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Influenza A(H1N1)pdm09 epidemiology in the Eastern Province of Saudi Arabia

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ABSTRACT

Objectives: The influenza A(H1N1)pdm09 virus caused a worldwide pandemic in 2009–2010 and has since remained in seasonal circulation. This study was conducted to determine any variations in the influenza A(H1N1)pdm09 status according to sex, age group, sample type, or location within the Eastern Province of the Kingdom of Saudi Arabia.
Methods: Samples from 749 patients with suspected Middle East respiratory coronavirus who presented to Johns Hopkins Aramco Healthcare facilities in the Eastern Province of Saudi Arabia were tested reflexively for influenza A/H1N1 2009 by the Ministry of Health using the RealTime ready Influenza A/H1N1 Detection Set for real-time PCR. The sample types included nasopharyngeal swabs (n = 677), expectorated deep cough sputum (n = 32), induced sputum (n = 17), and tracheal aspirates (n = 23).
Results: The incidence of influenza A(H1N1)pdm09 was higher among younger patients; 27.94% of patients in the 0–19-year age group tested positive compared to only 3.51% of patients in the ≥80-year age group. The incidence of influenza A(H1N1)pdm09 was higher in Ras Tanura city compared to other locations in the Eastern Province.
Conclusions: Younger individuals in the Eastern Province of the Kingdom of Saudi Arabia had a relatively higher risk of influenza A(H1N1)pdm09 infection. Additionally, an outbreak of influenza A(H1N1)pdm09 may have occurred in Ras Tanura city between April 2015 and February 2016.

Introduction

Seasonal influenza is an acute, contagious respiratory infection caused by one of the seasonal influenza viruses that circulate worldwide [1]. Although A, B, and C types of influenza exist, type C is detected much less frequently than the other two types and tends to cause only mild infection in humans [1]. In contrast, both A and B types cause outbreaks and epidemics; accordingly, circulating strains of both types are incorporated in seasonal influenza vaccines [1]. Human influenza is most commonly caused by type A viruses, which are the only type known to cause pandemics. Influenza A viruses are divided into subtypes based on surface hemagglutinin (H) and neuraminidase (N) proteins [1]. According to the World Health Organization (WHO), the H1N1 (A (H1N1) pdm09) and H3N2 subtypes are currently circulating among humans [1].

A pandemic-causing influenza A(H1N1)pdm09 virus (H1N1 2009; "swine flu") was first identified in humans in Mexico, Canada, and the United States during March/April 2009 [2]. Subsequently, the WHO declared a pandemic level phase 6 in June 2009. During the pandemic, viral spread via person-to-person contact affected at least 214 countries worldwide, including the Kingdom of Saudi Arabia (KSA) [1,3–6]. A total of 18,500 laboratory-confirmed deaths were recorded between April 2009 and the end of the pandemic on August 10, 2010 [7]. However, the actual number of respiratory and cardiovascular deaths was estimated to range between 151,700 and 575,500 [7]. After the pandemic, influenza A(H1N1)pdm09 replaced the previously circulating seasonal H1N1 and has since remained in seasonal circulation [6]. Currently, circulating influenza B viruses are divided into two main lineages: B/Yamagata and B/Victoria [1].

Unlike typical seasonal flu epidemics, 80% of deaths during the influenza A(H1N1)pdm09 pandemic occurred in people aged ≤65 years [7]. Consistent with this, the KSA observed a high prevalence of infection among younger people during the pandemic [8–12]. Specifically, a study of the first 100 cases revealed the highest inci-

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dence among people aged 20–30 years, followed by children aged 1–10 years [8].

Rapid and accurate diagnostic methods are essential for the prevention and control of influenza outbreaks, epidemics, and pandemics and for the initiation of appropriate and timely antiviral treatment. In the KSA, the Ministry of Health (MOH) currently uses the RealTime ready Influenza A/H1N1 Detection Set (Roche Diagnostics GmbH, Germany) for influenza A/H1N1 detection [13–16]. This real-time PCR-based method incorporates a generic influenza virus A PCR that targets the matrix protein-2 "M2 gene" (M2 PCR), as well as a specific PCR that targets the variable part of hemagglutinin in influenza A(H1N1)pdm09 (H1 PCR) [13–16]. Therefore, this study was designed to assess the epidemiology of influenza A(H1N1)pdm09 infection in the Eastern Province of KSA through a retrospective analysis of 749 samples obtained from patients with suspected Middle East respiratory coronavirus (MERS-CoV) infection between April 2015 and February 2016 and stratified according to sex, age group, sample type, and location.

