MOLECULAR CHARACTERISATION OF INFLUENZA VIRUSES DURING THE 2012-2016 SEASONS AT A TERTIARY UNIVERSITY HOSPITAL IN CATALONIA (SPAIN)

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Introduction and purpose

Influenza viruses are continuously evolving both by the RNA polymerase error-prone and the 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.

Materials and Methods

From October 2012 (week 40) to May 2016 (week 20), 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.

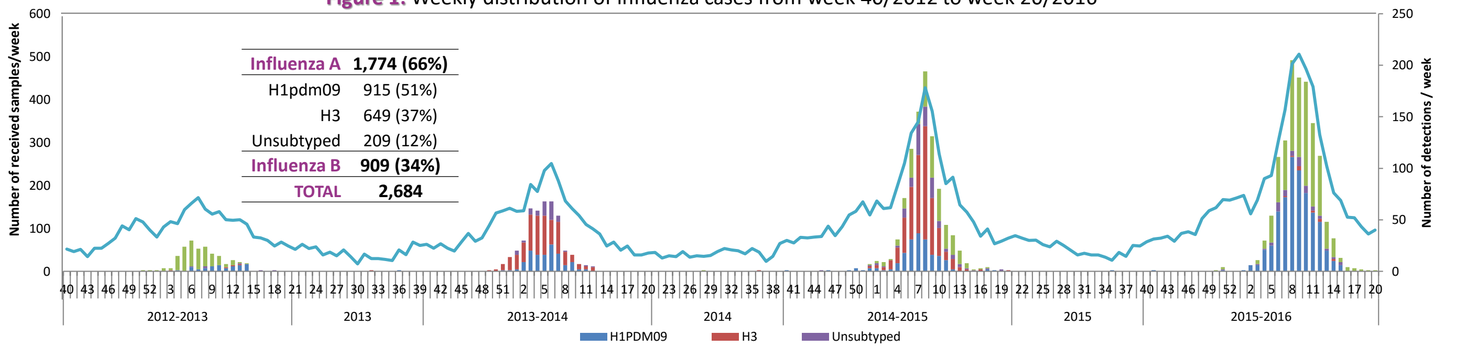
Results

A total of 18,405 specimens from 11,215 cases were studied, of which 2,866 (15.5%) samples from 2,684 (24%) patients were influenza laboratory-confirmed: 1,766 (66%) influenza A (FLUAV) and 909 (34%) influenza B (FLUBV) cases. Detections, hospitalisation rates and Intensive Care Unit admissions (ICU) 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	49 (27%)	132 (73%)	397 (100%)	-	667 (76%)	215 (24%)	661 (54%)	562 (46%)
	38 (78%) 6 (12%)		113 (28%) 236 (59%)		172 (26%) 394 (59%)		5932 (90%) 13 (2%)	
Hospitalisation	97 (54%)	68 (72%)	185 (47%)	-	719 (81%)	181 (25%)	493 (40%)	166 (33%)
	29 (30%)		185 (47%)		538 (75%)		327 (66%)	
	H1pdm09 H3		H1pdm09 H3		H1pdm09 H3		H1pdm09 H3	
	23 (24%) 4 (4%)		52 (28%) 102 (55%)		141 (20%) 307 (43%)		296 (60%) 6 (1,2%)	
ICU-admission	5 (22%)	12 (18%)	7 (13%) 10 (10%)		7 (5%) 4 (1,3%)	3 (1,6%)	17 (6%)	9 (5%)

Figure 1: Weekly distribution of influenza cases from week 40/2012 to week 20/2016



