27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P070 Update on respiratory viruses

Category: 1c. Influenza and respiratory viruses

24 April 2017, 13:30 - 14:30
P1416

Molecular characterization of influenza viruses during the 2012-2016 seasons at a tertiary university hospital in Catalonia (Spain)

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Background: Influenza viruses are continuously evolving both by the RNA polymerase error-prone and high replication rate. This explains the occurrence of seasonal influenza epidemics and the need to revise and to update the vaccine strain composition annually. The aim of this study was to describe the genetic diversity of influenza viruses detected at our hospital during the 2012-2016 seasons.

Material/methods: From October 2012 to May 2016, respiratory tract specimens were collected from patients attended at Vall d`Hebron University Hospital for respiratory viruses laboratory-confirmation. The detection of influenza viruses was carried out by either immunofluorescence or PCR-based assays. A specific real-time one-step multiplex RT-PCR was performed for influenza A subtyping (H1pdm09 or H3). The complete coding HA1-domain sequence from a representative sampling of influenza viruses was sequenced for molecular characterisation and phylogenetic analyses. Coding

neuraminidase protein sequence was further sequenced from randomly selected 2015-2016 influenza viruses to detect amino acid substitutions related to reduced antiviral susceptibility.

Results: A total of 18,411 specimens from 11,215 cases were studied, of which 2,866 (15.5%) samples from 2,674 (23.8%) patients were influenza laboratory-confirmed: 1.782 (66,7%) influenza A (FLUAV) and 892 (33,3%) influenza B (FLUBV) cases. Detections and hospitalisation rates by seasons are summarised in Table 1.

Table 1	2012-2013			2013-2014			2014-2015			2015-2016		
	FLUAV		FLUBV	FLUAV		FLUBV	FLUAV		FLUBV	FLUAV		FLUBV
% type/subtype circulation	44 (26%)		122 (74%)	393 (100%)		-	667 (76%)		207 (24%)	651 (55%)		532 (45%)
	35 (80%)	4 (10%)		111 (29%)	236 (60%)		173 (26%)	387 (58%)		583 (90%)	14 (2%)	
Hospitalisation	95 (53%)			212 (53%)			719 (81%)			489 (41%)		
	27 (28%)		68 (72%)	212 (53%)		-	538 (75%)		181 (25%)	326 (67%)		163 (33%)
	H1pdm09	H3		H1pdm09	H3		H1pdm09	H3		H1pdm09	H3	
	22 (23%)	3 (3%)		52 (28%)	102 (55%)		141 (20%)	307 (43%)		320 (65%)	6 (2%)	
ICU	5 (23%)		12 (67%)	7 (14%)	10 (10%)		7 (5%)	4 (1,3%)	3 (1,6%)	22 (7%)		9 (6%)

Regarding molecular characterisation, influenza viruses were genetically similar to the seasonal vaccine strains used in the three seasons. But most of characterised 2014-2015 influenza A(H3) viruses belonged to a genetic group with antigenic features different from the viruses represented by the vaccine strain. The amino acid substitutions D222G or D222N in HA1, which were previously described as virulence genetic markers, were found in 3 A(H1)pdm09 strains from respiratory specimens of patients admitted to Intensive Care Unit (ICU) during the 2012-2013 season. Three A(H1)pdm09 viruses detected during the 2015-2016 season were carrying the mutations H275Y (2) and S247N (1), which are related to resistance or decreased susceptibility to oseltamivir, respectively. While B/Yamagata lineage was the predominant during the first three seasons, B/Victoria viruses were the only detected during the last season, in which the corresponding strain was not included in the trivalent influenza vaccine.

Conclusions: The predominant influenza type or subtype was frequently detected in the largest number of hospitalisation, but A(H1)pdm09 subtype was usually associated with the high percentage of ICU-admissions. Mutations D222G/N in A(H1)pdm09 were only detected in 2012-2013 ICU-admitted patients, as reported in previous seasons, but no viruses carrying these were detected in the following seasons. The circulation of drifted A(H3) strains during the 2014-2015 season was related with high hospitalisation rate, but not with an increase of ICU-admissions. The predominance of a FLUBV lineage not included in the trivalent vaccine highlights once again the need to use the tetravalent vaccine in patients at high-risk of infection.