Antiviral resistance: influenza B

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Background:

Currently circulating influenza B viruses are resistant to adamantanes and, except for a low number of sporadic cases, most are sensitive to neuraminidase inhibitors (NI). Adamantanes are ineffective against influenza B viruses and although NI-resistant influenza B viruses have been rarely reported, recently in the United States was identified one cluster of influenza B viruses with reduced susceptibility to NI and with the I221V substitution in the active site of the neuraminidase (Garg et al.,2013). Despite the low prevalence of oseltamivir or zanamivir resistant influenza viruses, the constant evolution of influenza requires the monitoring of antiviral resistance among these viruses in the community. This is very important for the clinical management of severe influenza cases as to the detection of novel genetic markers associated with antiviral resistance.

This study reports the antiviral susceptibility to neuraminidase inhibitors of influenza B viruses isolated in Portugal during the 2010/2011, 2011/2012 and 2012/2013 seasons.

Material and Methods:

Over the period of 3 influenza seasons, 147 influenza B viral strains were analysed by phenotypic fluorescent assays (MUNANA) in order to assess their susceptibility to NI, oseltamivir and zanamivir. For this purpose, was determined the NI concentration required to inhibit 50% of each influenza virus neuraminidase activity (IC_{50}). The IC_{50} baseline of influenza B viruses was calculated for both oseltamivir and zanamivir using the Robust Excel programme. The neuraminidase gene segments were also monitored for the presence of the main molecular markers, associated with the resistance to neuraminidase inhibitors in influenza B viruses, by sequencing the neuraminidase (NA) gene.

Results:

All analysed influenza B strains proved to be susceptible to oseltamivir and zanamivir.

In the 2010/2011 season the determined IC_{50} values ranged from 5.58 to 71.88 nM for oseltamivir and from 0.83 to 10.94nM to zanamivir (Figures 1 and 2). The zanamivir IC_{50} median value was about 8-fold lower than oseltamivir IC_{50} median value (Table I). Statistical analysis revealed the presence of one minor outlier (B/Lisboa/13/2010, 71.08nM) for oseltamivir (2-fold reduction in susceptibility) and four minor outliers (B/Lisboa/15/2010: 9.52 nM; B/Lisboa/19/2010: 9.25 nM; B/Lisboa/51/2010: 10.94 nM and B/Lisboa/53/2010: 9.62 nM) for zanamivir (3-fold reduction in susceptibility) comparing to the median IC_{50} value (Figures 1 and 2).

During the 2011/2012 season IC_{50} values ranged from 23.0 to 38.38 nM (oseltamivir) and from 2.97 to 9.07nM (zanamivir). In this season were not found minor or major outliers for both neuraminidase inhibitors (Figures 1 and 2).

The IC_{50} values obtained in 2012/2013 ranged from 7.91 to 84.84 nM (oseltamivir) and from 1.48 to 5.88 nM (zanamivir). During this last season 3 viral strains were classified as major outliers for oseltamivir: B/Castelo Branco_PT/176/2013 (IC_{50}=49.61nM), B/Lisboa_PT/74/2013 (IC_{50}=50.93nM) and B/Lisboa_PT/50/2013 with the highest IC_{50} value of 84.84nM) (Figure 1). Were also found 6 minor outliers for oseltamivir and 13 minor outliers for zanamivir (Figures 1 and 2).

Along the three studied seasons, the median IC_{50} values for both NI were higher among the B/Victoria than B/Yamagata viruses (Table I).

None of the actually known mutations associated with resistance of influenza B viruses to NI was found in the NA gene (R150K, D197E/N/Y, I221T/V, N294S, R374K and G407S) of the analysed viruses.

Table I – Number of viruses analysed and oseltamivir and zanamivir IC_{50} baseline levels for influenza B in 3 last flu seasons, between 2010 and 2013 (total influenza B results and by lineage).

Influenza B lineage and total	2010/2011				2011/2012				2012/2013			
	Median ± Robust Standard Deviation				Median ± Robust Standard Deviation				Median ± Robust Standard Deviation			
	n	Oseltamivir	n	Zanamivir	n	Oseltamivir	n	Zanamivir	n	Oseltamivir	n	Zanamivir
B/Yamagata	2	10,55±2,57	2	1,73 ± 1,31	5	28,75±1,17	5	6,69 ± 1,57	80	14,85±1,38	80	2,82 ± 1,30
B/Victoria	49	31,25±1,55	46	3,79 ± 1,68	1	38,38	1	6,7	10	31,57±1,72	10	3,86 ± 1,52
B (Total)	51	30,73±1,61	48	3,75± 1,74	6	30,26±1,29	6	6,69 ± 1,42	90	15,14±1,41	90	2,85 ± 1,30

