV and Enterovirus (EV).

The detection and characterization of SaV was carried out by a conventional nested PCR targeting amplification of the RdRp-Capsid junction region (~420 bp) using universal primers for the detection of all four known SaV genogroups. HBoV detection and characterization was carried out using panbocavirus primers in a nested PCR reaction targeting amplification of the partial sequence (~575 bp) of the VP1/VP2 capsid region. Salivirus RNA was detected by PCR amplification of the 2C helicase (~345 bp). Molecular typing of these viruses was performed by PCR amplification of the 3D polymerase (~688 bp) and VP capsid (~852 bp) regions. Nucleotide and phylogenetic analyses were performed using the MEGA 6.0 software. Clinical parameters of SaV, HBoV and salivirus associated acute gastroenteritis cases were studied and scored according to the 20 point Vesikari scoring system. Statistical significance between groups was compared using the $\chi^2$ test, Fisher’s test, Student’s T test and Mann-Whitney U test, as applicable. A $p$ value of $\leq 0.05$ was considered to be statistically significant. Statistical analyses were performed using the Epi Info™ software.

Before the present study was undertaken, 54% (418/778) of the sporadic cases of acute gastroenteritis remained undiagnosed for an aetiological agent. During the five year study period, sapovirus was detected in 2.7% (21/778) of the sporadic acute gastroenteritis cases. The frequency of detection varied between 2.2% to 3.3% from 2007 to 2011. SaV was detected at a higher frequency in cases of unknown aetiology (3.6%, 15/418) than in
cases where at least one other enteric virus was previously detected (1.6%, 6/360). However, this difference was not found to be statistically significant (p=0.09, χ² test). None of the 315 clinical samples from the Solapur 2010 (n=267) and 2011 (n=47) outbreaks showed presence of SaV. Co-infection with other enteric viruses was observed in 28.6% (6/21) of the positive cases. SaV infections were observed to occur in children up to 36 months of age. Maximum proportion of the infections (95.2%) were reported up to 24 months of age with highest incidence (47.6%) occurring in children between 6-12 months of age. Although SaV infections were reported to occur throughout the year, peak activity was observed in summer (28.6%, 6/21) and monsoon (14.3%, 3/21) months. Among the SaV positive cases, mono-infection was observed in 71.4% (15/21) of the cases while mixed infection with other viruses was observed in 28.6% (6/21) of them. Severity assessment of SaV mono-infections revealed that majority of the cases caused severe gastroenteritis (67%) while moderate and very severe gastroenteritis occurred in 27% and 6% of the cases. The major clinical manifestations of SaV mono-infections were diarrhoea (100%), vomiting (73%) and dehydration (80%). Maximum proportion of cases with SaV mono-infection with severe dehydration resulted in longer duration of hospitalization than the mixed infection cases (p=0.039, Mann-Whitney U test). The present study identifies SaV as an important aetiological agent of acute gastroenteritis resulting in severe infection.

Genotypic characterization of the SaV study strains revealed the presence of all four human SaV genogroups. Overall, GII was predominantly detected during the study period at a frequency of 50%, followed by GI at 22%, GV at 17% and GIV at 11%. While the predominant genogroup, GII was detected throughout the study period, GI was present during years 2007 to 2009 and 2011. GIV was detected in 2008 and 2011 whereas GV emerged in 2010 and was found to be in circulation in 2011 as well. During the years 2007, 2008 and 2009, GII was detected at a frequency of 67%, 50% and 67%, respectively. In 2010, GII and GV were detected at equal frequency of 50% each while in 2010, GV was predominant at 50%. At least 7 SaV genotypes were identified in Pune between 2007 and 2011. Interestingly, the genotypic
circulation pattern varied greatly. GII.1 strains were predominant in 2007 (66.7%) and 2009 (75%) while GIV.1 was predominant in 2008 (33.3%). GV.1 emerged in 2010 and went on to be the predominant genotype in 2011 (50%).

