Novel human astroviruses: challenges for developing countries

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Abstract Astroviruses have been gaining widespread importance over the past few decades owing to their detection through advanced molecular techniques. The association of astrovirus-associated enteric infections have been reported from various settings among different age groups. The tremendous efforts of scientists from different countries to detect and characterize these star-like viruses in the course of surveillance has shown the emergence of novel astroviruses from varied host species, necessitating changes in the classification to update their taxonomy. The public health importance of these viruses implies new control measures are essential to reduce disease burden in developing countries.

Keywords Astrovirus · Genetic diversity · Emerging viruses · Disease burden

Introduction

Astroviruses are small, non-enveloped RNA viruses that were first identified by Appleton, Higgins, Madeley and Cosgrove in 1975 by electron microscopy (EM) from faecal specimens of diarrhoeic children. Astroviruses (named after the Greek word ‘astron’ meaning ‘star’) have a distinctive five or six pointed, star-like appearance detectable by negative stain EM. These small virus particles are approximately 27 nm in diameter. Astrovirus morphology and size is clearly different from some of the

other viruses like Rotavirus and Norwalk virus as these viruses do not show star-like capsid profiles. In the year 1982, Caul and Appleton visualized astrovirus using appropriate immunological reagents and observed that the star shape of the virion is pH dependent and may only be present in less than 10 % of the population. Gradually, specific tools were developed for the detection and genome-based identification of astroviruses from a wide variety of hosts [5]. The morbidity of astrovirus infection depends upon the season; a higher infection rate was reported during the winter season in temperate regions and rainy season in tropical regions [26]. In developing countries with tropical climate and poor sanitation, a higher prevalence of astrovirus infections was observed during the rainy season. Other than through the consumption of faecally contaminated food and water, astrovirus transmission occurs predominantly through the faecal–oral route or as person-to-person contact. The person-to-person spread or faecal–oral mode of transmission had occurred during nosocomial infection in hospitals, hotels, cruise ships, family outbreaks and in day care centers. Transmission through infectious vomit and environmental surfaces (such as hand-mouth contact) was also reported. Besides, the consumption of faecally contaminated astroviruses in shellfish also caused infection [33].

Astrovirus epidemiological studies were earlier generally based on electron microscopic studies and enzyme immunoassay techniques. During the past few decades, there was a tremendous increase in collection of information through astrovirus surveillance using molecular techniques, mainly reverse transcription-PCR (RT-PCR). Furthermore, seroprevalence studies showed that most children acquire astrovirus antibodies during the first few years of life. Consequently, a new appreciation for the role of astrovirus in diarrheal disease has evolved. In most

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Table 1 Reporting of astrovirus genera and species 1995–2012 from international committee meetings on taxonomy of viruses

Year	ICTV release ^a	Genus	Species	Name of species
1995	Sixth report	Astrovirus	10	Bovine AstV 1, 2; duck AstV 1; human AstV 1, 2, 3, 4, 5; ovine AstV 1; porcine AstV 1
1996–1998		Astrovirus	10	Bovine AstV 1, 2; duck AstV 1; human AstV 1, 2, 3, 4, 5; ovine AstV 1; porcine AstV 1
1999	Seventh report	Astrovirus	7	Astroviruses from bovine, duck, feline, human, ovine, porcine, turkey
2002		Astrovirus	7	Astroviruses from bovine, duck, feline, human, ovine, porcine, turkey
2004		Avastrovirus	3	Avian nephritis virus; astroviruses from duck turkey
		Mamastrovirus	5	Astroviruses from bovine, feline, human, ovine, porcine
2005	Eighth report	Avastrovirus	3	Astroviruses from chicken, duck, turkey
		Mamastrovirus	6	Astroviruses from bovine, feline, human, mink, ovine, porcine
2008		Avastrovirus	3	Astroviruses from chicken, duck, turkey
		Mamastrovirus	6	Astroviruses from bovine, feline, human, mink, ovine, porcine
2009	Ninth report	Avastrovirus	3	Astroviruses from chicken, duck, turkey
		Mamastrovirus	6	Astroviruses from bovine, feline, human, mink, ovine, porcine
2011		Avastrovirus	3	Avastrovirus 1–3
		Mamastrovirus	19	Mamastrovirus 1–19
2012		Avastrovirus	3	Avastrovirus 1–3
		Mamastrovirus	19	Mamastrovirus 1–19

^a Astrovirus was not included in ICTV first report-1971; ICTV second report-1976; ICTV third report-1979; ICTV fourth report-1982 and ICTV fifth report-1991

cases, astroviruses are the second most common cause of viral gastroenteritis in children in various geographical locations, behind rotavirus infection.