The hospitalisation rate was around 50% season by season (Table 1), with the exception of the 2014-2015 season, in which it was considerably high (81%). The number of hospitalisations was associated with the influenza type/subtype predominance during each season, but not with the admission to ICU because although the subtype A(H1)pdm09 among FLUAV was not the most detected in some seasons (2012-2014), it was more related to ICU admissions over A(H3). FLUAV was much more frequently detected in the study period than FLUBV, and while FLUAV circulated during all seasons, FLUBV did not during the 2013-2014 season. A particular influenza type, subtype or lineage was predominant in each studied season: FLUBV/YAM during the 2012-2013 season; A(H3) during the 2013-2014 and 2014-2015 seasons; and A(H1)pdm09 and FLUBV/VIC during the 2015-2016 season, as shown in Figure 1 and Table 2. Phylogenetic analyses of HA1 sequences revealed the genetic diversity of the different influenza viruses distinguishing different genetic clades and subclades (Table 2). While the A(H1)pdm09 sequences clustered into clades 6 and 7 during the 2012-2013 season, the sequences of strains detected during the following seasons fell within two subgroups (6B.1 and 6B.2) within clade 6B. All A(H3) sequences but one clustered in different genetic subgroups (3C.2a, 3C.3 and 3C.3a) within genetic clade 3 represented by A/Slovenia/537/2011. During the 2014-2015 season, A(H3) viruses with antigenic features different from vaccine strain used were mostly reported, and in parallel, a high activity by influenza viruses was noted showing a high hospitalisation rate (81%) as shown in Table 1. During the 2012-2013 and 2014-2015 the FLUBV viruses characterised belonged to FLUBV/YAM lineage whereas during the 2015-2016 season only FLUBV/VIC lineage was detected. Most of FLUBV/YAM sequences belonged to clade 3, while those belonging to clade 2 were only detected during the 2012-2013 season. Molecular characterisation of HA1 sequences of A(H1)pdm09 viruses revealed the mutations D222G and D222N mutations in the receptor binding site (RBS) from the viruses detected in respiratory specimens from 3 ICU-admitted patients (D222G: 1 case; D222N: two cases) during the 2012-2013 season, which were previously related to high severity. Regarding genetic markers related to neuraminidase inhibitors (NAIs) resistance, only 3 2015-2016 A(H1)pdm09 viruses were carrying the mutations H275Y (2) and S247N (1).

Table 2: Characterised FLUAV and FLUBV strains during the 2012-2016 season

	2012-2013	2013-2014	2014-2015	2015-2016
Influenza A Virus	**12**	**186**	**358**	**98**
H1pdm09 Subtype	**9**	**53**	**114**	**92**
Vaccine strain (trivalent)	A/California/7/2009	A/California/7/2009	A/California/7/2009	A/California/7/2009
A/St Petersburg/27/2011 (clade 6)				
A/South Africa/3626/2013 (subclade 6B)		53*	144*	5*
A/Michigan/45/2015 (subclade 6B.1)				86*
A/Israel/Q-204/2015 (subclade 6B.2)				1*
A/Dakar/04/2014 (subclade 6C)	7*			
A/St Petersburg/100/2011 (clade 7)	2*			
H3 Subtype	**3**	**133**	**244**	**6**
Vaccine strain (trivalent)	A/Victoria/361/2011	A/Texas/50/2012	A/Texas/50/2012	A/Switzerland/9715293/2013
A/Slovenia/537/2011 (Clade3)				
A/Victoria/361/2011 (subclade 3C)				
A/Hong Kong/146/2013 (3C.2)	1*	5		
A/Hong Kong/4801/2014 (subset 3C.2a)			189	5
A/Samara/73/2013 (subset 3C.3)	1*	128	20	
A/Switzerland/9715293/2013 (subset 3C.3a)			35	1*
A/Alabama/05/2010 (Clade 5)	1			
Influenza B Virus	**36**		**104**	**51**
B/Victoria/2/87 lineage	**5**		**1**	**51**
Vaccine strain (tetravalent)	B/Brisbane/60/2008	B/Brisbane/60/2008	B/Brisbane/60/2008	B/Brisbane/60/2008
B/Brisbane/60/2008 (clade 1)	5*		1*	51*
B/Yamagata/16/88 lineage	**31**		**103**	
Vaccine strain (trivalent)	B/Wisconsin/1/2010	B/Massachusetts /2/2012	B/Massachusetts/2/2012	B/Phuket/3073/2013
B/Massachusetts/02/2012 (clade2)	21*			
B/Phuket/3073/2013 (clade 3)			103	
B/Stockholm/12/2011-like	1			
B/St Petersburg/24/2012-like	9			
	48	**186**	**462**	**149**

Conclusions

The molecular characterisation of influenza viruses detected in our hospital helps to monitor their genetic diversity and their matching to the corresponding vaccine strains. The number of hospitalisations was associated with the influenza type/subtype predominance during each season, 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, 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. Despite their low prevalence, resistant viral variants in hospitalised patients should be closely monitored, to avoid their transmission and likely spreading.

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