Antiviral susceptibility of influenza A viruses isolated between 2009-2013, in Portugal

Raquel Guiomar[1], Patrícia Conde[1], Paula Cristóvão[1], Pedro Pechirra[1]

[1]National Influenza Reference Laboratory, Infectious Diseases Department, National Institute of Health, Portugal

Background:

Monitoring the influenza antiviral susceptibility had become an important issue in the recent years. The influenza A viruses are naturally susceptible to Neuraminidase inhibitors (NAI) and M2 inhibitors (adamantanes). The increased resistance to oseltamivir and adamantanes (since 2006), the availability and clinical use of influenza antivirals enhanced the need for a close monitoring for loss of susceptibility. Since 2009, at Portuguese National Influenza Centre, was developed in the scope of the National Influenza Surveillance Programme a systematic monitoring of antiviral susceptibility (oseltamivir, zanamivir and adamantine).

This study shows the results of antiviral susceptibility to NAI and M2 inhibitors for influenza A viruses characterized between 2009 and 2013.

Material and Methods:

The phenotypic method, fluorescence NA inhibition assay, considered the "gold standard", to determine the susceptibility to oseltamivir and zanamivir, measuring the concentration of the antiviral to inhibit 50% of NA activity (IC_{50}) was performed during the study period for 119 influenza A viruses: 26 and 60 A(H1)pdm09 from 2009/10 and 2012/13 season, respectively, and 33 A(H3) viruses from 2011/12, plus 2 A(H3) from the last season were also analysed. Genotypic screening methods were performed for the most common NAI inhibition-reducing substitution, H275Y in A(H1)pdm09 viruses (n= 340). Matrix gene sequencing, for adamantanes susceptibility surveillance, and neuraminidase gene sequencing were performed for 64 and 119 strains, respectively.

Results:

In the study period all A(H1)pdm09 and A(H3) viruses, carried the S31N substitution in the M2 protein, which confers resistance to the adamantanes to 100% of the isolates.

For A(H1)pdm09 viruses, the oseltamivir IC_{50} values (Figure 1) ranged from 0.45 to 2.82 (2009/2010) and from 0.15 to 2.27 (2012/2013).

The resistance to oseltamivir was identified in 3 A(H1)pdm09 viruses that carried the H275Y substitution (Table I). One case in 2009/2010 (pandemic season) from an 8 years old child (phenotypically showed a IC_{50} of 502.48nM, a value 700-fold higher when comparing to the susceptible viruses. Two other cases of resistance were detected in 2010/2011 (Table I) but they were not submitted to phenotypical assays. Both the 8 year old child from 2009/2010 and the 26 year old (pregnant) woman with a fatal outcome from 2010/2011 season were patients with chronic diseases. The second case from 2010/2011 is a community-acquired resistance detected in a ILI-case.

The A(H1)pdm09 IC_{50} values for zanamivir (Figure 2) ranged from 0.32 to 3.88 (2009/2010) and from 0.29 to 3.44 (2012/2013). The oseltamivir and zanamivir median IC_{50} values for A(H1)pdm09 viruses were stable between 2009 and 2013 (Table II).

None of the A(H1)pdm09 isolates presented the other substitutions associated with reduced susceptibility to NAI (D119N, I223R, N295S).