Astrovirus classification

The recognition of Astrovirus as a genus under the family Astroviridae was accepted by the International Committee on Taxonomy of Viruses (ICTV) in 1995. The detection of Astrovirus from different avian and mammalian species was recognized and the classification was modified during meetings of the ICTV, as shown in Table 1. From 2004, the family Astroviridae included two genera: Mamastroviruses (MAstVs) and Avastroviruses (AAstVs) that were known to infect mammalian and avian species respectively [15, 24]. Astroviruses associated with acute gastroenteritis cases have gradually been reported from humans and a wide range of animals covering different species or families, comprising domestic or wild animals as well as avian or mammalian species from terrestrial or aquatic environments. The ICTV has officially classified limited avian and mammalian astrovirus species. The avian astroviruses

include the following: duck astrovirus 1 (DAstV-1), turkey astrovirus 1 and 2 (TAsTV-1 and TAsTV-2) and avian nephritis virus (ANV). Mammalian astroviruses include bovine astrovirus (BoAstV), feline astrovirus (FeAstV), human astroviruses (HAstVs of genotypes 1–8), ovine astrovirus (OAstV), mink astrovirus (MiAstV) and porcine astrovirus (PoAstV), respectively.

Since 2011, the Astroviridae family has been officially classified by the ICTV based on the full length amino acid sequences of the ORF2 (capsid) region. The current ICTV official classification shows three species of *Avastrovirus* [Avastrovirus 1 (GI.A), Avastrovirus 2 (GI.B) and Avastrovirus 3 (GI.A)] and 19 species of *Mamastrovirus* [Mamastrovirus 1 (GI.A-Human); Mamastrovirus 2 (GI.B-Feline); Mamastrovirus 3 (GI.C-Porcine); Mamastrovirus 4 (GI.D-California sea lion); Mamastrovirus 5 (GI.E-Canine); Mamastrovirus 6 (GI.F-Human); Mamastrovirus 7 (GI.G-Bottlenose dolphin); Mamastrovirus 8 (GI.A-HMOAstV-A and HAstV-VA2); Mamastrovirus 9 (GI.B-HMOAstVs -B and -C and HAstV-Va1); Mamastrovirus 10 (GI.C-MiAstV); Mamastrovirus 11 (GI.D-California sea lion AstV-1); Mamastrovirus 12 (GI.E-BatAstV); Mamastrovirus 13 (GI.F-OAstV); Mamastroviruses 14–19 (GI.G to GI.L-BatAstVs)].

Astrovirus detection in various settings

In 1977, it was reported that virus like particles with 26 nm diameter were detected in faecal samples examined by EM during an outbreak of winter vomiting disease among pupils and staff of a primary school. Astrovirus infection was detected in severe combined immunodeficiency patients by Chrystie et al. [4]. Chronic astrovirus infections were detected in children with T cell immunodeficiencies [43]. Astroviruses were also associated with gastroenteritis among patients who had undergone bone marrow transplantation and among immunocompromised children. Astrovirus infections have been detected both in healthy and human immunodeficiency virus-infected children.

The prevalence of human astrovirus (HAstV) infections was shown to depend upon the place and test settings (urban hospital vs child-care centres). The infection rate ranged from 2 to 6 % for hospitalized children with acute diarrhoea and 5–17 % for acute diarrhoea cases from the community [3]. In both developed and developing countries, HAstV infection was associated with diarrhoea episodes and ranged from 3 to 12 and 2 to 26 % respectively [18, 23, 29, 34, 40, 41].

