Chapter 1: Introduction

Acute gastroenteritis is one of the leading causes of morbidity and mortality in children below 5 years of age (Murray and Lopez, 1997; Shek et al., 2004; Wilhelmi et al., 2003). Annually, it affects 3-5 billion children resulting in ~1.5-2.5 million deaths (Chow C. M. et al., 2010). The disease burden is greater in developing countries owing to additional risk factors such as lack of proper sanitation measures, poor hygiene practices, dearth of clean potable water and low socio-economic status. In such countries, children with poor nutrition are at an increased risk (Elliott, 2007; Fabiana et al., 2007). Highest child mortality rates have been reported from African (17.9%) and South Asian countries (24.5%), with 5,35,000 deaths occurring in India alone (Boschi-Pinto et al., 2008). Although the mortality rates in developed countries are lower, acute gastroenteritis results in hospital admissions which cause significant economic burden (Clark and McKendrick, 2004). The main clinical symptoms of the disease include watery diarrhoea and vomiting which may be accompanied with fever, abdominal pain, nausea and malaise.

Several bacteria, protozoa and viruses are responsible for causing acute gastroenteritis. In developing countries, improvement in hygiene and sanitation measures has reduced the burden of disease due to bacterial and parasitic infections however; there is an increase in hospitalizations due to viral gastroenteritis (Ramani and Kang, 2009). The aetiology of acute gastroenteritis has been studied extensively and it has been reported that parasitic infections account for 10% of the cases while bacteria are responsible for 10-20% of them (Elliott, 2007). The most common bacterial agents causing the disease are Salmonella spp, Campylobacter spp, Shigella spp, Yersinia spp and Vibrio cholerae. Giardia lamblia is the major protozoan agent which is generally associated with persistent diarrhoea while Cryptosporidium species and Entamoeba hystolytica are the other important protozoan agents associated with the disease.

Since 1940s, viruses have been implicated as being important causes of acute gastroenteritis, as the aetiology of most cases remained unknown (Kapikian, 1996; Parashar et al., 1998). Approximately 70% of the acute gastroenteritis cases are attributed to viruses (Sidoti et al., 2015; Webb and
Starr, 2005). Among enteric viruses, rotavirus (RV), caliciviruses (norovirus-NoV and sapovirus-SaV), adenovirus (AdV) and human astrovirus (HAstV) are known to be important aetiological agents worldwide (Wilhelmi et al., 2003). The viruses causing acute gastroenteritis are transmitted by faeco-oral route and have short incubation period between 1 to 6 days (Lee et al., 2013). Before 1970, over 80% of the acute gastroenteritis cases were attributed to malnutrition and idiopathic causes as no aetiological agent could be found (Glass, 2013). Since the discovery of NoV in an outbreak of acute gastroenteritis that occurred in 1972, several other enteric viruses have been reported to cause this disease (Kapikian et al., 1972). In the following year, Bishop and co-workers observed the presence of RV in the duodenal mucosa of children suffering with acute gastroenteritis (Bishop et al., 1973). Shortly after the discovery of these viruses, enteric AdV and HAstV were detected in faeces of children with watery stools (Madeley and Cosgrove, 1975; Morris et al., 1975). Among enteric viruses, RV remains to be the leading cause of severe acute gastroenteritis (Parashar et al., 2006; Tiku et al., 2014). Worldwide, RV is recognized as the most common cause of the disease and accounts for 30% to 72% of all hospitalizations (Parashar et al., 2003; Rivest et al., 2004). It is responsible for 26.6% to 50% of all the sporadic acute gastroenteritis cases (Zhang et al., 2016; Azaran et al., 2016). NoV is accountable for 50% to 90% of all outbreaks of gastroenteritis in young children and elderly persons and ~18% of all the sporadic cases occurring globally (Belliot et al., 2014; Hall et al., 2011; Lopman et al., 2016).

