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Group A rotavirus genotypes in hospital-acquired gastroenteritis in Italy, 2012–14

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SUMMARY

Background: Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (AGE) in young (aged <5 years) children, causing ~250,000 deaths worldwide, mostly in developing countries. Differences on nucleotide sequences of VP7 (G-type) and VP4 (P-type) genes are the basis for the binary RVA nomenclature. Although at least 32 G-types and 47 P-types of rotavirus are presently known, most RVA infections in humans worldwide are related to five major G/P combinations: G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8].
Aim: To provide the hospitals of the Italian surveillance network with update information on RVA AGE.
Methods: During RVA gastroenteritis surveillance in Italy in 2012–14, a total of 2341 RVA-positive faecal samples were collected from children hospitalized with AGE, and RVA strains were genotyped following standard EuroRotaNet protocols.
Findings: Most strains analysed belonged to the five major human genotypes and 118 out of 2341 (5.0%) were reported to be hospital-acquired. Comparison of the distributions of the RVA genotypes circulating in the community or associated with nosocomial infections showed a different distribution of genotypes circulating inside the hospital wards, with respect to those observed in the community. G1P[8] and G9P[8] RVA strains were detected frequently, whereas G12P[8] caused a single large nosocomial outbreak.
Conclusion: The information from this study will be useful to implement guidelines for preventing RVA AGE and optimizing the management of patients in hospital wards.

Introduction

Group A rotaviruses (RVA) are the leading cause of acute gastroenteritis (AGE) in young children worldwide, and are estimated to cause 250,000 deaths every year among children aged 0–5 years, mostly in developing countries of Sub-Saharan Africa and South East Asia [1].

Rotaviruses belong to the Reoviridae family, and possess a genome composed of 11 double-stranded RNA genomic segments encoding six structural (VPs), and five or six non-structural (NSPs) proteins. The RVA capsid is composed of

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three protein layers, without envelope. The outer layer comprises the VP7 (capsid) and VP4 (spike) proteins [2].

Differences in the intermediate layer protein VP6 define nine rotavirus antigenically distinct groups, causing gastroenteritis in humans (A, B, and C) and animals (A-I) [2,3]. RVA may be classified in G and P genotypes, based respectively on nucleotide differences of RNA sequences encoding the outer layer proteins VP7 and VP4, which represent the antigenic sites where the neutralization domains of rotavirus are located [4—6]. Nucleotide differences in gene 9 (VP7) and gene 4 (VP4) are currently used to classify RVA in 32 G- and 47 P-genotypes [7,8]. Although several G/P combinations have been reported, the five genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] RVA cause up to 75% of human RVA infections worldwide [9—11].

Hospital-acquired viral infections are a major concern for public health. Among them, AGE is one of the most prevalent and may severely affect susceptible children admitted for other causes [12]. Children admitted to hospital or neonatal intensive care units are at high risk of exposure to infection because of their vulnerable condition, including in some cases the lack of a mature microbiota. Nosocomial AGE is a cause of prolonged hospital stay, increasing both the costs for the public health system and the mortality in preterm neonates. Several reports have demonstrated that RVA are frequent causes of nosocomial AGE in hospitals [13—16]. Moreover, both symptomatic and asymptomatic RVA infections of adults have been described [17—19], which may sustain virus transmission between patients; asymptomatic healthcare staff have been reported as the souorce of ongoing transmission of nosocomial outbreaks [20].

These infections raise costs associated with duration of hospital stay, re-hospitalization, the ward closures/opening measures, and the loss of working days for parents and staff. Moreover, nosocomial rotavirus infections are associated with the worsening of symptoms and clinical conditions mostly in children affected by severe diseases [21].

The Istituto Superiore di Sanità in collaboration with several hospitals and universities across Italy (the RotaNet—Italy Study group) is part of the European Rotavirus Network (EuroRotaNet, http://www.eurorota.net/), a national surveillance network for RVA gastroenteritis. Following its establishment in January 2007 the network has been conducting rotavirus strain surveillance in 15 European countries. Epidemiological surveillance performed between 2007 and 2009 showed that RVA strains possessing the G1—G4 and G9 G-genotypes, and the P[4] and P[8] P-genotypes are the most widespread cause of RVA gastroenteritis in Italy [22], confirming previous studies conducted worldwide [9,23—25].

During the 2012—13 and 2013—14 RVA surveillance years, a remarkable number of hospital-acquired RVA infections was notified by the paediatric units participating in the Italian surveillance net. The percentage of these infections accounted for 5.6% (62 out of 1112 cases) in 2012—13 and for 4.6% (56 out of 1229) in 2013—14, for a total of 5.0% (118 out of 2341) in the two-year surveillance considered in this study. This pilot study was performed to compare the RVA genotype circulation in community and in nosocomial AGE cases, and to investigate possible correlations involved in the apparent increase of nosocomial (RVA) AGE cases notified.

Methods

Sample collection

Stool specimens were collected from children admitted to the paediatric units of public hospitals throughout Italy, between September 2012 and August 2014. The September to August years were selected to encompass two northern hemisphere rotavirus seasons. Rotavirus infection was diagnosed using commercial antigen detection methods. Clinical information was obtained from RotaNet—Italy questionnaires filled in by the hospital staff, in compliance with the Informed Consensus Agreement. Most samples were obtained from patients specifically hospitalized for AGE, enrolled in the molecular surveillance activities of RotaNet—Italy [22]. In the same sampling period, stool samples were also collected from patients who had been admitted to the hospital for other clinical reasons and who developed AGE symptoms between two and seven days after admission. These cases were defined as nosocomial RVA AGE following three main criteria: (i) the patient was admitted with a diagnosis other than gastroenteritis; (ii) the first enteric symptoms appeared at least 48 h after the admission; (iii) the RVA AGE was diagnosed by rapid enzyme-linked immunosorbent assays by the paediatric unit.

Nucleic acid extraction and RVA genotyping

Total viral RNA was extracted from 140 μL of 10% faecal suspensions in distilled water, using the Viral RNeasy Mini Kit (Qiagen, Milan, Italy), according to the manufacturers' instructions. RNA was eluted in 60 μL of RNase-free water, and stored at −80°C.

After an initial step of denaturation, the viral RNA was subjected to reverse transcription (RT) using the Invitrogen Superscript III reverse transcriptase kit (Life Technologies, Monza, Italy) with a single cycle at 37°C for 60 min and 95°C for 5 min. The obtained DNA was then used as template for polymerase chain reaction (PCR) amplification of VP7 (primers Beg9—End9) and VP4 (primers Con3—Con2) segments. The reactions were performed with the Invitrogen Platinum Taq kit (Life Technologies), following the manufacturers' instructions. RVA genotyping was carried out by a multiple semi-nested PCR using a mixture of primers specific for G- and P-types, as previously described [5,26].

Reverse transcription and all PCR reactions were accomplished following standardized EuroRotaNet protocols (http://www.eurorota.net/docs.php). PCR products were visualized on 2% agarose gel, stained with gel red, for genotype assignment.

Statistical analysis

Correlations between community and nosocomial rates of RVA infections were analysed using Student's *t*-test on the whole set of data with a 95% confidence interval (CI). The statistical correlation between the different RVA genotypes circulating in community and nosocomial cases was also analysed applying the chi-square test, with a 95% confidence interval (95% CI). Data were analysed with the SocSciStat online statistical calculators (http://www.socscistatistics.com/).