Human astroviruses gradually gained importance as another etiological agent and are presently considered as the second most common cause of diarrhoea among infants and children aged below 5 years. Human astroviruses were detected in 23 stools samples collected from 365 diarrhoea episodes among 214 children (<18 months old) who were monitored for diarrhoea in Mexico City. In this prospective study, these stool samples were first tested by EIA and then by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis which showed that six different HAstV antigenic types co-circulated in the community. Human astrovirus was reported from Brazil, among samples collected from children with acute gastroenteritis and admitted to three hospitals in Rio de Janeiro during the period of January to December 2004. In this study, astrovirus infection was reported in 43 cases. Diarrhoea, vomiting, and fever were the main clinical manifestations observed during the study period [39]. An astrovirus associated gastroenteritis outbreak was reported in a primary immunodeficiency unit at Newcastle General Hospital in the UK. In this study, astroviruses were detected from four environmental swabs and two patient faecal samples using heminested reverse transcription and PCR. HAstV-3 genotypes were detected in environmental swabs and faecal specimens and these strains were responsible for the outbreak [13]. Astrovirus infection was reported from 335 infants with diarrhoea in Wuhan, China. The report indicated that the reported astrovirus HAstV_G1 showed highest identity to a Mongolian strain [19]. In Japan, higher prevalence of astrovirus-associated gastroenteritis was

detected in infants in a day care center [2]. In Spain, the overall incidence of astrovirus was found to be 4.9 % in children. In Korea, a total of 10,603 stool samples were collected from 2004 to 2008 and tested by RT-PCR or ELISA technique; the study had shown 1.8 % of human astrovirus positivity that was responsible for acute gastroenteritis [28]. In Novosibirsk, a total of 1,107 faecal samples collected from young children admitted to hospital for acute enteric infection from January to December 2007 were tested for astroviruses. In this study, astroviruses were detected in 64 (5.8 %) cases, 50 % of which were found as sole infection [36]. From Hungary, astrovirus-associated outbreak of viral gastroenteritis was reported and clinical manifestation of diarrhoea and vomiting was observed in 4.2–7.3 % cases comprising of infants and children, and rarely in elderly patients [27].

In a separate study from France, astroviruses were detected in 1.5 % cases of children hospitalized with acute gastroenteritis [21]. In Saudi Arabia, astrovirus associated diarrhoea cases were reported in 1.5 % of pediatric population and faecal specimens were characterized for genotype by using molecular methods (PCR, RT-PCR, and RFLP) [35]. Outbreak of neonatal gastroenteritis associated with astrovirus serotype 1 was observed at a maternity hospital in Inner Mongolia, China. During this study, 40 specimens were tested for astrovirus and 28 faecal samples showed positivity. This study indicated that poor hygiene was responsible for the spread of HAstV in the neonatal care room [17]. Astrovirus-associated acute gastroenteritis and predominant dual serotype strains have been detected in western India [38].

A preliminary study on astrovirus infections among children as an effort to understand their seasonal distribution, clinical characterization and co-infection with bacterial, viral and parasitic pathogens was reported from Kolkata [3]. Human astrovirus classical serotypes 1–8 and three novel astroviruses [AstV-VA2, AstV-VA3, and AstV-MLB2] have been detected in Vellore, Southern part of India [7].

Astroviruses mostly caused acute watery diarrhoea in infants or children and infections were not common in adults. Nevertheless, gastroenteritis outbreaks associated with astroviruses were also reported among students and teachers and from adult military recruits. In Kolkata there were more adult astrovirus positives than infants among the hospitalized cases of acute gastroenteritis [25, 29, 30]. In the course of this study, twelve recombinant-like astroviruses with three different inter-genotype combinations were detected among hospitalized infants, children and adults in Kolkata, India. Four cases showed unique, hitherto unreported amino acid changes within the conserved stretch of ORF1a and ORF2 fragment, indicating the emergence of HAstV variants in this setting that was

different from all the eight different genotypes of astroviruses known to date. This was the first evidence of inter-genotype recombinant-like astrovirus strains associated with acute gastroenteritis in adults involving two different genotypes (ORF1aG8 with ORF2G2, and ORF1aG8 with ORF2G1) and two inter-genotype recombinants (ORF1aG8 with ORF2G1, and ORF1aG2 with ORF2G3) among infants or children from Kolkata. The conserved stretch of amino acids spanning the 289 bp fragment of ORF1a (in four strains) and 449 bp fragment of ORF2 (in three strains) showed unique amino acid changes indicating emergence of unique variants of HAsVs, hitherto unreported among the eight recognized genotypes of astroviruses [29].

