Short communication

Characterization of influenza outbreaks in Lebanon during the 2013/14 and 2014/15 seasons

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ABSTRACT Despite the significant burden of influenza outbreaks, active disease monitoring has been largely absent in the Middle East, including Lebanon. In this study we characterized influenza virus in 440 nasopharyngeal swabs collected from patients with acute respiratory infections during two influenza seasons in Lebanon. Influenza A(H3N2) was dominant in the 2013/14 season while the A(H1N1)pdm09 and B/Yamagata strains were most prevalent in the 2014/15 season. All tested isolates were susceptible to 4 neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir). Genetic analysis of the haemagglutinin gene revealed multiple introductions of influenza viruses into Lebanon from different geographic sources during each season. Additionally, large data gaps were identified in the Middle East region, as indicated by the lack of current influenza sequences in the database from many countries in the region.
Introduction

Influenza A and B viruses cause significant morbidity and mortality during their annual outbreaks (1). Two influenza A subtypes, A(H1N1)pdm09 and A(H3N2), and 2 influenza B lineages, B/Yamagata and B/Victoria, circulate in humans (2, 3). Influenza A viruses are also associated with periodic pandemics that result in a significantly higher disease burden than annual outbreaks (4).

Currently, neuraminidase inhibitors (e.g. oseltamivir) are the mainstay for treatment and prophylaxis of influenza infections (5). Maintaining the efficacy of these drugs and vaccines requires close monitoring of viruses circulating in the community on a global scale.

In this study, we genetically characterized influenza viruses that circulated in Lebanon during the 2013/14 and 2014/15 seasons. We also report their susceptibility to antiviral drugs.

Methods

Patients presenting with acute respiratory infections at 2 sentinel sites in Beirut, Lebanon were recruited to the study. The sentinel sites were Makassed General Hospital in Beirut and the American University of Beirut Medical Center, which together serve ethnically and socioeconomically varied patient populations. Two nasopharyngeal swabs were collected from each patient by a nurse or physician. We collected 444 swabs between July 2013 and June 2015, spanning the 2013/14 and 2014/15 seasons; 90 of these were collected during the 2013/14 season. One swab was tested immediately at the clinic or hospital using a rapid-antigen detection test (RDT) (Quick Navi Flu+RSV, Denka Seiken, Japan). The second swab was suspended in viral transport medium and stored at –80 °C until shipped to the laboratory for further analysis. Subtype-specific real-time polymerase chain reaction (PCR) analysis was carried out on all samples testing positive by RDT. The PCR method used in this study employs cycling probes that can simultaneously detect the H275Y mutation in the A(H1N1)pdm09 neuraminidase protein (6), which confers resistance to the neuraminidase inhibitors oseltamivir and peramivir. The phenotypic antiviral drug susceptibilities were determined for 9 isolates from the 2013/14 season and 17 from the 2014/15 season using a fluorescent-based neuraminidase inhibition assay (7,8).

All patients consented to participate in the study, which was approved by the ethics committee at the American University of Beirut. The haemagglutinin (HA) genes of 24 influenza A and B isolates were sequenced as described in a previous study (9). Sequences were aligned in BioEdit 7.5.5 (10) and phylogenetic trees were inferred using maximum-likelihood method based on the best fit nucleotide substitution model with 1000 bootstrapped iterations implemented in MEGA 6.0 (11).

Results

Of the 90 samples that were collected during the 2013/14 season, 31 (34.4%) were positive for influenza A and none for influenza B (Figure 1). Influenza activity peaked in January during the 2013/14 season but was delayed till March during the 2014/15 season. Analysis of all samples testing positive for influenza showed 6 (19.3%) were A(H1N1)pdm09, 16 (51.6%) were A(H3N2), and 3 (9.7%) were A(H1N1)pdm09 and A(H3N2) mixed infections.

Of the 350 swabs collected during the 2014/15 season, 51 (14.5%) were influenza A and 37 (10.5%) were
Table 1: Antiviral drug susceptibility to neuraminidase inhibitors

