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and Immunology, Faculty of Medicine, University of Ljubljana, for the routine detection of respiratory viruses, including respiratory syncytial virus (RSV), human rhinoviruses (hrRV), human metapneumovirus (hMPV), human coronaviruses (HCoVs), human bocavirus (HBoV), adenoviruses (AdV), parainfluenza virus (PIV) and influenza viruses A and B (Flu A-B) by real-time RT-PCR. HCoVs positive samples with high viral load (low Ct value) and those negative for all other respiratory viruses were included into further testing by amplifying a 440-bp-long fragment of the highly conserved polymerase gene.

Results: From December 2013 to February 2016, a total 16686 nasopharyngeal swabs from patients with acute respiratory tract infections were enrolled in the study. From these 976 (5.8%) were positive for HCoVs and 523 (32.6%) were negative for RSV, hRV, hMPV, HCoVs, HBoV, AdV, PIV, Flu A and FluB by real-time RT-PCR. From 523 HCoVs positive samples 129 were further tested for all HCoVs species, including 47 HCoV-HKU1, 44 HCoV-OC43, 24 HCoV-NL63, 11 HCoV-229E, 1 HCoV-HKU1/HCoV-229E and 1 HCoV-NL63/HCoV-229E. Only HCoVs positive samples (HCoV-HKU1 and HCoV-OC43) with high viral load (Ct-value less than 30) were included into further testing. To characterize the overall diversity of coronavirus sequences, 65 sequences have been included in phylogenetic analysis; 31 sequences of HCoV-OC43 and 34 sequences of HCoV-HKU1.

Conclusions: Among four circulating HCoVs, HCoV-HKU1 and HCoV-OC43 seem to show the highest prevalence and incidence in hospitalized patients. The phylogenetic analysis shows that Slovenian human coronavirus strains from this study belong to the four clusters, two grouping HCoV-OC43 and two HCoV-HKU1. The present study draws genetically diversity of human coronaviruses in Slovenian hospitalized patients.

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Rapid diagnosis of respiratory viral infections in primary health care

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Background: Respiratory tract infections (RTI) are the most common acute problems in primary health care. RTIs are mainly of viral origin. The epidemiology of respiratory viruses in primary health care settings is scarcely reported, as diagnostic tests for RTIs are sporadically used by general practitioners (GP). Rapid, sensitive and specific identification of viral RTIs might assist diagnostic interpretation and potentially prevent inappropriate use of antibiotics.

Aim: To increase our insight in the epidemiology of viral RTIs in primary health care; to evaluate the feasibility and diagnostic accuracy of a new rapid test for respiratory viruses (mariPOC® test system, ArcDia International, Turku, Finland) in primary health care.

Methods: Patients with RTI symptoms presenting to a primary healthcare practice in the neighborhood of the Academic Medical Center (AMC) Amsterdam were asked to complete a small questionnaire about his/her symptoms and undergo nasopharyngeal swab sampling. The swab was immediately tested at the point-of-care with the automated mariPOC® test. The mariPOC® test is a simple to perform test for the detection of nine respiratory viruses (influenza A and B, parainfluenza type 1, 2 and 3 viruses, respiratory syncytial virus (RSV), human adenovirus, human bocavirus, and human metapneumovirus) and Streptococcus pneumoniae, with preliminary results ready within 20 min and final results within 2 h. The remaining sample solution was transferred on the same day to the Laboratory of Clinical Virology at the AMC for reference testing with multiplex PCR. Clinical and epidemiological data were collected including age, gender, underlying illness, presenting symptoms, time from onset of symptoms and detected viruses. The sensitivity and specificity of the mariPOC® as compared to PCR was calculated. The clinical feasibility of the mariPOC® test was evaluated using a questionnaire for the study participants and GPs.

Results: From November 11 2015 till March 30 2016 a total of 371 patients (59.3% female, median age 45 years) were included. One or more respiratory viruses were detected by PCR in 43.4% (n = 161) of the collected nasopharyngeal swabs. Rhinovirus (RV) was the most frequently detected virus with a prevalence of 11.8%. When reporting samples with Ct up to 40 as positive findings in PCR, the sensitivity and specificity of the mariPOC® test were respectively for influenza A virus (n = 24), 54.2% and 98.9%; for influenza B virus (n = 18), 72.2% and 99.5%; and for RSV (n = 12), 50.0% and 100%. In samples with higher viral load (i.e. Ct-value < 30) sensitivity for influenza A, influenza B and RSV was 85.7%, 78.6% and 87.5%, respectively. The availability of a diagnostic test for respiratory viruses in primary healthcare was appreciated by both patients and GPs.

Conclusion: Respiratory viruses are frequent causes of RTIs in primary health care. Acute infections with high viral loads were accurately detected by the mariPOC test and for these infections a rapid test would be a helpful tool for GPs. Both doctors and patients were positive about the availability of a rapid test in primary health care. The development of a rapid test for rhinovirus would be valuable as rhinovirus was the most frequently detected virus.