Astroviruses have been recognized as another leading cause of infantile gastroenteritis and in a variety of other host species [5]. Astrovirus infection in adults was observed in two clinical settings, namely nosocomial diarrhoea and epidemic diarrhoea, but generally, human astrovirus infection in adults was uncommon. A small study from South Africa showed astrovirus infection in children and adults with diarrhoea. Astrovirus incidence rate was reported as 3–9 % in patients hospitalized with diarrhoea. HAsV-associated large outbreaks of viral gastroenteritis infection was reported in infants and children under age of 0–6 years among Maxakali Indians in Southeast Brazil [11, 12]. During the study, higher rate of infection was detected in infants than among children or adults.

Molecular epidemiology data of HAsV infection suggests that HAsV1 is the most predominant genotype reported worldwide, although the predominant astrovirus genotype may vary in different geographical locations [14, 24]. HAsV genotypes 1, 3 and 4 were predominant in Australia and HAsV_G2 was the predominant genotype found in Mexico. The higher prevalence of HAsV_G1 genotype was reported among 2-year old children hospitalized with acute gastroenteritis in Goiânia-Goiás, Brazil. HAsV genotype 1 associated with a neonatal gastroenteritis outbreak was observed at a maternity hospital in Inner Mongolia from China [17]. A HAsV_G1 associated outbreak of gastroenteritis was detected in a home from England. The HAsV_G3 genotype was detected from rectal swabs among patients of various age groups in the Central West Region of Brazil. HAsV_G3 genotype infection was also detected in faecal samples of children hospitalized with acute gastroenteritis in Chiang Mai, Thailand [22]. The new HAsV subtype 3 was detected from infants with diarrhoea in Wuhan, China [20]. The HAsV_G3 genotype circulation was detected in 4.5 % of faecal samples collected from children with and without diarrhoea at São Paulo in Brazil [31]. The prevalence of HAsV_G3 infection with diarrhoea in children below

3 years of age was reported from Argentina. Encephalitis is a major cause of death worldwide and >100 pathogens were listed as the panel of causative agents; however these causative agents could not be determined in 75 % of the cases due to diagnostic failures. Although astroviruses are mostly recognized as a common cause of acute gastroenteritis, recent unbiased pyrosequencing method indicated that astrovirus was associated with central nervous system disease, it was detected as the causative agent for encephalitis in a 15-year old boy with agammaglobulinemia which indicates that astrovirus can also cause encephalitis in humans.

A highly divergent human astrovirus MLB1 strain detected in a child with acute diarrhoea was reported from Australia [8] and in stool samples of children in USA [9]. A novel human astrovirus (Ast-VA1) strain that was associated with acute gastroenteritis outbreak was reported from USA [10] and highly divergent strains (AstV-VA2, AstV-MLB2 and Ast-VA3) were reported in children from USA [16].

Three novel human astrovirus species (HMOAstV-A–B–C) found in human stool samples showed close genetic relatedness to mink and bovine astroviruses. Novel astroviruses (HAsV, MLB1 and HMOAstV) detected in human stool samples displayed close genetic relatedness to animal astroviruses and indicated the zoonotic transmission of human astroviruses in humans [16]. Recently, novel astroviruses found in urban rats (*Rattus norvegicus*) showed close relation to human astroviruses (AstV-MLB1 and AstV-MLB2) which indicated that both these strains possibly shared a common ancestor.

Genomic diversity among astroviruses

The astrovirus virions are 28–30 nm in diameter, icosahedral and non-enveloped with single stranded positive (+) sense genomic RNA of approximately 6.8–7.9 kb. The genome consists of untranslated (UTR) regions at 3' and 5' ends, and a poly(A) tail at 3' end. The viral genome comprises three overlapping open reading frames (ORFs): ORF1a, ORF1b and ORF2. In Astroviruses, the ORF1a encodes the non-structural serine protease (nsP1a) and ORF1b encodes the RNA dependent RNA polymerase (nsP1b) and both are flanked by ribosomal frameshift mechanism event. In astroviruses, the ORF2 region is translated from a sub genomic RNA and encodes capsid protein precursor. Genome analysis of astroviruses has shown that ORF1b is most conserved and ORF2 is highly divergent due to selective pressure in this region. To date, eight serotypes of human astroviruses identified in humans displayed good correlation with eight known human astrovirus genotypes in the phylogenetic grouping of

nucleotide sequence analyses of limited partial ORF2 region. Previous studies in different countries around the world indicated that HAsV1 was the most common serotype, which caused acute gastroenteritis in infants, children and immunocompromised individuals. Generally, HAsV-6 and HAsV-7 serotypes have been detected in rare cases.