A(H1)pdm09

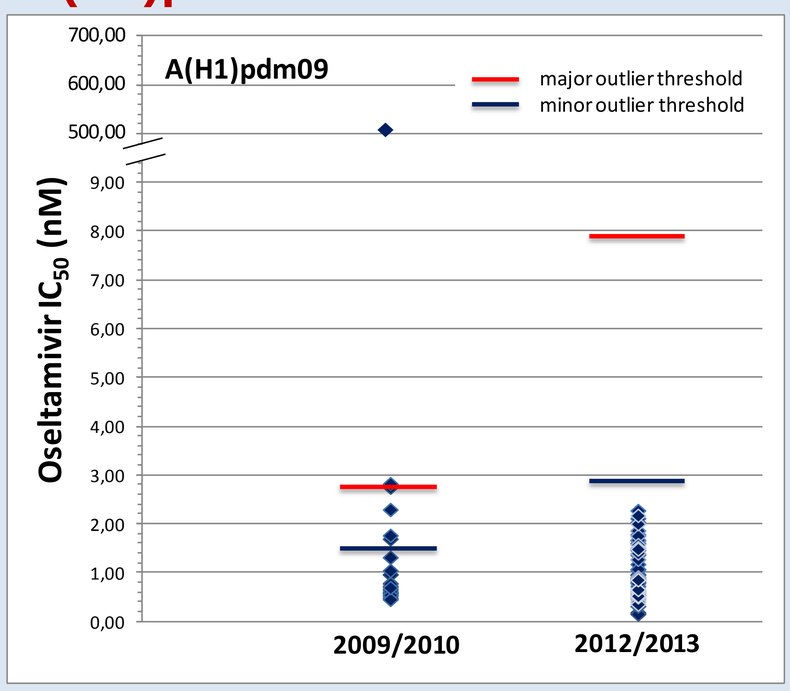

Figure 1 – Oseltamivir IC_{50} values for influenza A(H1)pdm09 in 2009/2010 and 2012/2013 flu seasons .

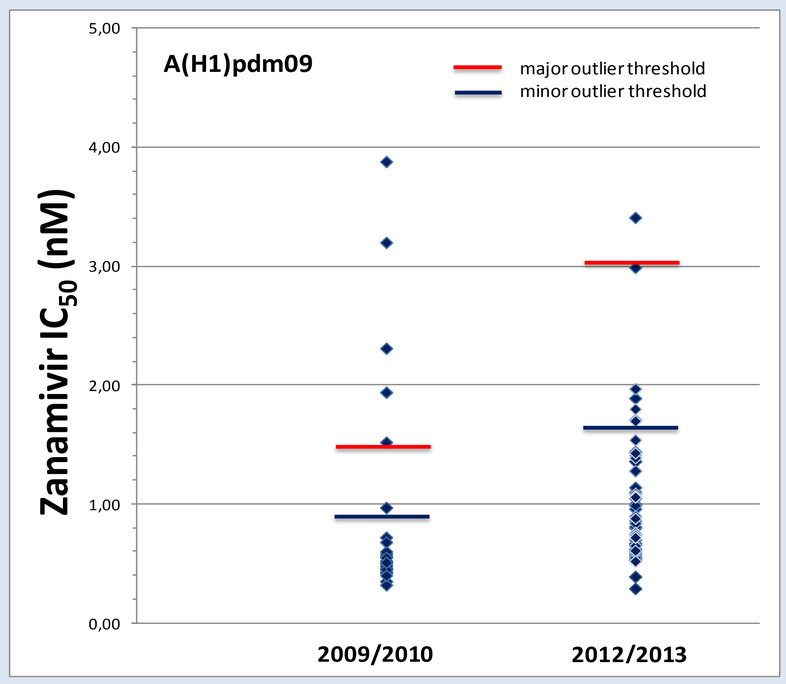

Figure 2 – Zanamivir IC_{50} values for influenza A(H1)pdm09 in 2009/2010 and 2012/2013 flu seasons .

A(H3)

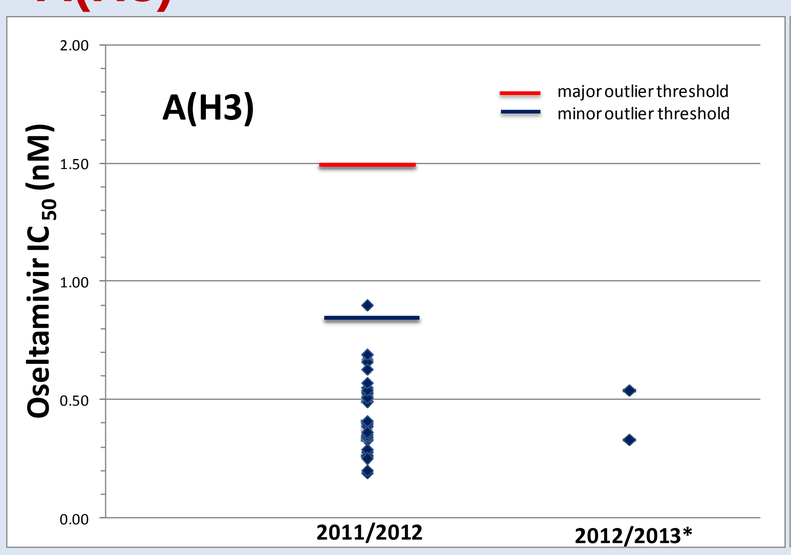

Figure 3 – Oseltamivir IC_{50} values for influenza A(H3) in 2011/2012 and 2012/2013 flu seasons.

