Abstract no: 62
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Surveillance of a severe A(H1N1)pdm09 dominated influenza season in N. Greece, 2015–2016

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Objects of the research: The objective was the epidemiological and virological analysis of a severe influenza A(H1N1)pdm09-dominated season, 2015–2016 in N. Greece.

Materials and methods: 686 pharyngeal swabs/washes from patients with influenza-like-illness were tested up to week 17. Influenza viruses were typed and their haemaglutinin was sequenced. (CDC and WHO protocols). All of the samples were non-sentinel, mostly originating from outpatient and inpatient hospital clinics.

Results: 246 samples were positive for influenza. A and B viruses were detected in 220 and 24 samples respectively. B viruses appeared during the first and the last weeks of the season. Out of the A viruses, 206 were H1N1pdm09 and 8 were H3N2. Molecular analysis of B viruses revealed that B-Victoria lineage viruses dominated this season. A(H1N1)pdm09 viruses were A/California/7/2009(H1N1)pdm09-like, but with accumulating variations at antigenic and other HA sites, that designated them into two distinct phylogenetic clades, 6B.1 and 6B.2.

Samples ranged between 0 and 86 years of age, with an average 40.6 years. Sixty-seven ICU patients had an average age of 54.5 years and in all of them A(H1N1)pdm09 was detected and most suffered from underlying medical conditions, were obese or pregnant. Most common complications were ARDS and pneumonia. In total, 39 fatalities have been reported in northern Greece. All of them were attributed to A(H1N1)pdm09. Interestingly, 6 of the decedents did not suffer from any underlying medical condition, 10 of those were obese (26.%) and 25 were suffering from cardiological problems (64%). Complications and underlying medical conditions are mentioned in detail in Table 1.

Conclusions: Compared to the findings from previous studies from Greece, it seems that it was a severe A(H1N1)pdm09-dominated influenza season. This subtype seems to cause more severe influenza illness, in a younger age group, more often causing hospitalization to otherwise healthy individuals. Circulating strains are increasingly more divergent. Our findings confirmed the genetic instability of influenza type A(H1N1)pdm09 viruses and highlighted the importance of continuous surveillance for the effective management of viral epidemics. Variation observed in Greek and also in European B viruses prompted WHO to change the B vaccine component to B/Victoria.

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Enhancement of respiratory virus isolation from specimens using centrifugation and interferon inhibitors

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Virus isolation from clinical specimens is inevitable to identify the etiological agent and to investigate the epidemiological analysis. In addition, the viruses isolated are used as important sources in the fields from the basic research to the bioindustry, including therapeutics, and vaccines. However, the respiratory viruses in clinical specimens are not easily isolated in cell cultures. Thus, methods to speed or enhance virus isolation are urgently required. Many previous reports have proved that centrifugation during virus inoculation to cells increased virus yields and speeded the virus detection time. Recently, interferon inhibitors treatment has also been used to enhance virus infection by blocking the expression of interferons, modulators inhibiting virus replication in cells. We used interferon inhibitors with centrifuged cultures for the detection of metapneumovirus, human respiratory syncytial virus, and Middle East respiratory syndrome coronavirus. Combination of centrifugation and interferon inhibitor treatment significantly increased the virus replication and viruses detected earlier than the routine method. We also test and compare the virus isolation rates between the centrifugation/interferon inhibitor treatment culture and the mock-treated culture.

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Molecular characterisation of human coronaviruses from patients with respiratory disease in Slovenia

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Background: Coronaviruses (CoVs) are the largest enveloped single-strand RNA viruses and belong to the *Coronaviridae* family in the *Nidovirales* order and are divided into four genera named *Alphacoronaviruses*, *Betacoronaviruses* (divided into the four clades A to D), *Deltacoronaviruses* and *Gammacoronaviruses*, based on the phylogenetic distance of highly conserved domains. Until now six human coronaviruses have been identified and HCoV-OC43 is the most common human coronavirus and has high genetically diversity. Five genotypes of HCoV-OC43 (A to E) have been identified and genotype D has been dominant from 2004 to 2012. Until now only 90 complete genome sequences of HCoV-OC43 were available in GenBank. In this study, we investigate of the presence of different genotypes among HCoVs strains and comparison their potential similarity.

Methods: Patients hospitalized with acute respiratory tract infections were included in the study. All nasopharyngeal swabs were sent to the laboratory of the Institute of Microbiology

and Immunology, Faculty of Medicine, University of Ljubljana, for the routine detection of respiratory viruses, including respiratory syncytial virus (RSV), human rhinoviruses (hRV), 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 include 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 (58.6%) were negative for RSV, hRV, hMPV, HCoVs, HBoV, AdV, PIV, Flu A and FluB by real-time RT-PCR. From 523 HCoVs positive sample 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 include 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 11.9%. 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