Methods

Patient population and specimens

Clinical specimens from 749 patients with suspected MERS-CoV who presented at Johns Hopkins Aramco Healthcare facilities in the Eastern Province of KSA between April 2015 and February 2016 were subjected to reflexive influenza A/A(H1N1)pdm09 testing. In total, 23, 614, 50, 53, and one samples were collected from Abqiq City (AB), Dhahran City (DH), Al-Hasa (AH), Ras Tanura City (RT), and Udhailya City (UC), respectively; the remaining eight were from unknown locations. Reflex testing was performed to fulfil the KSA MOH guidelines for patients who met the Category I [acute respiratory illness with clinical and/or radiological evidence of pulmonary parenchymal disease (pneumonia or acute respiratory distress syndrome)] or Category II (hospitalized patient with healthcare-associated pneumonia based on clinical and radiological evidence) criteria for possible MERS-CoV infection [17]. The MOH guidelines require that samples from such patients should be simultaneously tested for other common viral and bacterial causes of community-acquired pneumonia [17]. The sample types included nasopharyngeal swabs (NASPH) (n = 677), expectorated deep cough sputum (SPUEX) (n = 32), induced sputum (SPUIN) (n = 17), and tracheal aspirate (TRAC) (n = 23). Of all the samples, 359 and 390 were collected from female and male subjects, respectively. The patients' ages ranged from 1 to 108 years (median: 63 years).

The MOH conducted testing using the RealTime ready Influenza A/H1N1 Detection Set for real-time PCR. Samples were collected according to the standard procedures of the collecting institution. Samples were kept at 2–8 °C until transportation to the MOH Dammam regional laboratory. Samples were transferred on a daily basis.

Specimen type and processing

One volume of mucoid sample was mixed with two volumes of bacterial lysis buffer, incubated at room temperature (22–25 °C) for 5 min and centrifuged at 15,000 rpm for 5 min. Subsequently, viral nucleic acid was extracted from the supernatant.

Nucleic acid extraction

Nucleic acid extraction was performed using MagNA Pure 96DNA and a viral nucleic acid small volume kit on a Magna Pure 96 instrument (Roche Diagnostics) according to the manufacturer's instructions. The Pathogen Universal-200 purification protocol was used.

Reverse transcription and DNA amplification: RealTime ready Influenza A/H1N1 Detection Set

The influenza A/A(H1N1)pdm09 virus was detected using the RealTime ready Influenza A/H1N1 Detection Set. The generic influenza A M2 target and the influenza A(H1N1)pdm09 subtype-specific H1 target was amplified via one-step RT-PCR with the RealTime-ready RNA virus master, according to the manufacturer's instructions. PCRs were performed using the LightCycler 2.0 instrument (Roche Diagnostics). All PCRs were performed according to the following conditions: 58 °C for 8 min; 95 °C for 30 s; 45 cycles of 95 °C for 1 s, 60 °C for 20 s, and 72 °C for 1 s; and cooling to 40 °C for 30 s. For the M2 PCR, two sets of probes and primers (targeting human nucleic acid and influenza A/M2 gene) and four controls were used [extracted control for human nucleic acid (internal sample control), commercial positive plasmid control for the whole PCR, negative extracted control (water), and no template negative control]. For H1 PCR, one set of probes and primers and three controls were used [commercial positive plasmid control for the whole PCR, negative extracted control (water), and no template negative control].

The results were interpreted as positive if the crossing point (Cp) value was ≤40 in the presence of a sigmoid curve. Negative results were reported if no value or Cp value was >40 and a sigmoid curve was absence. A sample was reported as positive (POS) for influenza A(H1N1)pdm09 if positive results were reported for both generic M2 and subtype-specific H1 PCRs. A sample was reported as influenza A (FluA) if only the generic M2 PCR yielded a positive result. If a sample was negative for M2, the H1 PCR was not performed, and the sample was reported as negative (NEG).

Statistical methods

A chi-square test was used to compare the distribution of RealTime ready Influenza A/H1N1 Detection Set results with respect to sex, sample type, location and age group; a p value of ≤0.05 was accepted as significant.

Results

Influence of age group

To determine any variation in the incidence of influenza A(H1N1)pdm09 according to the age of the patient from whom the sample was obtained, the subject group was stratified into the following age groups: 0–19 (n = 68), 20–39 (n = 105), 40–59 (n = 162), 60–79 (n = 243), and ≥80 years (n = 171) (Table 1). We compared the distributions of POS vs. NEG results from Table 1 (n = 738) across the age groups using a chi-square test. The chi-square statistic was 41.9976 ($p < 0.00001$), which confirmed a significant variation in the incidence of A(H1N1)pdm09 by age.

Influence of location

Of the 749 samples, 23 samples were from AB, 614 from DH, 50 from AH, 53 from RT and one from UC, whereas the remaining eight were from an unknown location. Table 2 presents the distribution of influenza A/A(H1N1)pdm09 statuses according to the location. To determine whether the location affected the likelihood of a positive influenza A(H1N1)pdm09 result, we compared the distribution of POS v. NEG results for AB, DH, AH, and RT (n = 729; Table 2) using a chi-square test. The chi-square statistic was 68.7453 ($p < 0.00001$), which confirmed a significant variation in the incidence of influenza A(H1N1)pdm09 by location.

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