Diagnostic Microbiology and Infectious Disease xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

# Molecular detection of gastrointestinal viral infections in hospitalized patients

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ARTICLE INFO

*Article history:*
Received 15 March 2013
Received in revised form 10 July 2013
Accepted 31 July 2013
Available online xxxx

*Keywords:*
Gastrointestinal viruses
Real-time RT-PCR
Gastroenteritis
Stool samples

ABSTRACT

Gastrointestinal viral syndromes are a common cause of morbidity and mortality in humans worldwide. Etiological agents include a large number of viruses encompassing several orders, families, and genera. During the period April 2011 to April 2012, 689 stool samples from as many patients hospitalized at the Fondazione IRCCS Policlinico San Matteo of Pavia exhibiting gastrointestinal syndromes were examined for the presence of rotavirus, norovirus, astrovirus, adenovirus, rhinovirus, enterovirus, parechovirus, bocavirus, coronavirus, sapovirus, cosavirus, and aichi virus using polymerase chain reaction assays. Gastrointestinal viral agents were detected in 246 (36%) patients of the 689 analyzed. Adenovirus and norovirus were the most common viruses in this cohort, while aichi virus was the only gastrointestinal agent not detected. Surprisingly, rhinovirus was one of the most frequently detected viruses. However, a potential association with gastroenteritis remains to be confirmed.



## 1. Introduction

Viral gastroenteritis is a common cause of morbidity and mortality in humans worldwide, affecting all age groups. Etiological agents include a number of viruses encompassing several orders, families, and genera.

Viral pathogens causing acute gastroenteritis include *Rotavirus* (RV), members of the *Caliciviridae* family such as *Norovirus* (NoV) and *Sapovirus* (SaV), *Adenovirus* (HAdV) and *Astrovirus* (HAstV) (Eckardt and Baumgart, 2011). Viral gastroenteritis can be more severe in young children, the elderly, and immunocompromised patients. RV causes 600,000–875,000 deaths per year (Clark and McKendrick, 2004), the majority of which occur in developing countries, and it is the most severe and common cause of diarrhea in children under 5 years of age (Wilhelmi et al., 2003). Frequently, NoVs are responsible for outbreaks and sporadic cases of nonbacterial gastroenteritis in children and adults worldwide (Kele et al., 2011). SaV is considered an important cause of gastroenteritis in children under 5 years of age, while it is of minor importance in adults (Eckardt and Baumgart, 2011). HAstVs and enteric HAdVs cause gastroenteritis, primarily in children less than 4 years of age (Dennehy, 2011).

Human bocavirus and human coronavirus, mainly involved in infections of the respiratory tract, are also implicated in gastrointestinal infections (Clark and McKendrick, 2004; Khan and Bass, 2010).

Members of the *Picornaviridae* family, for example, enterovirus (EV), parechovirus (HPeV), aichi virus (AiV), and human cosavirus (HCoSV), are causative agents of gastroenteritis (Harvala et al., 2010; Holtz et al., 2008). Recently, also rhinoviruses (HRV) have been detected in stool samples (Harvala et al., 2012).

Considering the highly contagious nature of these viruses, surveillance of new cases is needed for outbreak prevention and control. Unfortunately, the number of agents implicated in gastrointestinal infections makes the construction of a comprehensive diagnostic panel very challenging. The present study is aimed at evaluating the circulation of gastrointestinal viruses in hospitalized patients using polymerase chain reaction (PCR) assays.

## 2. Methods

### 2.1. PCR assays

Overall, 689 stool samples stored in the period April 2011 to April 2012 from as many patients (356 pediatrics and 333 adults) with gastrointestinal syndromes hospitalized at the Fondazione IRCCS Policlinico San Matteo of Pavia (a teaching and university hospital with 50,000 admissions, 2,500,000 outpatients visits, and 94,000 emergency consultations per year) were systematically examined for the presence of gastroenteric viruses. Gastrointestinal syndrome was defined as the rapid onset of 2 or more of the following symptoms: diarrhea, vomiting, nausea, fever, or abdominal pain. In the present study, stool samples collected from patients with diarrhea during the acute phase of gastroenteritis were retrospectively analyzed.

