Evaluation and Characterization of Influenza Antiviral Drug Resistance in Portugal: Major Results and Achievements of a 5-Year Study

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Abstract:
In 2007 started to be carried out for the first time in Portugal a study focused on influenza antiviral drug resistance. Three main objectives were established:(1)to determine the antiviral profile of influenza viruses to oseltamivir, zanamivir and amantadine;(2)to determine and monitor the baseline level of susceptibility along winter seasons and for each influenza sub(type);(3)to analyse and characterize the whole genome of viruses that showed phenotypic levels of inhibition to neuraminidase inhibitors(NAIs).
NAIs profile was determined phenotypically, using a fluorescence MUNUNA assay, and genotypically by NA and HA sequencing. A total of 340 seasonal viruses(117 A(H3N2),93 A(H1N1),130 B) were tested for oseltamivir and of 297(112 A(H3N2),68 A(H1N1),117 B) for zanamivir. Additionally, 142 A(H1N1)pdm09 viruses were evaluated for both NAIs. Whole genome sequencing was performed in 27 of the A(H1N1)pdm09 viruses. Amantadine profile was determined through M2 pyrosequencing or conventional sequencing in a total of 205 seasonal A viruses(138 A(H3N2),84 A(H1N1)) and of 117 A(H1N1)pdm09 viruses.
Main results are:
-Resistance to oseltamivir in 27 A(H1N1) seasonal viruses(29%,N=93) from 2007/2008 and 2008/2009 and in one A(H1N1)pdm09 virus(0.7%,N=142) from 2010/2011. These viruses exhibited a highly reduced level of inhibition to oseltamivir by phenotypic analysis (170-650 IC50 fold-change) and NA H275Y mutation;
-One suspected case of clinical resistance to oseltamivir with a mixed population of H275Y viruses(73,8%H275,26.2%Y275);
-No resistance to zanamivir;
-Dual reduced susceptibility to oseltamivir and zanamivir in one B virus(0,85%,N=117) and in two A(H1N1)pdm09 viruses(1,41%,N=142). These viruses exhibited a 2-4 IC50 fold-change level of inhibition to both NAIs. A mixed population of D197N viruses was found in the B virus(56%D197,44%N197) and the two A(H1N1)pdm09 viruses shared NA I223V and PB2 V480I mutations;
-Resistance to amantadine in 49 A(H3N2) viruses(35,5%,N=138) from 2005/2006 to 2008/2009(46 S31N,3 S31N+V27A), and in all A(H1N1)pdm09 viruses(S31N).
This 5-year study allowed to establish a technical platform for influenza antiviral drug resistance evaluation, to timely detect the emergence of resistant viruses, to acquire know-how on the natural variation of virus susceptibility, and to contribute for the management of cases suspected of clinical resistance. Additionally, it allowed the gathering of a large amount of data that will be used in more advanced studies, focused on evolutionary analysis and on detailed characterization of specific mutations.