Although the global distribution and epidemiology of these four enteric viruses has been well established in literature, ~40% of the acute gastroenteritis cases still remain undiagnosed for any causative agent (Denno et al., 2005; Simpson et al., 2003). In recent years, advanced molecular detection techniques like Real time PCR (RT-PCR) and Next generation sequencing (NGS) have led to discovery of an increasing number of viruses which have been associated with gastroenteritis. Novel picornaviruses (aichivirus, enterovirus, cosavirus, saffoldvirus and salivirus), human bocaviruses (HBoV) and others such as coronavirus, picobirnavirus, pestivirus and torovirus, which are reported to cause diarrhoea in animals, have been
identified as emerging causes of the disease (Kapoor et al., 2010a; Nielsen et al., 2013; Wilhelmi et al., 2003).

SaV, a member of the family Caliciviridae, genus Sapovirus, is known to cause acute gastroenteritis in humans and animals. SaV infections are a matter of public health concern as they are responsible for both, sporadic cases and outbreaks of gastroenteritis (Svraka et al., 2010). On the basis of nucleotide sequence variation in their capsid region, they are divided into five genogroups (G): GI-GV. Among these, GI, GII, GIV and GV strains are known to cause acute gastroenteritis in humans while GIII strains infect porcine hosts (Oka et al., 2012). Epidemiological surveillance studies have identified the presence of genetically diverse SaV strains in sporadic cases of gastroenteritis, at prevalence rates ranging between 2.2% and 12.7% (Oka et al., 2015). GI and GII are the predominant SaV genogroups reported worldwide (Dey et al., 2007; Pongsuwanna et al., 2016; Ren et al., 2013). SaV have been responsible for 1% to 17.6% of all outbreaks occurring across USA, Europe and Asia (Sala et al., 2014; Svraka et al., 2010). Majority of these outbreaks are reported to occur in semi closed settings like schools, kindergartens, nursing and elderly homes (Hansman et al., 2007; Lee et al., 2012). While SaVs are mainly transmitted via faeco-oral route; foodborne and waterborne outbreaks have also been documented (Iizuka et al., 2013; Shibata et al., 2015). The severity of SaV infections is reported to be lower than NoV infections however; severe cases requiring hospitalization have also been documented (Zintz et al., 2005). Previous reports from northern, southern and eastern regions of India have documented the prevalence of SaVs in acute gastroenteritis patients between 0.9% to 10.2% and reported GI and GII strains to be in circulation between 2006 to 2008 (Gupta et al., 2015; Monica et al., 2007; Nayak et al., 2009; Rachakonda et al., 2008). These studies have focused on detection of these viruses in children suffering with acute gastroenteritis however; there is no information available regarding the clinical presentation of SaV infections and their epidemiology. Till date, there is no data available on SaV in acute gastroenteritis from western India.
HBoV, a recently discovered single stranded DNA virus, belongs to the family *Parvoviridae*, genus *Bocavirus*. Like other members of this genus, HBoV has been reported to cause respiratory and gastrointestinal infections (Kapoor et al., 2010a; Lüsebrink et al., 2009). Apart from this, few studies have documented the presence of HBoV in cases of acute flaccid paralysis and encephalitis (Kapoor et al., 2009; Mori et al., 2013). HBoV strains have been divided into four genetic subtypes: HBoV1-4 on the basis of >5% nucleotide difference in their capsid (VP1) region. Among these, HBoV1 is considered to be a respiratory pathogen while HBoV2-4 are recognized as enteric pathogens (Schildgen, 2013). The overall frequency of detection of HBoV in acute gastroenteritis cases ranges between 0.9% to 25.6% (Chieochansin et al., 2008; Jin et al., 2011). Young children below 2 years of age are found to be most susceptible to HBoV infections (Chhabra et al., 2013). Although HBoV has been detected frequently in diarrhoeal samples of gastroenteritis patients, its causative role is still not clearly understood (Chieochansin et al., 2008; Nawaz et al., 2012). Few studies have established a clinical as well as a statistical association between HBoV2 and acute gastroenteritis but similar data regarding other enteric HBoV subtypes is still lacking (Arthur et al., 2009; Chow B. D. et al., 2010; Jin et al., 2011). In a single outbreak study reported from Germany, HBoV was not found to be responsible for any of the outbreaks of acute gastroenteritis (Campe et al., 2008). Data on epidemiological and clinical features of HBoV infections is largely lacking especially from India. In earlier reports from India HBoV has been detected in respiratory samples at a frequency of 0.67% (Vellore, south India) and 7.6% (New Delhi, north India) however, genotypic data of circulating strains is not available so far (Bharaj et al., 2010; Narayanan et al., 2013). Till date, no attempts have been made to study the epidemiology and clinical features of HBoV in acute gastroenteritis cases from India.

