European countries to avoid an increase in the incidence of RS in the current context of the novel A/H1N1 influenza virus pandemic. Because self-administration of aspirin or aspirin-containing medications is frequent specifically during the winter season, it is of utmost importance to disseminate messages to the public concerning the possible dramatic consequences of aspirin intake during viral infections.

Transparency Declaration

None of the authors have conflict of interest to declare.

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Surveillance of human astrovirus circulation in Italy 2002–2005: emergence of lineage 2c strains

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Abstract

By screening faecal samples collected over four consecutive years (2002–2005) from hospitalized children with diarrhoea in Palermo, Italy, astroviruses (HAstVs) were detected in 3.95% of the patients. The predominant type circulating was HAstV-1 but, in 2002, only HAstV-2 and -4 were identified. Interestingly, the HAstVs-2 detected appeared to be consistently different in 5′ end of their open reading frame 2 from the previously described subtypes. These novel type 2 strains were included in a new 2c lineage based on the phylogenetic analysis and the presence of nine peculiar substitutions.

Keywords: Astrovirus, gastroenteritis, genotyping, Italy, sequence analysis

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Human astroviruses (HAstVs) are enteric viruses associated with gastroenteritis in young children in both developed and developing countries [1]. Their prevalence is usually in the range 2–9%, although some studies in developing countries report rates of up to 28.2% [1–5]. The pathogenic role of HAstVs is still disputed because they are frequently found (33–65% of the cases) in conjunction with other enteric

viruses [5,6]. HAstVs have a single-stranded RNA containing three open reading frames (ORFs). ORF1a and ORF1b encode for nonstructural proteins, whereas ORF2 encodes for the capsid proteins precursor. Sequence analysis of ORF2 is commonly used for prediction of HAstVs serotypes (HAstV-1 to - 8) [1,7–9]. In general, HAstV-1 is the predominant type, whereas the frequency of types 2–5 varies with time and location, and types 6–8 are rare [1,7]. Recently, novel HAstV strains that are highly divergent from the known types have been described in Australia, India, USA and Mexico [10–12].

Epidemiological data on HAstV in Italian children with gastroenteritis are limited [13,14]. A 1-year study conducted in Palermo in 1999–2000, revealed a 3.1% of prevalence, with most strains being HAstV-1 and one HAstV-3 [15]. In the present study, we extended the investigation on the prevalence and genetic diversity of HAstVs in Palermo to 2002–2005. From January 2002 to December 2005, a total of 708 faecal samples (106 of 2002, 215 of 2003, 199 of 2004 and 188 of 2005) were obtained from children aged less than 5 years, hospitalized with acute gastroenteritis at the 'G. Di Cristina' Children's Hospital of Palermo. The samples were screened for the presence of HAstVs by enzyme immunoassay (EIA) (IDEIA, Dako Cytomation, Angel Drove, UK). The EIA-positive specimens were analyzed by RT-PCR with HAstV-specific primers Mon269 and Mon270 [9]. The obtained amplicons were sequenced and phylogenetic analysis was performed using a selection of reference sequences with the software MEGA, version 3.0 [16]. All stool specimens were also tested for presence of rotaviruses and noroviruses by RT-PCR and positive strains were submitted to genotyping [17,18].

HAstVs were found in 28 (3.95%) patients; in particular, five HAstVs (4.7%) were detected in 2002, 15 (7%) in 2003, seven (3.5%) in 2004 and one (0.5%) in 2005. These values are similar to those observed in other countries [1,2,19]. Mixed infections with other enteric viruses were found in 50% of the HAstV-positive samples. Co-infections by HAstV-rotavirus were the most frequent (64.3%), followed by HAstV-norovirus (21.4%) and HAstV-rotavirus-norovirus (14.3%). There were no significant differences in the symptoms and severity of diarrhoea between patients with HAstV single infection and patients with mixed infections (data not shown).

Fourteen HAstV strains, representative of each year, generated good quality sequence data for genotyping analysis and HAstV types-1, -2, and -4 were detected. In particular, HAstV-1 strains were identified in the years 2003–2005, whereas HAstV-2 and HAstVs-4 circulated only in 2002. In the phylogenetic tree, all Italian HAstVs-1 clustered within lineage 1d, following the classification scheme proposed by Guix *et al.* for lineage designation [1] (Fig. 1). The Italian HAstV-1d viruses showed 98–100% nucleotide (nt) identity to each other and 93.9–100% nt identity to HAstVs-1 previously detected [8,15,20,21]. Three HAstV strains were characterized as type-4 and clustered within lineage 4b. They displayed 99.4–99.7% nt identity to each other and 97.5–100% nt identity to HAstVs-4b identified in Brazil, Japan and Venezuela [8,19,22].

The two Italian HAstVs-2 displayed 99.3% nt identity to each other, 81.4–81.9% nt identity to HAstV-2a prototype (AF348771) and 90.3–93.5% nt identity to HAstV-2b prototype (L13745). In the phylogenetic tree, the Italian type-2 viruses segregated along with a group of HAstVs-2 identified in Norway, Ghana, Thailand and Australia [22–25]. This group of viruses forms a well defined genetic cluster (99% bootstrap value) and shares >95.1% nt identity. At present, there are no clearly defined criteria in the literature for classification of HAstV lineages.

Guix *et al.* [1] proposed that viruses belonging to different lineages within the same type diverge by at least 7% nt. Gabbay *et al.* [20] used a 5% nt divergence cut-off value, coupled with a high bootstrap value, to define a new lineage (1e) of type-1 HAstV. Because this novel group of type-2 HAstVs appears to fulfil these criteria, designation of this discrete lineage as 2c was believed to be appropriate and was adopted. Detailed visual inspection of the ORF2 sequence of HAstVs-2c revealed conserved nt polymorphisms (Table 1), allowing clear differentiation of HAstVs-2c from HAstVs-2a and -2b, although these nucleotide substitutions did not affect the deduced amino acid sequence.

The data obtained in the present study demonstrate that HAstV infection is common in children admitted to hospital with acute gastroenteritis and that HAstV-1 is predominant in Italy. Interestingly, when considering the HAstV-1 lineages, a pattern of temporal fluctuations was observed. Lineage 1d HAstVs were circulating in Palermo in 1999, and were replaced by lineage 1b strains in 2000 [15]. In 2003, HAstVs-1d re-emerged and continued to circulate until 2005. Prolonged circulation of HAstV-1d has been also described in Brazil and Spain where, respectively, they replaced HAstVs-1a in 2004 and were replaced by HAstV-1a and -1b strains in 1999–2000 [1,20]. High rates of seroprevalence to HAstV-1-specific neutralizing antibodies have been reported in many studies [26]. The population immunity against HAstV-1 could exert continual pressure on the viruses and drive the emergence and re-emergence of strains belonging to different lineages over time. This could account for the continual predominance of HAstV-1 in human population.