<table>
<thead>
<tr>
<th>Type/ Subtype</th>
<th>No. tested</th>
<th>Oseltamivir IC50 (SD) (nM)</th>
<th>Zanamivir IC50 (SD) (nM)</th>
<th>Peramivir IC50 (SD) (nM)</th>
<th>Laninamivir IC50 (SD) (nM)</th>
<th>Resistance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013/14 season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(H1N1)pdm09</td>
<td>3</td>
<td>0.87 ± 0.07</td>
<td>0.49 ± 0.03</td>
<td>0.11 ± 0.01</td>
<td>0.45 ± 0.01</td>
<td>0</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>6</td>
<td>0.7 ± 0.08</td>
<td>0.73 ± 0.21</td>
<td>0.11 ± 0.01</td>
<td>0.72 ± 0.33</td>
<td>0</td>
</tr>
<tr>
<td>2014/15 season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A(H1N1)pdm09</td>
<td>6</td>
<td>1.20 ± 0.16</td>
<td>0.54 ± 0.05</td>
<td>0.12 ± 0.01</td>
<td>0.43 ± 0.05</td>
<td>0</td>
</tr>
<tr>
<td>A(H3N2)</td>
<td>3</td>
<td>1.20 ± 0.16</td>
<td>0.62 ± 0.13</td>
<td>0.15 ± 0.03</td>
<td>0.87 ± 0.09</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>1.20 ± 0.16</td>
<td>13.57 ± 5.25</td>
<td>1.10 ± 0.27</td>
<td>5.56 ± 1.67</td>
<td>0</td>
</tr>
</tbody>
</table>

*SD = standard deviation.*

influenza B. Real-time PCR analysis showed that 17 (19.3%) were A(H1N1)pdm09, 10 (11.3%) were A(H3N2), 13 (14.7%) were B/Victoria, and 16 (18.8%) were B/Yamagata by (8). Therefore, 64–81% of the RDT-positive samples were detectable by PCR.

None of the A(H1N1)pdm09 viruses possessed the H275Y mutation. All of the influenza isolates were susceptible to the 4 neuraminidase inhibitors tested (Table 1). The IC50 values of peramivir and laninamivir against influenza B isolates were much lower than those for oseltamivir and zanamivir.

Phylogenetic analysis of the HA gene showed that the Lebanese A(H1N1)pdm09 isolates belonged to clade 6B, which is characterized by mutations K163Q, A256T, and K283E (Figure 2). Five isolates from the 2014/15 season formed a sub-cluster that was defined by the N38D and K142R amino acid substitutions. In the case of A(H3N2), the isolates from the 2013/14 season belonged to subclade 3C, which is defined by the T128A amino acid substitution (Figure 2). The 2014/15 Lebanese A(H3N2) isolates fell into the 3C2 subclade, characterized by an N144S mutation. Lebanese influenza B isolates from the 2014/2015 season belonged to genetic groups 3 of the B/Yamagata-lineage and 1A of the B/Victoria lineage (Figure 2).

Discussion

We demonstrated that influenza viruses from the 2013/14 and 2014/15 seasons remain largely susceptible to neuraminidase inhibitors; this is consistent with the findings of other studies (12–15). Influenza A(H1N1)pdm09 was the most prevalent subtype circulating during the 2013/14 season, while influenza B was not detected. In contrast, influenza B was predominant in the 2014/15 season. In this study, 64–81% of the RDT-positive samples were detectable by PCR. However, other data from our laboratory (unpublished data, 2015) indicate that for the RDT test used in our study, specificity is >99%. The discrepant results between RDT and PCR results may have been a result of deterioration of the specimens during storage at the clinic or during transfer to the laboratory. Overall, our results emphasize on the clinical value of point-of-care RDT as it provides an easily accessible diagnostic tool enabling clinicians to make immediate decisions on whether to prescribe antivirals to their patients.

The phylogenetic analysis revealed that influenza outbreaks in Lebanon are seeded through introductions of multiple strains within the same season. Our ability to track the origins of influenza outbreaks in Lebanon and their movement across the region was limited by the lack of influenza sequences from most of the Middle Eastern countries, especially those neighbouring Lebanon. Our database search of influenza virus sequences from countries neighbouring Lebanon returned very few results, an indication of the large gaps in influenza surveillance in this highly interconnected region. A full understanding of the dynamics of influenza in Lebanon and the region will require better monitoring of influenza outbreaks and sequencing of the viruses isolated in these countries. The Middle East region is facing numerous wars and conflicts that have displaced millions of people within and across borders causing many people to be out of reach of the health system. At the time of our study, Lebanon was hosting more than 1.5 million Syrian refugees (one third of the overall population), many of whom were living in substandard shelters and were thus out of reach of surveillance activities (16). Further studies are urgently needed to assess the burden of respiratory infections among refugees and how they affect the disease dynamics among the local population.

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Competing interests: None Declared
Figure 2. Phylogenetic tree analysis of the HA genes of human influenza viruses isolated in Lebanon. The trees were inferred using maximum-likelihood analysis based on the best-fit nucleotide substitution model for each gene. Bootstrap support values >70%, which corresponds to a >95% probability that a given clade is real, are shown. Key amino acid substitutions are shown near the respective nodes. The WHO-recommended vaccine strains were obtained from the Influenza Resource Database and are indicated in boldface italics. Lebanese isolates are in bold fonts.
References