Oseltamivir

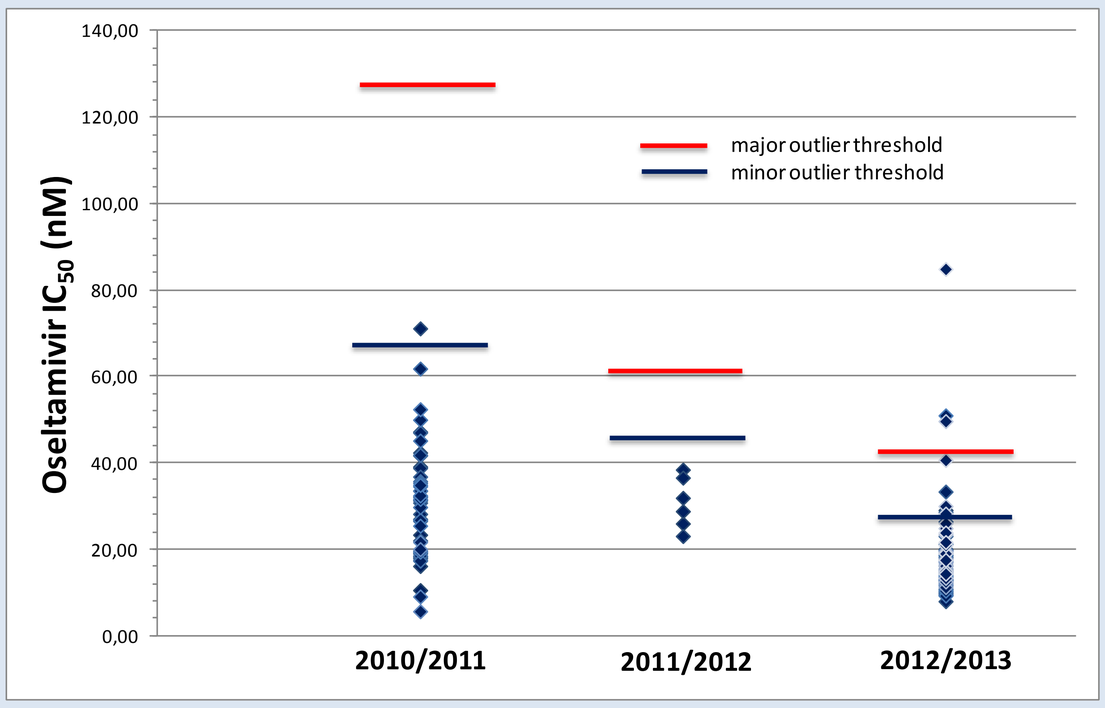

Figure 1 – Oseltamivir IC_{50} values for influenza B in 3 last flu seasons, between 2010 and 2013.

Zanamivir

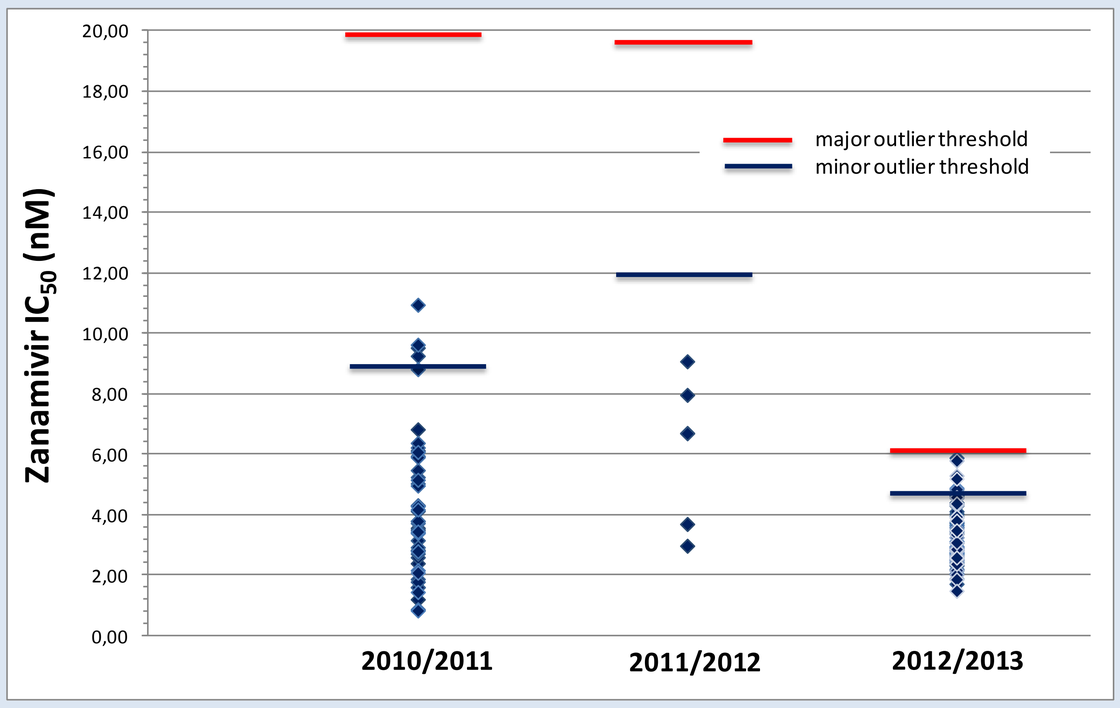

Figure 2 – Zanamivir IC_{50} values for influenza B in 3 last flu seasons, between 2010 and 2013.

Conclusions:

Influenza B viruses isolated in Portugal during the last three seasons were susceptible to the neuraminidase inhibitors. Portuguese influenza B strains revealed higher susceptibility to zanamivir than to oseltamivir, as observed in other studies (Kawai et al., 2009).

The oseltamivir IC50 values were also different between viruses from the B/Victoria and B/Yamagata lineages, however this was not statistically demonstrated due to the small number of analysed viruses. Among B/Yamagata strains the oseltamivir and zanamivir IC50 values were higher in 2011/2012 than in 2010/2011 and 2012/2013 seasons.

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Options for the Control of Influenza VIII, Cape Town, South Africa, 5-10 September 2013