In more detail, all 689 samples were tested by: i) real-time reverse transcriptase polymerase chain reaction (RT-PCR) for NoV, RV, HAstV, EV, HRV, HPeV, SaV, human coronavirus (hCoV); ii) real-time PCR for HAdV; iii) nested RT-PCR for AiV and HCoSV; and iv) nested PCR for human bocavirus (hBoV).

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http://dx.doi.org/10.1016/j.diagmicrobio.2013.07.020

**Table 1**
Molecular parameters used for detection of different gastrointestinal viruses in stool samples.

| Virus | molecular test | Gene target | Thermal profile | Cycle no. | Oligonucleotide sequence (5′→3′) | References |
|---|---|---|---|---|---|---|
| NoV GI | Real-time RT-PCR | capsid | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>45 | QNIF4: CGCTGGATGCGNTTCCAT<br>NV1LCR: CTTAGACGCCATCATCATTTAC<br>NV1LCpr probe: TGGACAGGAGAYCGCRATCT | Da Silva et al. (2007) |
| NoV GII | Real-time RT-PCR | ORF1-ORF2 junction | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>45 | QNIF2d: ATGTTCAGRTGGATGAGRTTCTCWGA<br>COG2R: TCGACGCCATCTTCATTCACA<br>QNIFS probe: AGCACGTGGGAGGGGATCG | Da Silva et al. (2007) |
| RV | Real-time RT-PCR | non-structural protein 3 | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>50 | ROTAs (fwd1): ACCATCTTCACGTAACCCTC<br>ROTAs (fwd2): ACCATCTACACATGACCCTC<br>ROTAas (rev): CACATAACGCCCCTATAGCC<br>ROTA (probe): ATGAGCACAATAGTTAAAAGCTAACACTGTCAA | Van Maarseveen et al. (2010) |
| HAstV | Real-time RT-PCR | ORF-1a | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>50 | ASTVs: TCTYATAGACCGYATTATTGG<br>ASTVas: TCAAATTCTACATCATCACCAA<br>ASTV probe: CCCCADCCATCATCATCTTCATCA | Van Maarseveen et al. (2010) |
| EV | Real-time RT-PCR | 5′-noncoding region | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>40 | rhientfwd: CCTCCGGCCCCTGA<br>P1.4taq: GATTGTCACCATAAGCAGCC<br>Entpr1 probe: CGGAACCGACTACTTTGGGT | Van Doornum et al. (2007) |
| HRV | Real-time RT-PCR | 5′-noncoding region | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>40 | primer fwd: CPXGCCZGCGTGGC<br>primer rev: GAAACACGGACACCCAAAGTA<br>probe: TCCTCCGGCCCCTGAATGYGGC | Lu et al. (2008) |
| HPeV | Real-time RT-PCR | 5′-NTR | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 58 °C[a]/30″ 72 °C/10″ | 1<br>50 | AN345: GTAACASWWGCCTCTGGGSCCAAAAG<br>AN344: GGCCCCWGRTCAGATCCAYAGT<br>AN257 probe: CCTRYGGGTACCTYCWGGGCATCCTTC | Nix et al. (2008) |
| SaV GI<br>GII<br>GIV | Real-time RT-PCR | polyprotein | 50 °C/10′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>40 | sapo.fwdA: ACCAGGCTCTCGCCACCTA<br>sapo.fwdB: ATTTGGCCCTCGCCACCTA<br>sapo.rev: GCCCTCCATYTCAAACACTAWTTT<br>sapo.probeA: CTGTACCACCTATGAACCA<br>sapo.probeB: TTGTACCACCTATGAACCA<br>sapo.probeC: TGTACCACCTATAAACCA<br>sapo.probeD: TGCACCACCTATGAAC | Logan et al. (2007) |
