Genetic influenza surveillance

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Influenza genetic characterization:

- To determine how closely related or similar Flu viruses are to one another genetically.

- To monitor how Flu viruses are evolving.

- To identify genetic changes that affect the virus' properties. For example, to identify the specific changes that are associated with influenza viruses spreading more easily, causing more-severe disease, or developing resistance to antiviral drugs.

- To assess how well an influenza vaccine might protect against a particular influenza virus based on its genetic similarity to the virus.

- To monitor for genetic changes in influenza viruses circulating in animal populations that could enable them to infect humans.

https://www.cdc.gov/flu/about/professionals/genetic-characterization.htm
MATERIALS Y METHODS

NPS/ NPS from pediatric and adult, inpatient and outpatient with ARI. The Flu diagnostic developed at the Laboratories of the National Influenza and ORV Network

Flu A/B positive samples sent to the NRL /WHO NIC-Buenos Aires Argentina

- Viral isolation
- Antigenic Characterization
- Subtyping and \( \text{Influenza B Lineages} \)
- Phylogenetic Analysis/Genomic Characterization
- Antiviral Susceptibility

Information is sent to:

- SNVS
- FLUNET
- OMS
- PAHO WEEKLY FLU REPORT
Influenza positive samples received at the NIC EW 44 / 2020

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of samples</th>
</tr>
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<tbody>
<tr>
<td>2014</td>
<td>1.139</td>
</tr>
<tr>
<td>2015</td>
<td>1.203</td>
</tr>
<tr>
<td>2016</td>
<td>2.988</td>
</tr>
<tr>
<td>2017</td>
<td>2.520</td>
</tr>
<tr>
<td>2018</td>
<td>1.889</td>
</tr>
<tr>
<td>2019</td>
<td>2.972</td>
</tr>
<tr>
<td>2020 (SE 44)</td>
<td>101</td>
</tr>
<tr>
<td>Total</td>
<td>12.812</td>
</tr>
</tbody>
</table>

2019, n= 2207  
2020, n= 101
6 out of 9 viruses presented the substitution N156K, shown to be less well recognized by the A/Brisbane/02/18 antiserum.

6 out of 27 presented the two amino acid deletion in HA1 (Δ162-163 viruses) and 21 of them had the triple amino acid deletion in HA1 (Δ162-164 viruses).
Influenza genetic characterization in the surveillance of influenza viruses, examples. Argentina-Buenos Aires NIC

- Intra-season evolution of seasonal viruses and their relationship with vaccine viruses
- Confirmation of resistance to antivirals
“Characterization of influenza A(H3N2) strains circulating in Argentina during the 2017 season”

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Awarded as the second best work presented “in extenso” at the 18th International Congress of Infectious Diseases / XVII Congreso Sociedad Argentina de Infectología 2018. Buenos Aires, Argentina

INTRODUCTION

- Influenza viruses are grouped into 4 Genera: A, B, C, D.
- Characterized by their ability to change: Drift / Shift.
- The viral surface glycoproteins HA and NA are the most important antigens.
- It is an important cause of ARF in humans and responsible for around 500 thousand deaths a year in the world.
- Genus A viruses have caused pandemics of significant morbidity and mortality: 1918 A (H1N1), 1957 A (H2N2), 1968 A (H3N2) and 2009 A (H1N1) pdm

AIMS

- To describe the genetic diversity of the FLUA (H3N2) strains circulating in Argentina.
- To estimate its temporal distribution throughout 2017.
- To try to explain the relationship between the characteristics of the circulating strains, the vaccination status of infected patients and the severity of the disease.
- To use genetic characterization in real time to contribute to decision making in Public Health.
MATERIALS Y METHODS

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RESULTS I

- IRA events studied, 2017: 84,437
- Influenza viruses detected: 6,562
- % Positivity for influenza: 8
- Influenza A viruses detected: 5,492
- Influenza B viruses detected: 1,070
- Samples received at the LNR: 2,705
- Virus A characterized: 1,961
- Characterized B viruses: 453
- Other condition: 291
- A: (H3N2) 100%
- B-Yam: 87.4%
- B-Vic: 12.6%
RESULTS II

Cocirculation of 3 H3N2 genetic clades:

3C.2a related to the vaccine component A / Hong Kong / 4801/2014

3C.2a1 related to a new variant, A / Bolzano / 7/2016

3C.3a related A / Switzerland / 9715293/2013 vaccine component of the southern hemisphere 2015

% Clados genéticos identificados de virus A(H3N2)
Relationship between influenza virus circulation, ILI and SARI frequencies in the population. Argentina 2017

RESULTS III

Genetic Shift of the circulating influenza A(H3) viruses. Argentina, 2017
Early onset and peak of viral activity.
Circulation prevalence of A (H3N2) strains.
Co-circulation of three genetic clades, 3C.2a1, 3C.3a and 3.C2a, the latter being the predominant one.
At the beginning of the season, 70% of the circulating strains were associated with the strains of the Bolzano clade (3C.2a1), (SE 13-19).
During the peak of higher viral activity, the circulating strains were closely related to the clade that includes the vaccine component (3.C2a), (SE 20-30).
42% of viruses characterized as Bolzano variant were isolated from vaccinated patients.
Antibodies raised against the Hong Kong vaccine component would not efficiently limit the disease caused by the Bolzano variant.
Information was provided to the Ministry of Health in real time to contribute to decision-making and formulate prevention and control measures to minimize the impact.
Surveillance of antiviral resistance markers in Argentina: detection of E119V neuraminidase mutation in a post-treatment immunocompromised patient

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INTRODUCTION

The neuraminidase inhibitors (NAIs) oseltamivir and zanamivir have been the cornerstone of antiinfluenza therapy in recent years.