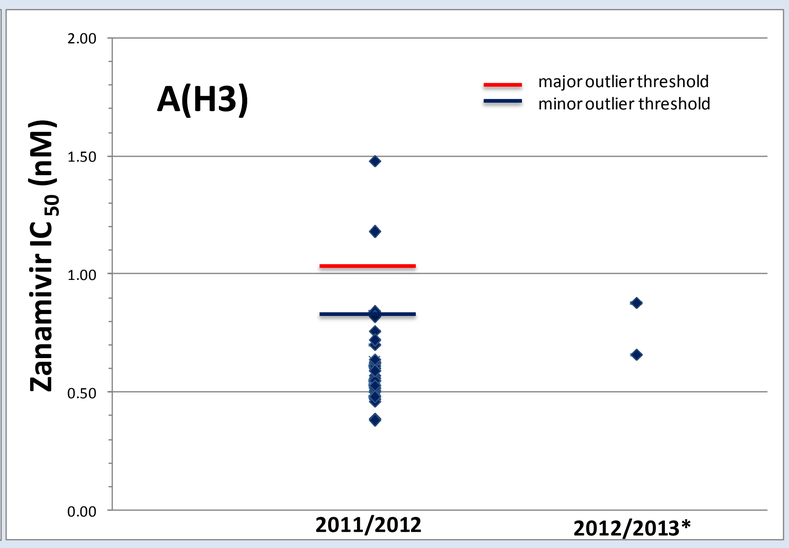

Figure 4 – Zanamivir IC_{50} values for influenza A(H3) in 2011/2012 and 2012/2013 flu seasons.

* In 2012/2013 season only 2 virus A(H3) were tested. Were considered the minor and major outlier threshold of the previous season for analysis.

Table I – Oseltamivir resistant influenza A(H1)pdm09 virus and epidemiological and laboratory data from cases detected in 2009/201 and 2010/2011 seasons.

Season	Isolate or sample name	Virus	Method	IC50		NA H275Y mutation	Age	Sex	Underlying Conditions
				Oseltamivir	Zanamivir				
2009/2010	A/Lisboa/171/2009	A(H1)pdm09	Phenotypic (MUNANA)	502,48	2,31	275Y	8	M	chronic disease
2010/2011	VIR1204918	A(H1)pdm09	Genotypic (PCR H275Y)	NA	NA	275Y	26	F	chronic disease
	EVA216	A(H1)pdm09	Genotypic (PCR H275Y)	NA	NA	275Y	61	F	UNK

NA- non available; UNK- unknown

Table II – Number of viruses analysed and oseltamivir and zanamivir IC_{50} baseline levels for influenza A in 3 flu seasons (2009/2010, 2011/2012 and 2012/2013).

Influenza A	2009/2010			2011/2012			2012/2013		
	Median± Robust Standard Deviation			Median± Robust Standard Deviation			Median± Robust Standard Deviation		
	n	Oseltamivir	Zanamivir	n	Oseltamivir	Zanamivir	n	Oseltamivir	Zanamivir
A(H1)pdm09	26	0,70±1,58	0,53±1,40	0	NA	NA	60	0,87±2,08	0,80±1,56
A(H3)	0	NA	NA	33	0,41±1,55	0,59±1,21	2	0,42±1,44	0,76±1,24

NA- non available

The A(H3) viruses analysed in 2011/2012 season showed IC_{50} values for oseltamivir (Figure 3) that ranged from 0.19 to 0.90 and for zanamivir from 0.39 to 1.48 (Figure 4). The median IC_{50} values for oseltamivir and zanamivir were 0.41±1.55 and 0.59±1.21 respectively (Table 1). In the last season 2012/2013 only two A(H3) isolates were analysed and the IC_{50} values were in the same range of the 2011/2012 A(H3) viruses. Since 2009 all the A(H3) viruses are susceptible to oseltamivir and zanamivir.

None of the A(H3) viruses presented the amino acid substitutions known to reduce susceptibility to NAI (E119V/I, R229K, N294S and H274Y).

Conclusions:

Influenza A viruses isolated in Portugal since 2009 are resistant to adamantanes, which are no longer indicated for influenza A treatment. Otherwise, the resistance to oseltamivir was only observed in a reduced number of strains and all the viruses show susceptibility to zanamivir. The use of conventional sequence analysis and genotypic screening methods for monitoring the molecular markers of antiviral resistance in influenza A virus provides a valuable tool for an early detection of antiviral resistant strains.

For further information on this poster please contact: Raquel Guiomar, Laboratório Nacional de Referência para o Vírus da Gripe, raquel.guiomar@insa.min-saude.pt; Tel.: 00351217519216, Fax.: 00351217526400

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