| hCoV OC43 | Real-time RT-PCR | nucleoprotein | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>40 | OC43 fwd: CGATGAGGCTATTCCGACTAGGT<br>OC43 rev: CCTTCCTGAGCCTTCAATATAGTAACC<br>OC43 probe: TCCGCCTGGCACGGTACTCCCT | Dare et al. (2007) |
| hCoV 229E | Real-time RT-PCR | nucleoprotein | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>40 | 229E fwd: CAGTCAAATGGGCTGATGCA<br>229E rev: AAAGGGCTATAAAGAGAATAAGGTATTCT<br>229E probe: CCCTGACGACCACGTTGTGGTTCA | Dare et al. (2007) |
| hCoV NL63 | Real-time RT-PCR | nucleoprotein | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>40 | NL63fwd: GACCAAAGCACTGAATAACATTTTCC<br>NL63 rev: ACCTAATAAGCCTCTTTCTCAACCC<br>NL63 probe: AACACGCTTCCAACGAGGTTTCTTCAACTGAG | Dare et al. (2007) |
| hCoV HKU1 | Real-time RT-PCR | replicase 1b | 45 °C/10′ 95 °C/10′<br>95 °C/15″ 55 °C/1′ | 1<br>40 | HKU1 fwd: CCTTGCGAATGAATGTGCT<br>HKU1 rev: TTGCATCACCACTGCTAGTACCAC<br>HKU1 probe: TGTGTGGCGGTTGCTATTATGTTAAGCCTG | Dare et al. (2007) |
| HAdV | Real-time PCR | hexon | 50 °C/2′ 95 °C/10′<br>95 °C/15″ 60°/1′ | 1<br>40 | AQ1: GCCACGGTGGGGTTTCTAAACTT<br>AQ2: GCCCCAGTGGTCTTACATGCACATC<br>AP probe: TGCACCAGACCCGGGCTCAGGTACTCCGA | Heim et al. (2003) |
| AiV | Nested RT-PCR | 3CD junction | 50 °C/10′ 95 °C/10′<br>95 °C/30″ 55 °C/30″ 72°/1′ | 1<br>40 | 6261: ACACTCCCACCTCCCGCCAGTA<br>6779: GGAAGAGCTGGGTGTCAAGA | Yamashita et al. (2000) |
| | | | 94 °C/10′<br>94 °C/30″ 65 °C/30″ 72 °C/1′<br>72 °C/10′ | 1<br>35<br>1 | C94b: GACTTCCCCGGAGTCGTCGTCT<br>246k: GACATCCGGTTGACGTTGAC | Kaikkonen et al. (2010) |
| HCoSV | Nested RT-PCR | 5′NTR | 45 °C/20′ 94 °C/5′<br>94 °C/1′ 55 °C[b]/1′ 68 °C/45″<br>94 °C/1′ 53°/1′ 68°/45″<br>68 °C/10′ | 1<br>10<br>40<br>1 | DKV-N5U-F1: CGTGCTTTACACGGTTTTTGA<br>DKV-N5U-R2: GGTACCTTCAGGACATCTTTGG | Kapoor et al. (2008) |
| | | | 95 °C/10′<br>95 °C/45″ 57 °C[b]/1′ 72 °C/30″<br>95°C/30″ 54 °C/30″ 72°C/30″<br>72 °C/10′ | 1<br>10<br>30<br>1 | DKV-N5U-F2: ACGGTTTTTGAACCCCACAC<br>DKV-N5U-R3: GTCCTTTCGGACAGGGCTTT | |
| hBoV 1-2-3-4 | Nested PCR | VP1/2 | 95 °C/10′<br>95 °C/35″ 58 °C[b]/1′ 72 °C/1′<br>95 °C/30″ 54 °C/45″ 72 °C/45″<br>72 °C/10′ | 1<br>10<br>30<br>1 | AK-VP-F1: CGCCGTGGCTCCTGCTCT<br>AK-VP-R1: TGTTCGCCATCACAAAAGATGTG | |
| | | | 95 °C/10′<br>95 °C/35″ 60 °C[b]/1′ 72 °C/1′<br>95 °C/30″ 58 °C/45″ 72 °C/45″<br>72 °C/10′ | 1<br>10<br>30<br>1 | AK-VP-F2: GGCTCCTGCTCTAGGAAATAAAGAG<br>AK-VP-R2: CCTGCTGTTAGGTCGTTGTTGTATGT | Kapoor et al. (2010) |

ORF = open reading frame; NTR = nontranslated region; 3CD = C terminus 3C-N terminus 3D junction.
[a] Probe detection during the 58 °C annealing step.
[b] A decrease of 0.5 °C in annealing temperature each cycle.

Please cite this article as: Rovida F, et al, Molecular detection of gastrointestinal viral infections in hospitalized patients, Diagn Microbiol Infect Dis (2013), http://dx.doi.org/10.1016/j.diagmicrobio.2013.07.020