Their effectiveness has been compromised by the emergence of resistance among some A(H3N2) and A(H1N1) viruses circulating in different geographic regions.

One of the most frequent changes in A(H3N2) viruses during or soon after oseltamivir administration has been reported to be the amino acid substitution of glutamic acid (E) to valine (V) at position 119 of neuraminidase (NA).

Immunocompromised host is a patient who does not have the ability to respond normally to an infection due to weakened immune system. Antiviral prophylaxis and therapy are particularly important in these patients because the influenza vaccine is often poorly immunogenic and unlikely to be fully protective.

These patients are at risk of developing antiviral resistance and subsequent complications.

AIM

In this report, we describe the emergence of the E119V substitution in the NA protein of an influenza A(H3N2) isolate detected in a clinical specimen collected from an immunocompromised patient after oseltamivir treatment in Argentina during 2014 epidemic season.
MATERIALS Y METHODS

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Molecular antiviral assays –
In 2014, a rapid genotypic screening was implemented at the NIC to identify the single Nucleotide polymorphism (SNP) encoding E119V substitution in clinical specimens that contained A(H3N2) influenza virus.
This technique allows the differentiation between wild-type (WT) and oseltamivir-resistant viruses. It was essentially a qRT-PCR assay conducted using a reaction mixture that included specific primers for influenza A(H3N2) NA segment with the E119V coding region and a pair of dual-labelled probes for the detection of the E119 and V119 variants. The original protocol was provided by Dr Adam Meijer, National Institute for Public Health and the Environment, The Netherlands.

Sanger sequencing and phylogenetic analysis –
The NA segment (1-1052 bp) of the viruses from pre- and post- treatment samples was sequenced to confirm the results obtained by E119V SNP screening.
The haemagglutinin (HA) segment of the E119V variant was also sequenced (1-986 bp). The sequencing PCR amplicons were purified with the MinElute® Gel Extraction Kit (QIAGEN). Sequencing reactions were performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™), and the products were analysed on an ABI PRISM® 3700 Genetic Analyser (Applied Biosystems™). Sequencing reaction conditions and primer sequences are available on request.
Sequences were analysed using BioEdit software, version 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/ bioedit.html). MEGA 4 (Tamura et al. 2007) and MEGA 5 (Tamura et al. 2011) programmes were used to build the phylogenetic trees using the neighbour-joining distance method. Tree topology was supported by bootstrap analysis with 1000 replicates. Vaccine strain sequences, as well as sequences from viruses collected in other countries, were obtained from the EpiFlu database available via the Global Initiative on Sharing All Influenza Data (GISAID) website, https://www.gisaid.org, and included in the analysis.
RESULTS

Out of 888 A(H3N2) samples received at the NIC, **842 were tested for the E119V substitution.**

836 samples were collected from untreated patients and six samples were obtained from six patients (four of them immunocompromised) after treatment.

WT E119 genotype was found in 841 specimens and **one sample contained a mixture of viral E119/V119 subpopulations.** This sample came from a post-treatment specimen collected from an immunocompromised patient. This specimen was from a 12-year-old girl with a history of acute B-cell lymphoblastic leukaemia. On September 15th, the first NPA tested positive for influenza A virus by qRT-PCR, and oseltamivir therapy (75 mg twice a day orally) was administered from that date until September 20th. On September 25th, the second NPA also tested positive for influenza A virus, none could be recovered in tissue culture and phenotypic studies could not be performed.

**Sanger sequencing of both pre- and post-treatment samples confirmed** the results obtained by E119V SNP screening and further quantitative assays will be necessary to confirm this finding (Fig. 2). Nucleotide sequences of all the genes sequenced have been deposited in the GISAID database under accession numbers EPI_ISL_172694 and EPI_ISL_172636. The HA sequence analysis of the resistant virus demonstrated that this strain belongs to group 3C.3 and is different from the A(H3N2) strain included in the 2014 influenza vaccine (McCauley et al. 2015).
Fig. 2: (A) a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) amplification plot corresponding to the wild-type E119 virus; (B) a qRT-PCR amplification plot corresponding to the mixture of E119 and V119 viral subpopulations; (C) a Sanger sequencing chromatogram showing base substitutions in the codon for residue 119 of the neuraminidase gene in the pre-treatment sample; and (D) Sanger sequencing chromatogram of the post-treatment sample; sensitive/resistant E119E/V subpopulations.
Although the frequency of the E119V substitution is low (0.01%) (WHO 2015), in this study, we have been able to detect a mixture of E119/V119 subpopulations in an influenza A(H3N2) virus from a post-treatment immunocompromised patient.

Because the resistant variant was not recovered in tissue culture, phenotypic assays could not be performed. Molecular detection of the E119V SNP cannot alone inform about oseltamivir resistance without complementary studies to confirm the decrease of the susceptibility to antiviral drugs.

The WHO working group on the surveillance of influenza antiviral susceptibility recommends that any detection of V119 variant is critical to the evolution of the patients and should result in a prompt treatment review (WHO 2015).

The absence of the E119V change in the pre-treatment sample suggests that the V119 variant arose as a consequence of the oseltamivir therapy. This finding is in agreement with previous reports about the emergence of E119V substitution in oseltamivir-treated immunocompromised patient.

This first study represents the beginning of the National systematic surveillance of the antiviral susceptibility of circulating influenza A(H3N2) viruses in Argentina in order to contribute to better decisions in health policies and help in selecting optimal medical treatment.

CONCLUSIONS

Although the frequency of the E119V substitution is low (0.01%) (WHO 2015), in this study, we have been able to detect a mixture of E119/V119 subpopulations in an influenza A(H3N2) virus from a post-treatment immunocompromised patient.

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Members of the National reference laboratory for respiratory viral diseases, WHO Argentina-Buenos Aires NIC.

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