Human bocavirus was detected at an overall prevalence of 6% (46/778) in sporadic cases of acute gastroenteritis from Pune, western India. During the years 2007 and 2011, the frequency of detection ranged between 3.2% and 9.5%. HBoV prevalence was observed to be significantly higher in cases that remained undiagnosed for aetiology (9%, 38/418) than in cases where at least one enteric agent was detected (2.2%, 8/360) (p=0.00001, \( \chi^2 \) test). None of the asymptomatic controls tested positive for HBoV. Also, HBoV was detected in one of the two outbreaks of acute gastroenteritis that occurred in Solapur during 2011 at a frequency of 4.2% (2/47). Co-infection with other enteric viruses was observed in 17.4% (8/46) of the positive cases. HBoV infections occurred in children up to 36 months of age with highest number (84.8%) of them occurring by 12 months of age. Children between 6-12 months of age were found to be most susceptible (52.2%) to HBoV infection. The infections were observed to occur throughout the year however, peak activity was reported in the monsoon months (50%, 23/46). Severity assessment of HBoV mono-infections revealed that maximum number of them resulted in severe gastroenteritis (61%). Moderate and very severe gastroenteritis were observed in 34% and 5% of the HBoV mono-infection cases. The major clinical symptoms of mono-infections included diarrhoea (100%), fever (84%), dehydration (80%) and vomiting (71%). The occurrence of fever was significantly higher in mono-infections (84%) than mixed infections (38%) (p=0.005, \( \chi^2 \) test). Other clinical parameters did not differ significantly between mono-infections of HBoV and other enteric viruses known to cause acute gastroenteritis (NoV, AdV and HAstV). It was noteworthy to find that the morbidity (duration of hospitalization) associated with HBoV mono infections was notably higher than AdV and AstV mono infections (p=0.04 and p=0.03, Student’s T test). This data suggests that in absence of such aetiological agents, HBoV could cause severe acute gastroenteritis resulting in long duration of hospitalization.
Genotypic characterization of HBoV study strains detected in sporadic cases and outbreaks of acute gastroenteritis indicated presence and circulation of all four HBoV genotypes in the study region. HBoV1 was predominantly detected in sporadic cases of acute gastroenteritis during the study period at an overall frequency of 50% while HBoV2, HBoV3 and HBoV4 were detected at a frequency of 33%, 11% and 4%, respectively. While HBoV1 was detected consistently throughout the study period, HBoV2 was detected from 2007-2010, HBoV3 between 2007 and 2009 and in 2011 while HBoV4 was detected in the years 2009 and 2011 alone. The predominant genotype was also observed to vary between these years. In the year 2007, HBoV2 was found to be predominant (54%) while in 2008, HBoV2 and HBoV1 were detected at similar frequency (44.5%). HBoV1 was the predominant genotype detected in 2009 (64%), 2010 (87%) and 2011 (60%). Among the two outbreak samples which tested positive for HBoV, HBoV3 was characterized from both of them. HBoV1 study strains were highly homologous (>99% nucleotide identity) while HBoV2-4 strains showed greater level of variation. HBoV2 strains shared a nucleotide identity of 91.9% to 99.2% among themselves and HBoV3 and HBoV4 strains were ~95% identical.

Salivirus was detected in sporadic cases of acute gastroenteritis at an overall prevalence of 2.6% (20/778). During the years 2007 and 2011, frequency of detection ranged between 1.5% and 2.9%. The frequency of detection was not found to differ significantly in previously diagnosed (2.7%, 10/370) and undiagnosed cases (2.4%, 10/418) (p=0.78, χ² test). In the asymptomatic control group, salivirus was detected in 1.93% (4/207) of the samples. The frequency of SaV detection in cases and controls did not differ significantly (p=0.57, χ² test). None of the 315 samples from the Solapur 2010 (n=267) and 2011 (n=47) outbreaks showed presence of salivirus. Co-infections with other enteric viruses were observed in 50% (10/20) of the positive cases. Salivirus infections mainly occurred in children up to 30 months of age. Children below 24 months of age showed highest incidence of salivirus (95%) with those between 6-12 months of age observed to be most susceptible. Peak salivirus activity was observed in monsoon months (60%,
12/20). Severity assessment of salivirus positive mono-infection cases revealed that maximum proportion of them showed moderate gastroenteritis (60%) while mild and severe gastroenteritis was observed in 20% of the cases each. Occurrence of vomiting was significantly higher in the mixed-infections (80%) than the mono-infections (30%) (p=0.034, χ² test). The frequency of diarrhoea was significantly higher in the mixed infections than mono infections (p=0.02, Student’s T test). Comparison of other clinical parameters between mono-infections of salivirus and other known enteric viruses (RV, NoV, AdV and HAstV) indicated that salivirus was mainly associated with moderate infections. Based on these clinical correlations, salivirus alone was not found to exacerbate the severity of acute gastroenteritis.

Genotypic characterization of salivirus study strains revealed vast genetic diversity among them. Phylogenetic analysis based on 3D and VP regions indicated that all study strains belonged to salivirus A1 however, they were grouped into two distinct clusters (Cluster1 and Cluster2) in the phylogenetic trees. Between themselves, the two clusters shared a nucleotide identity of 94.1% to 96.2% in the 3D region and 91.8% to 93.7% in the VP region. Interestingly, the more divergent Cluster2 strains shared a low nucleotide identity with the closest reference strain in both regions (~95% in 3D and ~92% in VP) suggesting they could represent a variant type of salivirus A1.

The present study, for the first time, determines the prevalence of SaV, HBoV and salivirus in sporadic cases and outbreaks of acute gastroenteritis from western India. It also explores the genetic diversity and circulation pattern of SaV, HBoV and salivirus strains detected in sporadic cases and identifies a changing trend in genotypic circulation for SaV and HBoV. The study highlights the clinico-epidemiological features of these viruses, indicating that SaV and HBoV are strongly associated with the disease. In summary, it has helped in understanding the contribution of these viruses to acute gastroenteritis which will further help in devising interventional strategies for better management of the disease.