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Original Article

Molecular diagnosis of H1N1 virus

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ABSTRACT

Background: Influenza A viruses are medically significant pathogens responsible for higher mortality and morbidity throughout the world. Swine influenza is known to be caused by influenza A subtypes H1N1, H1N2, and H3N2, which are highly contagious, and belongs to the family Orthomyxoviridae. Efficient and accurate diagnosis of influenza A in individuals is critical for monitoring of a constantly evolving pandemic. A rapid result is important, because timely treatment can reduce disease severity and duration. Rapid antigen tests were among the first-line diagnostic tools for the detection of pandemic H1N1 (2009) virus infection during the initial outbreak. Current study focuses on the significant approach of the usage of molecular method utilizing real-time PCR for the detection of type A influenza virus (H1N1 subtype) in humans.

Methods: A total of 2000 mixed nasal/throat swab specimens collected in commercial viral transport from Apollo hospitals, Hyderabad were submitted to Institute of Preventive Medicine for molecular testing by reverse transcriptase polymerase chain reaction (RT-PCR) from 2009 to 2015 from its affiliated primary care clinics.

Results: Among the 2000 samples collected, 700 samples were positive for Human Inf A, swine Inf A, and Swine Inf H1 (fourth table in the article). One thousand two hundred samples were negative for Human Inf A, swine Inf A, and Swine Inf H1, and 100 samples were positive for Influenza A only.

Conclusion: The molecular testing of H1N1 patients helped the clinicians in timely diagnosis and treatment of these patients during the pandemic surveillance. The RT-PCR test has higher sensitivity and specificity; hence it is considered to be the best tool to use during the pandemic surveillance, as compared to the any other commercial antigen-based tests, which show a variable performance, with the sensitivities of tests from different manufacturers ranging from 9 to 77%.

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1. Introduction

Since the identification of the pandemic influenza (H1N1) 2009 virus and its subsequent antigenic and genetic characterization, this new influenza virus strain has rapidly spread worldwide.[1–10] As of December 2009, >600,000 cases and at least 8768 deaths were reported.[11] In June 2009, the outbreak was officially declared a pandemic by the World Health Organization (WHO). The pandemic (H1N1) 2009 strain evolved from the family of swine triple-reassortant viruses, which contain genes derived from avian, swine, and human influenza viruses. The pandemic (H1N1) 2009 strain acquired the hemagglutinin (H) gene from a swine H1N2 virus and the neuraminidase (N) and matrix protein genes from the Eurasian swine lineage, and it evolved into a pathogen capable of sustaining efficient human-to-human transmission.[4]

Frontline pandemic surveillance relies on rapid diagnosis of suspected cases and timely treatment of infected individuals. The current diagnostic tests for pandemic (H1N1) 2009 virus include qualitative reverse transcriptase polymerase chain reaction (RT-PCR) and antigen-based assays. The antigen-based assays provide rapid diagnosis (within 15 min) but with a sensitivity of only 56%, when compared with the results of RT-PCR (74%), which is more sensitive.[12,13] The qualitative RT-PCR analysis has the primers specific for the hemagglutinin or neuraminidase gene (or both) of the pandemic virus. Due to its high sensitivity and specificity, RT-PCR is the preferred diagnostic platform in individual labs or in centralized lab settings. Nowadays along with the routine antigen testing, RT-PCR is also accessible in resource-limited setting and has been used frequently in diagnosis.

This manuscript focuses on the importance of the molecular tools and its use in point-of-care testing at an affordable cost in critically needed pandemic surveillance. Confirmation of novel influenza A (H1N1) infection may be necessary for surveillance purposes and for special situations, e.g. severely ill patients, patients with immune-compromising conditions, and pregnant and breast feeding women, which is possible by molecular methods only.

2. Materials and methods

2.1. *Clinical specimen preparation*

A total of 2000 mixed nasal/throat swab specimens were collected in commercial viral transport Media (Himedia) and submitted to the Molecular Biology and Cytogenetics Department, Apollo hospitals, Hyderabad from 2009 to 2015 from its affiliated primary care clinics. These included patients, who attended the emergency medicine department at Apollo Hospitals, Hyderabad, public and private primary care clinics, in-patients with influenza-like symptoms, as well as patients or staff with compatible contact or travel histories. These samples were submitted to Institute of Preventive Medicine (IPM), which is the centralized laboratory recognized by the Telangana and Andhra Pradesh state Governments for molecular RT-PCR testing.

Patient ages ranged from 19 days to 85 years, and the male-to-female ratio was 1.2 to 1 (Table 1). A duly filled in form along with clinical history and the previous vaccination details were collected and submitted to IPM along with the sample.

Extraction was performed with QIAamp® Viral RNA Mini Kit. The primer and probe sequence used are shown in Table 2. The PCR primers are designed to target these three viruses: Human Inf A, Swine Inf A, and Swine Inf H1. The PCR conditions are shown in Table 3. Applied biosystems real time PCR 7000 equipment was used.

Table 2 – Primer and probe sequence for the Inf A, SW Inf A and SW H1virus.

Primers and probes	Sequence (5′>3′)
Inf A Forward	GAC CRA TCC TGT CAC CTC TGA C
Inf A Reverse	AGG GCA TTY TGG ACA AAK CGT CTA
Inf A Probe	TGC AGT CCT CGC TCA CTG GGC ACG
SW Inf A Forward	GCA CGG TCA GCA CTT ATY CTR AG
SW Inf A Reverse	GTG RGC TGG GTT TTC ATT TGG TC
SW Inf A Probe	CYA CTG CAA GCC CA“T”' ACA CAC AAG CAG GCA
SW H1 Forward	GTG CTA TAA ACA CCA GCC TYC CA
SW H1 Reverse	CGG GAT ATT CCT TAA TCC TGT RGC
SW H1 Probe	CA GAA TAT ACA “T”CC RGT CAC AAT TGG ARA A
RnaseP Forward	AGA TTT GGA CCT GCG AGC G
RnaseP Reverse	GAG CGG CTG TCT CCA CAA GT
RnaseP Probe	TTC TGA CCT GAA GGC TCT GCG CG

Table 3 – RT-PCR amplification conditions.

Reaction volume	25 μl
Program the thermo cycler as follows:	
Reverse transcription	50 °C for 30 min
Taq inhibitor activation	95 °C for 2 min
PCR amplification (45 cycles)	95 °C for 15 s
	55 °C for 30 s (FAM is used for fluorescence data)

3. Results

Among the 2000 samples collected, 700 samples were positive for Human Inf A, Swine Inf A, and Swine Inf H1 (Table 4). One

Table 1 – Age groups and gender of the patients.

Gender\Age group	0–25 years	25–50 years	50–75 years	>75 years
Male	177	589	365	104
Female	123	453	137	52
Mean age (years)	22	35–40	60	79