Circulation of Aichi virus genotype B strains in children with acute gastroenteritis in India

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SUMMARY
Acute gastroenteritis (AG) is considered as one of the major health problems affecting humans of all ages. A number of viruses have been recognized as important causes of this disease. Recently, Aichi virus has been shown to play an aetiopathological role in sporadic infections and outbreaks of AG. A study on surveillance of enteric viruses was conducted during 2004–2008 in three cities in Maharashtra state, western India. A total of 1240 stool specimens from children aged ≤8 years hospitalized for AG were screened for the presence of Aichi virus by RT–PCR of the 3C–3D junction region followed by sequencing for the identification of genotype. Aichi virus was detected at a prevalence of 1.1% in the <5 years age group and characterized as genotype B. This is the first report on the circulation of Aichi virus genotype B in India.

Key words: Acute gastroenteritis, Aichi virus, RT–PCR, genotype B.

INTRODUCTION
Acute gastroenteritis (AG) is considered as a cause of morbidity and mortality worldwide. Group A rotavirus (GAR), calicivirus, human astrovirus (HAstV) and enteric adenovirus (AdV) have been recognized as major aetiopathological agents [1]. However, many of the AG cases remain undiagnosed. Recently, other entero-viruses such as picornaviruses, toroviruses and Aichi virus have been also shown to cause AG [2]. Aichi virus of the genus Kobuvirus, family Picornaviridae was first isolated in 1989 from a Japanese patient and reported to cause oyster-associated non-bacterial gastroenteritis [3]. The complete nucleotide sequence of Aichi virus was described in 1998 [4]. The genome consists of a single-stranded positive-sense RNA molecule of 8251 nt excluding a poly (A) tail. The single open reading frame of 7302 nt encodes a polyprotein of 2433 amino acids that is cleaved into structural proteins VP0, VP3, VP1 and non-structural proteins 2A, 2B, 2C, 3A, 3B, 3C and 3D [4]. Three genotypes A, B and C of Aichi virus have been identified on the basis of phylogenetic analysis of the 3C–3D junction and VP1 regions of the virus genome [5, 6].

Aichi virus was detected at low (0.5–4.1%) prevalence rates with its distribution mainly in A and B genotypes in countries from Asia, South America, Africa and Europe [6–10]. However, seroprevalence of antibodies to the Aichi virus has been estimated to be 55–76%, with an increase up to 95% with age [8, 11, 12]. Thus the epidemiology of AG caused by Aichi viruses is not clearly understood. This report documents for the first time prevalence and genotype distribution of Aichi virus strains detected in AG patients from India.

METHODS

Clinical specimens
In a 5-year retrospective study, a total of 1240 faecal specimens were collected from children aged ≤8 years hospitalized for AG in Pune, Aurangabad...
and Nagpur, three cities in Maharashtra state, western India. These included 912 specimens from Pune (January 2004–December 2008), 100 from Aurangabad (May 2005–February 2006) and 228 from Nagpur (June 2006–August 2007).

All patients were examined for fever, number of episodes, and duration of vomiting and diarrhoea, extent of dehydration, and treatment for the assessment of severity of disease by a 20-point scale of the Vesikari scoring system [13].

**RNA extraction and RT–PCR**

Viral RNA was extracted from 30% (v/v) stool suspension using QIAamp Viral RNA kit (Qiagen Co., Germany) according to the manufacturer’s instructions.

Presence of Aichi virus RNA in faecal specimens was determined by using nested RT–PCR targeting the 3C–3D junction region [5]. The nested PCR was performed using the outer F6261 and R6779 primers (F5’-ACACTCCCACTTCCGCCAGTA-3’, R5’-GGAAGAGCTGGTGTAAGA-3’) to amplify a 519-bp segment and the inner FC94b and R246k primers (F5’-GACTTCGGAGTGGTGTAAGA-3’, R5’-GACATCCGGTTGACGTTGAC-3’) to amplify a 265-bp segment within the 3C–3D region (nu-

**Detection of other enteric viruses**

All of the faecal specimens (n = 1240) were investigated for the presence of GAR by rotavirus antigen detection ELISA [15], and norovirus (NoV), AdV and HAstV, by RT–PCR/PCR using RdRp gene, hexon gene and ORF1a region-specific primers, respectively.

