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Viral gastroenteritis in rotavirus negative hospitalized children <5 years of age from the independent states of the former Soviet Union

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ABSTRACT

Purpose: Rotavirus causes nearly 40% of all hospitalizations for AGE among children <5 years of age in the NIS of the former Soviet Union. The etiologic role of other established gastroenteritis viruses in this age group is unknown.
Methods: Laboratory-confirmed rotavirus negative fecal specimens ($N = 495$) collected between January and December 2009 from children in 6 NIS (Armenia, Azerbaijan, Belarus, Georgia, Republic of Moldova and Ukraine) were tested for norovirus, sapovirus, enteric adenovirus and astrovirus by real-time RT-PCR. Genotyping was carried out by sequencing and phylogenetic analysis.
Results: Norovirus, enteric adenovirus, sapovirus and astrovirus were detected in 21.8%, 4.0%, 3.2%, and 1.4% of the rotavirus negative specimens, respectively. Mixed infections were identified in 4.1% of the specimens. Phylogenetic analysis showed co-circulation of several different genotypes with GII.4 Den Haag (2006b) norovirus, GI.2 sapovirus, adenovirus type 41, and astrovirus type 1 causing majority of the infections.
Conclusion: Norovirus, enteric adenovirus, sapovirus and astrovirus account for a significant proportion (30.5%) of AGE in hospitalized children <5 years of age in 6 NIS.

1. Introduction

Viral gastroenteritis is the most common cause of acute diarrheal infections in infants and young children (Svraka et al., 2007); (Chhabra et al., 2013). Globally, rotavirus is the leading cause of severe diarrhea in children <5 years of age and is associated with ~450,000 deaths worldwide (Tate et al., 2012). Since the introduction of Rotarix in 2006 and RotaTeq in 2008, there is a substantial decline of rotavirus-associated acute gastroenteritis (AGE) in many industrialized countries including Latin America and in few developing countries in Africa and Asia (Curns et al., 2010; Molto et al., 2011; Braeckman et al., 2011; Dennehy, 2012; Patel et al., 2011).

The clinical impact of rotavirus disease has been well studied over the last couple of decades in the newly independent states (NIS) of the former Soviet Union (Ginevskaya et al., 1991; Spynu et al., 1991; Mirzayeva et al., 2009). Hospital based rotavirus surveillance established in six NIS (Armenia, Azerbaijan, Georgia, Republic of Moldova, Tajikistan, and Ukraine) with the assistance of the WHO and financial support from the Global Alliance for Vaccines and Immunization Alliance (GAVI) found that 38% of more than 13,000 specimens tested during 2008–2010 were positive for rotavirus (personal communication by Annemarie Wasley).

Abbreviations: AGE, acute gastroenteritis; NIS, newly independent states.

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Rotavirus surveillance in seven neighboring NIS (Belarus, Estonia, Kazakhstan, Latvia, Lithuania, Russian Federation and Turkmenistan) that could potentially introduce rotavirus vaccine in the near future showed that nearly 40% of all hospitalizations for acute gastroenteritis among children <5 years were rotavirus positive (Mirzayeva et al., 2009).

Apart from rotavirus, other established gastroenteritis viruses include norovirus, sapovirus, enteric adenovirus and human astrovirus. Norovirus is the primary cause of epidemic gastroenteritis in people of all ages globally and has emerged as the most important cause of medically-attended acute gastroenteritis in children <5 years of age in the United States of America (Payne et al., 2013). Sapoviruses also infect both children and adults and has also been associated with outbreaks as well as sporadic infections of AGE in humans (Lee et al., 2012; Chhabra et al., 2013). Noroviruses and sapoviruses belong to separate genera of the family *Caliciviridae*. They are non-enveloped single stranded, positive sense RNA viruses with genome size of 7.5–7.7 kb. Genetically, noroviruses can be divided into six genogroups (GI-GVI) of which viruses from GI, GII, and GIV can infect humans (Glass et al., 2009). Sapoviruses are grouped into at least 5 genogroups of which GI, GII, GIV and GV infect humans. To date 55 human adenovirus serotypes have been identified and classified into six subgenera A-G based on their biological and genetic characteristics. Subgenus "F" includes adenovirus serotypes 40 and 41 which cause acute gastroenteritis. Human astroviruses belong to family *Astroviridae* and can be classified into eight types (AstV-1 to 8) (De Benedictis et al., 2011).

The prevalence and genotype distribution of these four established AGE viruses in NIS of the former Soviet Union is unknown and therefore the aim of the current study is to determine the contribution of these viruses in causing AGE among hospitalized children <5 years of age in 6 NIS (Armenia, Azerbaijan, Belarus, Georgia, Republic of Moldova and Ukraine).