Genetic recombinant events were reported earlier in a number of single-stranded positive-sense RNA viral genomes. Recombination events have been observed in astroviruses which infect both human and animals. Novel recombinant human astrovirus strains were first described in children with HAsV_G3 (ORF1b) and HAsV_G5 (ORF2) combination in the ORF1b/ORF2 junction [40]. Human astrovirus wild-type strain obtained from a Kenyan child showed genetic recombination event in the sequence analyses of ORF1a (HAsV_G6/7), ORF1b (HAsV_G3) and ORF2 region (HAsV_G2) [42].

In Egypt, novel recombinant human astrovirus strains collected from children displayed close genetic relatedness to VA2 (ORF1b) and MLB1 strains (ORF2) [1]. In 2010, the study conducted by Rivera et al. [32] suggested a possibility of a recombination event between Human and California sea lion astrovirus, suggestive of zoonotic transmission or a common ancestral origin. Furthermore, Ulloa and Gutiérrez [37], described the recombination event between Porcine astrovirus (PAstV) and human astrovirus genotype 3 (HAsV_G3) in faecal samples obtained from piglets and children from different parts of Colombia. They showed a high prevalence of PAstVs in pigs and existence of different PAstV types which are closely related to human astroviruses.

Recently, two novel Mamastrovirus 6 species (GI.F and AstV-MLB2) identified in patients displayed close genetic relatedness to urban brown rat astroviruses identified in Hong Kong [42]. Further, three novel Mamastrovirus 8 and 9 species (GII.A and GII.B) were detected in human stool samples from Nepal, Pakistan and Nigeria [16].

Genetic analysis of astroviruses

At present, there is no clear defined information for classification of HAsV lineages and reports described that a variant could be considered a new subtype if it had less than 95 % nucleotide (nt) identity or homology match to the reference strains and >0.05 distance by phylogenetic analysis at the 3' end of the ORF2. It is also recognized that a group of strains with 97–100 % nucleotide homology are to represent a single strain and scientists have proposed that the strains showing sequence diversity of at least 7 % nucleotide (nt) could be considered as new lineages (new subtypes).

A 5 % nucleotide (nt) divergence cut-off value, coupled with a high boot strap value was used to define a new

lineage (1e) of HAsV-1 genotype [12]. Based on the previous reports, they defined HAsV_G1 into six lineages (HAsV_G1a_1f) [12]. Further HAsV_G2 was divided into three lineages (HAsV_G2a_2c) [6]. HAsV_G3 was classified into two lineages (3a and 3b) and HAsV_G4 was classified into two lineages (4a and 4b) [20]. The human astrovirus genotype 1 is the most prevalent and common genotype reported worldwide. The genotypes 2, 3, 4 are also common whereas HAsV6 and HAsV7 are less common. Translation of the structural polyprotein from ORF2 (782–794 amino acids, depending on the specific genotype) occurs from the sub genomic RNA. The upstream conserved RNA sequence from the ORF2 initiation codon might play a key role in the regulation of the capsid gene expression. This region was identified as a hotspot for RNA recombination [41].

Recently two more clades of astrovirus strains viz. MLB1 and novel astrovirus VA1 (HAsV-VA1) were reported from stool samples of children in Australia and USA and these were highly divergent from the 'classic' types [9, 10]. In this study, the nucleotides deletion was found in highly conserved 52nt region of upstream sequence of the ORF1b/ORF2 junction in MLB1 strain. Novel multiple astrovirus species were reported from human stool samples [16]. A divergent sequence in the ORF1 region has been reported from bat guano under a mixed species in North America.

Concluding remarks

The accumulation of evidence that astroviruses are a medically important enteric pathogen and that host susceptibility covers the same age group as that of rotaviruses is an indicator that one should envisage in designing a candidate vaccine that would protect young children against both the viruses. Vaccines are necessary for protection from infection and vaccinees will be protected from severity of infection in future, especially in developing countries. The constant monitoring and stringent surveillance measures will enable epidemiologists and clinicians to meet the challenges and advise policy makers to step up the public health measures in the country.

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