**RESULTS**

Aichi virus RNA was detected in 1.3% (12/912), 1% (1/100), and 0.4% (1/228) of the faecal specimens from children with AG from Pune, Aurangabad and Nagpur, respectively, with an overall prevalence of 1.1%. Aichi virus-infected children included 64.3% (9/14) males and 35.7% (5/14) females. Of these children, co-infections with GAR and HAstV were identified in 14.3% (2/14) and 7.1% (1/14), respectively. The highest Aichi virus positivity (35.7%) was detected in children aged between 25 and 36 months. During the 5-year study period, the infection rate was found to be higher in June and July while no infection was detected during January–May. Aichi virus-positive children suffered from moderate 57.1% (8/14) and severe 42.9% (6/14) AG infections.

Phylogenetic analysis of the sequences obtained for the 3C–3D junction region of the 14 strains of Aichi...
virus showed 94.2–99.5% nt identity with genotype B strains [B-171/05 (EF079157), Qld/2008/204 (EU715251)] isolated in Bangladesh and Australia. Six of the 14 strains could be amplified by RT–PCR of the VP1 gene. Sequencing of the amplicons revealed 92.6–98.3% nt identity with genotype B strain B-302 (EU143276) from Bangladesh (Fig. 1a, b).

**DISCUSSION**

The study describes for the first time the detection of Aichi virus genotype B strains in AG patients from India. Overall, the detection rate of Aichi virus was variable in the study regions (0.4–1.3%) and significantly low compared to other enteric viral aetiological agents GAR (36%), NoV (8.6%), AdV (6.2%) and HAstV (3.1%) of AG in the same set of specimens and/or previously reported from India [16–19]. These observations are in accord with those of earlier studies describing similar incidences of enteric viruses from countries in Asia, Europe and South America and differed from Aichi virus mono infections demonstrated in Tunisia [7–10, 20, 21]. GAR, NoV, AdV and HAstV infections have been reported to be

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**Fig. 1.** Phylogenetic trees of Indian Aichi virus strains based on (a) partial nucleotide sequences of 3C–3D junction region (200 bp) and (b) VP1 region (630 bp). The study strains are underlined. All strains are identified by abbreviations – accession number or name of city/strain name/country name. Nag, Nagpur; Aur, Aurangabad.
significantly higher in the first 2 years of life [16–19]. On the contrary, in the present study Aichi virus infections were more prevalent in children aged 2–3 years. In western India, GAR and HAstV infections prevailed in winter months, NoV infections were higher in summer months and AdV infections continued to occur throughout the year. By comparison, Aichi virus infections identified in the present study peaked in the rainy season [16–19].

VP1 sequence comparisons have been described as a valuable criterion in classification of picornaviruses [22]. Hence, phylogenetic analysis of the VP1 coding region of Aichi virus strains was performed. The distribution of the strains according to the VP1 sequences was similar to that obtained using analysis of the 3C–3D junction region indicating utility of both genomic regions for typing. While the Aichi virus strains from Japan and Europe were placed in genotype A and those from Africa in genotype C, all Indian strains were closer to genotype B strains from Australia and Bangladesh indicating circulation of genetically similar Aichi virus strains in these countries [6–10, 21].

In the present study, children (n = 153) hospitalized for reasons other than AG were detected negative for Aichi virus infections (data not shown). Moreover, a considerable proportion of co-infections with other enteric viral pathogens in Aichi virus-positive children were also noted. These data emphasize the need for further studies on epidemiology, transmission pattern and pathogenesis of Aichi virus to ascertain its aetiological role as a viral pathogen in AG.

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DECLARATION OF INTEREST

None.

REFERENCES