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False-negative detection of respiratory syncytial virus as an example that regular update of RT-PCR is required for reliable molecular detection of respiratory viruses

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Objectives: A respiratory sample that was RT-PCR adenovirus positive and negative for other tested respiratory viruses was cultured for adenovirus serotyping in December 2013. Surprisingly, shell vial culture was positive for respiratory syncytial virus (RSV).

Methods: In June 2013 an update of the RT-PCR that was used to detect respiratory viruses was started [1]. The update included the following steps: updating alignments of every target with sequences retrieved from GenBank, amplification and sequence
analysis of primer and probe regions from positive clinical materials, selection and validation of new, updated primers and probes.

Results: Analysis of sequences obtained from GenBank and clinical materials showed that primer and probe sequences for detection of influenza (Flu) A virus, parainfluenza virus (PIV) 1, human rhinovirus (HRV) and human coronaviruses (HCoV) 229E, NL63, and HKU-1 still showed a 100% match to the circulating virus genomes. However, primer and/or probe sequences for detection of Flu B, RSV, PIV2, PIV3, PIV4, human metapneumovirus (HMPV), and HCoV OC43 required some adjustment. The RSV assay, that detects both RSV-A and RSV-B, consisted of two sense primers, one antisense primer and two probes. All sequences obtained from RSV positive clinical isolates from 2013 contained a mismatch to both probe sequences. This mismatch was also observed in two sequences from GenBank (both from The Netherlands, 2012) but not in other L gene sequences from GenBank. Validation of adapted RSV probes was ongoing at the moment that a RSV RT-PCR negative sample resulted in a RSV positive culture. The cultured RSV strain was negative in the diagnostic RT-PCR, but positive in the updated RSV RT-PCR that was validated at that moment. However, the relative fluorescent unit (RFU) signal of the RSV strain was lower than that of positive control material and sequence analysis of the strain showed a mismatch with the new probe. This additional mismatch was not observed in sequences obtained from GenBank, but in January and February 2014 several clinical samples tested in our setting showed RSV signals with low RFU and turned out to have the same mismatch. Therefore, a new update of the RSV RT-PCR was started. Another target of the RT-PCR was considered, but alignment of over 100 whole genomes of RSV showed that the current target region in the L gene remained the target of choice. The two relatively short tagman probes were replaced by a longer tagman probe that should be able to better tolerate mismatches. The new RSV assay will be used next RSV season.

Conclusion: Due to the high mutation frequency in RNA viruses, regular update of RT-PCR assays is mandatory for reliable molecular detection of respiratory viruses.

Reference

[1] Templeton et al., Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4.

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Genetic characterization of human respiratory syncytial virus (hRSV) infecting children in France during two winter seasons

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Background: Worldwide, the human respiratory virus (hRSV) genetic characterization takes a significant place and highlights the importance to monitor the circulation of different genotypes and/or the emergence of new variants. These can affect the susceptibility to the current or future treatments of hRSV infection. There is no information to date regarding the molecular epidemiology of hRSV in France. The aim of this study was to investigate the genetic diversity of group A and B hRSV isolates, obtained from children under 1 year old, during two recent consecutive epidemic periods.

Material and methods: Nasopharyngeal swabs or aspirates obtained from children included in a study who evaluate the efficacy of the use of salt solution 3% in the management of non-complicated bronchiolitis, the “GUERANDE” study, were analyzed. The samples were collected in hospital centers in France who participate to the study during two winter seasons, 2012–2013 and 2013–2014. Viral ARN was extracted using Qiasymphony DSP Virus/Pathogen Mini kit®. All samples were tested by a real time RT-PCR for the detection and group typing of the hRSV A/B. The amplification and sequencing of the second variable region (HRV-2) of the G gene were performed using One-step RT-PCR kit (Qiagen, Hilden, Germany) and specific primers and protocols. The sequences obtained and reference sequences for different genotypes were analyzed with BioEdit® software and phylogenetic trees were constructed by the neighbor-joining method in MEGA7 software.

Results: A total of 719 samples were included in the “GUERANDE” study. These samples were collected from children under 1 year old consulting for a non-complicated bronchiolitis in 24 hospital centers distributed in 12 different French regions. The hRSV group typing identified 375(52.16%) hRSV-A, 247(34.35%) hRSV-B, as well as 14(1.95%) hRSV-A/B co-detections and 83(11.54%) were negatives for hRSV detection. The amplification and sequencing of the HRV-2 G gene were successfully undertaken for 228(60%) of the 375 hRSV-A and 215(87%) of the 247 hRSV-B.

The analyzed sequences of hRSV-A fell within different clusters genotypes, corresponding to ON1 in the majority of cases, but also NA1 and GA2. The ON1 identified sequences were closely related to GA2. The sequences that had been sampled in different epidemics did not form distinct clusters.

The phylogenetic analysis of hRSV-B sequences allows the identification of 3 genotypes, BA-9, BA-10 and BA-C. A distinct BA-9 cluster was observed for the sequences sampled in Toulouse. This cluster was confirmed by different phylogenetic analysis. The