Detection of mutations in hemagglutinin and neuraminidase genes coding for oseltamivir resistance and enhanced virulence/transmissibility among Indian strains of swine influenza A/H1N1 viruses

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Introduction

Influenza A virus evolves continuously by acquiring many mutations and multiple molecular determinants to cause increased disease severity. It is crucial to monitor the genetic make-up of this virus specially the surface glycoproteins; hemagglutinin and neuraminidase, to understand their adaptability and evolutionary dynamics in different regions.

Hemagglutinin (HA) is a major surface glycoprotein of influenza virus. It is shown to be under high selection pressure to evade the host immune response that makes it hyper variable. Any mutational changes in this region may affect the transmission and pathogenicity of influenza virus.

Neuraminidase (NA) is a sialidase protein in the surface of influenza virus which helps in viral replication. NA is the important target for anti-influenza drugs. As a result of drug use and /or natural variation in NA, viruses with reduced drug susceptibility can emerge. But molecular markers of resistance are not well defined, and the clinical relevance of some identified mutations remains uncertain.

Therefore, continued epidemiological and genetic surveillance is important for monitoring changes in virulence and resistance over seasons. This can be critical to study the molecular mechanisms involved in the emergence, global spread and resistance mutations in Indian isolates particularly after the controversial commentary regarding under reporting of the same from India.

Acknowledgement:DST-INSPIRE, NCDC &IDSP (New Delhi) , JIPMER, Immunology foundation

Table 1. Molecular characterization of influenza isolates

Gene	Amino acid changes	Probable reaction
Hemagglutinin	T200A D225N K166Q	Enhanced virulence and transmissibility
Neuraminidase	H275Y I223R N295S D199N S247N Q136K	Oseltamivir resistance

Methods

Based on the highly conserved sequences of the hemagglutinin and neuraminidase genes of influenza A(H1N1)pdm09, forward and reverse primers were designed to amplify target of 659bp and 727bp respectively, which included regions with potential mutations influencing virulence and resistance to neuraminidase inhibitors (Table.1). Total 45 isolates of influenza A(H1N1)pdm09 were selected from different years (2011-1, 2012-16, 2015-28). After PCR amplification and purification, DNA sequencing was done using ABI 3730 Genetic analyzer (Applied Biosystems, USA).

Multiple sequence alignment and phylogenetic analysis was performed with reference to A/California/07/2009 vaccine strain using MEGA6 software using neighbor-joining method. Translated protein sequences were aligned with sequences of reference strain to find the point mutations in HA and NA genes.

Results

• All influenza A(H1N1)pdm09 isolates fell within clade 7 and all possessed H275, indicating the common marker of neuraminidase inhibitor sensitivity

• Six strains had N295S mutation (2012-2, 2015-4); three had Q136K (2015-3) which are also considered to be markers of oseltamivir resistance though not as well established as the H275Y mutation.

Table 2. Comparative amino acid sequence analysis of NA gene

Residue number	A/California/07/2009	SW 1207/2012	KIM 49/2012	JIP 1062/2015	PIP 538/2015	SW 1761/2015	SW 1774/2015	PIM 555/2015	JIM 1042/2015	SW 1811/2015
275	H	H	H	H	H	H	H	H	H	H
223	I	I	I	I	I	I	I	I	I	I
295	N	S	S	S	S	S	S	N	N	N
199	D	D	D	D	D	D	D	D	D	D
247	S	S	S	S	S	S	S	S	S	S
136	Q	Q	Q	Q	Q	Q	Q	K	K	K

Table 3. Comparative amino acid sequence analysis of HA gene

Residue number	A/California/07/2009	JIM 167/2012	JIM 134/2012	PIM 21/2012	JIM 901/2015	JIP 1062/2015
200	S	S	S	S	S	P
225	D	N	N	N	N	D
166	K	K	K	K	K	K

•All these strains were from patients who had received oseltamivir as therapy and recovered (Table.2)

•Four strains had D225N (2012-3, 2015-1) mutations in HA gene, one of which were isolated from a fatal case (2012) (Table.3)

• Additional mutations were found in HA in position 100 and 220 in two different strains (2012-1, 2015-1). Of them S100P co-occurred in one of the isolates with N295S mutation (2015).

Conclusion

Although the common mutation associated with oseltamivir resistance was not found in any of our isolates, it is worrying that we encountered some of the less common mutations associated with it. Whether this will translate into clinical, full blown resistance in future needs to be closely monitored. The additional finding of multiple markers of virulence is also a cause for concern.

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