Salivirus, a member of the family *Picornaviridae*, was discovered as recently as 2009 and has been classified into a new genus *Salivirus* A in 2014 (Wong et al., 2015). Two groups simultaneously reported the detection of novel viruses which shared a nucleotide identity of 38%, 34% and 43.3% and 42.6%, 35.2% and 44.6% in the P1, P2 and P3 region with their closest
relative. These novel viruses which were discovered by deep sequencing method were found to be most closely related to aichivirus from the genus *Kobuvirus*. Since the viruses were detected from stool samples and closely resembled kobuviruses, they were temporarily classified as Stool Aichi Like Virus (Sali) and Kobu Like Virus Associated with Stool and Sewage (Klasse) ([Greninger et al., 2009; Holtz et al., 2009]). Following their discovery, these viruses have been named as sali/klassevirus and there is no consensus on the usage of these terms. Till date, they are identified by both names by several groups ([Haramoto and Otagiri, 2013; Kitajima et al., 2014; Yip et al., 2014]). Recently, with the detection of a second genotype of this virus and its classification under genus *Salivirus* A, they are unanimously referred to saliviruses ([Aldabbagh et al., 2015; Fei et al., 2016]). Salivirus has been detected in diarrhoeal samples from acute gastroenteritis cases globally at a frequency of 0.1% to 8.1% ([Li et al., 2009; Yip et al., 2014]). Higher prevalence has been reported in Asian countries as compared with their European counterparts ([Han et al., 2010; Nielsen et al., 2013; Shan et al., 2010]). Salivirus A1 is the predominantly detected genotype while A2 has been reported to be circulating in Germany, Thailand and China alone ([Aldabbagh et al., 2015; Ng et al., 2012; Yu et al., 2015]). Earlier reports have identified these viruses as causative agents of acute gastroenteritis on the basis of case control studies but, the clinical features of salivirus infections remain to be known ([Li et al., 2009; Yu et al., 2015]). Currently, there is no detailed study defining the epidemiological features of salivirus infections. In the Indian context, salivirus has been detected in 4.2% of the children hospitalized with acute gastroenteritis ([Greninger et al., 2010]). However, this study did not attempt genotypic characterization of the salivirus strains detected in the study region.

With the advent of newer molecular detection techniques like RT-PCR and NGS (Next generation sequencing), several viruses have been detected in faecal samples of acute gastroenteritis cases. This has helped in understanding the aetiology of the undiagnosed cases of the disease and led to an increased understanding of the role of these viruses in development of acute gastroenteritis. However, the causal association of newly discovered
emerging enteric viruses is still poorly understood. In India, major aetiological agents such as RV, NoV, AdV and HAstV have been studied in detail however; besides these, other enteric viruses associated with the disease have not received much attention. There is no detailed epidemiological study on SaV and salivirus in acute gastroenteritis cases from India so far. Also, data on their genetic diversity and circulation pattern of SaV genotypes is largely unavailable. Emerging HBoV has not yet been studied in association with gastroenteritis from the country so far. Given the substantial disease burden of infectious diarrhoea in our country, information regarding prevalence and genetic diversity of these enteric viruses in sporadic cases and outbreaks of acute gastroenteritis along with collective epidemiological and clinical data with respect to viral infections will help public health and other healthcare professionals in better management of the disease.