2. Materials and methods

2.1. Specimen information

In 2009, approximately 14,925 specimens were collected from children with AGE from six NIS (Armenia, Azerbaijan, Belarus, Georgia, Republic of Moldova and Ukraine) (personal communication by Annemarie Wasley). All NIS except Belarus belong to a WHO coordinated network of sentinel surveillance sites that followed a standard protocol for the collection of stool samples. Although Belarus was not a part of the network, a similar protocol of case definition and sample collection was followed. Children <5 years of age who were admitted for AGE ($\geqslant$3 loose stools and/or $\geqslant$1 episode of vomiting within 24 h period, with a duration of <7 days from the day of hospital admission) and who were hospitalized for at least 1 night were enrolled in the study (Mirzayeva et al., 2009). One fecal specimen per child was collected and written informed consent was obtained from the parents/guardians. The majority of the specimens were collected from children with AGE within 48 h of admission to the hospital. A total of 495 rotavirus negative fecal specimens were available for this study.

2.2. Laboratory testing

All specimens had been tested previously for group A rotavirus by a commercial IDEIA enzyme-linked immunosorbent assay kit (Oxoid Ltd., Ely, United Kingdom). Viral nucleic acid was extracted from 10% clarified fecal suspensions prepared in phosphate buffered saline using MagMax-96 Viral RNA Isolation Kit (Ambion, Foster City, CA, USA) according to the manufacturer's instructions on an automated KingFisher extractor (Thermo Fisher Scientific, Pittsburgh, PA, USA). The presence of norovirus, sapovirus, enteric adenovirus and human astrovirus was detected by real-time (RT) PCR assay using the AgPath-ID One-Step RT-PCR kit (Applied Biosystems, Foster City, CA, USA) and virus-specific oligonucleotide primers and probes as described previously (Vega et al., 2011; Oka et al., 2006; Podkolzin et al., 2009; Lyman et al., 2009). For the RNA viruses, cDNA synthesis was carried out at 45 °C for 10 min followed by inactivation of the reverse transcriptase at 95 °C for 10 min. Cycling conditions for PCR amplification included 40 cycles of denaturation at 94 °C for 15 s, and annealing/extension at 60 °C for 1 min.

Genotyping of positive samples was carried out by conventional nested (RT) PCR using the OneStep RT-PCR kit (QIAGEN, Valencia, CA, USA) and virus-specific oligonucleotide primers (Kojima et al., 2002; Okada et al., 2006; Noel et al., 1995; Allard et al., 1992). RT-PCR products were separated on 2% agarose gels and the amplicons of appropriate sizes were purified with the QIAquick gel extraction kit (QIAGEN) followed by cycle sequencing using BigDye® Terminator v1.1 and 3130XL automated DNA sequencer (Applied Biosystems). Multiple sequence alignments were carried out using ClustalW (Thompson et al., 1994) and phylogenetic analysis was done using MEGA 4 (Tamura et al., 2007). Phylogenetic trees were generated using the neighbor-joining algorithm and Kimura 2-parameter distance model. The reliability of the phylogenetic trees was tested by applying 1000 bootstrap replicates.

3. Results

Of 14,925 specimens collected from 6 NIS, rotavirus was detected in 5900 (39.5%) specimens. Out of 9025 rotavirus negative specimens a small subset of 495 specimens was tested for norovirus, sapovirus, enteric adenovirus and astrovirus. At least one virus was detected in 145 of 495 rotavirus-negative specimens of which norovirus was detected in 108 (74.4%) of the virus-positive specimens (Table 1). Overall, GI and GII norovirus, enteric adenovirus, sapovirus and astrovirus were detected in 108 (21.8%), 20 (4.0%),

Table 1
Positivity rate of norovirus, sapovirus, adenovirus and astrovirus in rotavirus-negative fecal specimens of hospitalized patients from 6 NIS in 2009.

Newly independent states	No. of samples tested	GI norovirus no. positive (% positivity)	GII norovirus no. positive (% positivity)	Sapovirus no. positive (% positivity)	Adenovirus no. positive (% positivity)	Astrovirus no. positive (% positivity)
Belarus	259	1 (0.3%)	50 (19.3%)	8 (3%)	4 (1.5%)	2 (0.7%)
Azerbaijan	66	1 (1.5%)	13 (19.6%)	0	2 (3.0%)	0
Armenia	32	0	3 (9.3%)	1 (3.1%)	4 (12.5%)	0
Georgia	37	1 (2.7%)	8 (21.6%)	1 (2.7%)	4 (10.8%)	3 (8.1%)
Ukraine	67	0	17 (25.3%)	5 (7.4%)	4 (5.9%)	2 (2.9%)
Republic of Moldova	34	0	14 (41.1%)	1 (2.9%)	2 (5.8%)	0
Total	495	3 (0.6%)	105 (21.2%)	16 (3.2%)	20 (4.0%)	7 (1.4%)