Original Article

Molecular characterization of influenza A(H1N1)pdm09 virus circulating during the 2009 outbreak in Thua Thien Hue, Vietnam

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Abstract

Introduction: The influenza A(H1N1)pdm09 virus arrived in Vietnam in May 2009 via the United States and rapidly spread throughout the country. This study provides data on the viral diagnosis and molecular epidemiology of influenza A(H1N1)pdm09 virus isolated in Thua Thien Hue Province, central Vietnam.

Methodology: Nasopharyngeal swabs and throat swabs from 53 clinically infected patients in the peak of the outbreak were processed for viral diagnosis by culture and RT-PCR. Sequencing of entire HA and NA genes of representative isolates and molecular epidemiological analysis were performed.

Results: A total of 32 patients were positive for influenza A virus by virus culture and/or RT-PCR; of these 22 were positive both by viral isolation and RT-PCR, 2 only by virus culture and 8 only by RT-PCR. The novel subtype of influenza A(H1N1)pdm09 was present in 93.4% of the isolates. Phylogenetic analysis of the HA and NA gene sequences showed identities higher than 99.50% in both genes. They were also similar to reference isolates in HA sequences (> 99% identity) and in NA sequences (>98.50% identity). Amino acid sequences predicted for the HA gene were highly identical to reference strains. The NA amino acid substitutions identified did not include the oseltamivir-resistant H275Y substitution.

Conclusion: viral isolation and RT-PCR together were useful for diagnosis of the influenza A(H1N1)pdm09 virus. Variations in HA and NA sequences are similar to those identified in worldwide reference isolates and no drug resistance was found.

Key words: influenza A(H1N1)pdm09; genetic characterization; viral isolation; RT-PCR; Thua Thien-Hue

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Introduction

In 2009, cases of novel influenza A(H1N1)pdm09 virus were first identified in Mexico and the United States; the virus rapidly spread and caused a worldwide diffused pandemic within a few months. In Vietnam the first novel A(H1N1)pdm09 case was confirmed in a 23-year-old student returning from the United States [1,2]; confirmed cases quickly increased in the following months reaching the first peak in June [1]. According to the report by the Ministry of Health (MOH), 11,047 cases of novel A(H1N1)pdm09 infection were identified up to 21 December 2009, with 50 deaths[3]. In the province of Thua Thien Hue, the outbreak started at the end of June 2009, and the clinically reported cases increased in the following months, reaching the peak in October and November. The total number of clinically identified cases from the beginning of the outbreak to 30 December 2009 was 2,051, and no deaths were reported from the Provincial Health Service [4]. At the microbiology laboratories of the Carlo Urbani Centre in Hue University of Medicine and Pharmacy, we set up cell culture and reverse transcription polymerase chain reaction (RT-PCR)procedures to detect the virus from the suspected cases and identify the new subtype of influenza A(H1N1)pdm09virus causing the outbreak in Thua Thien Hue. This paper reports the results of our study on viral diagnosis of novel influenza A(H1N1)pdm09 virus and molecular characterization of hemagglutinin (HA) and neuraminidase (NA) sequences of representative strains isolated in Thua Thien Hue in the 2009 outbreak of A(H1N1)pdm09 infection.

Methodology

Patients and samples

A total of 53 patients suspected of having A(H1N1)pdm09 virus infection, who were hospitalized between 8 October and 19 November 2009, in the isolation department of Hue City Hospital during the 2009 pandemic were sampled for virus detection. All patients had acute respiratory symptoms as described by the World Health Organization (WHO)[5]. The patients ranged in age from 4 years to 40 years (median = 14), and there were 25 males and 28 females. At the time of sample collection, the majority of the patients were on the second day after the onset of symptoms. Naso-pharyngeal swabs or throat swabs were taken and put into 3ml of viral transport medium (VTM) (Becton Dickinson, Franklin Lakes, NJ, USA); the samples were then placed in an ice box and immediately transferred to the laboratory.

Sample preparation and viral RNA extraction

In the laboratory, 3ml VTM was mixed well and divided into four tubes, two of 1ml for isolation and two of 0.5ml for the amplification assay. For amplification, one 0.5ml tube of each sample was used for RNA extraction using the Qiagen viral RNA mini kit for RT-PCR per the manufacturer's instructions. Viral RNA in RNase-free water was kept at -20°C until testing.

Isolation

The 1ml portion of samples in VTM was filtered through 0.4 μm size filters (Millipore, Billerica, MA, USD), then inoculated into a 25-ml T-flask with confluent monolayers of Madin-Darby Canine Kidney (MDCK) cells grown in minimal essential medium (MEM; pH 7.2) (Invitrogen, Grand Island, NY, USA) containing 0.2% bovine serum albumin (BSA) and 2μg/ml TPCK-Trypsin, penicillin [10U/ml], streptomycin [10μg/ml], and amphotericin B [0.25mg/ml]. The samples were then incubated in a 5% CO_2 incubator at 35.5°C and followed daily for typical cytopathic effects (CPE) such as the presence of enlarged granular cells and swollen cells, destruction of the cells, and detachment of swollen cells from the surface under an inverted microscope (10x and 20x) for seven days [6]. When the CPE was visualized, the supernatant was harvested for viral RNA extraction with the QIAamp Viral RNA Mini Kit (Qiagen, Germantown, MD, USA) for typing and subtyping by reverse transcription-polymerase chain reaction.

RT-PCR for typing and subtyping

Primers selections for typing and subtyping influenza A virus are shown in Table 1. Two conventional RT-PCR protocols were used for typing and subtyping. The first multiplex RT-PCR was a modified WHO protocol [7] for identifying influenza A(H1N1)pdm09 virus by using the one-step RT-PCR assay (Invitrogen). PCR was performed using the Veriti Gradient thermal cycler (Applied Biosystems, Grand Island, NY, USA) with the following programme: 30 minutes at 50°C for cDNA synthesis, then 95°C for 15 minutes for initial denaturation, followed by 45 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for one minute, and a final extension step of 72°C for 10 minutes. The second RT-PCR multiplex protocol was performed with the primers for subtyping H3N2 and seasonal H1N1 as described previously [8]. Positive controls were kindly provided by the Pasteur Institute, HCM City [influenza A(H1N1)pdm09 virus] and the Virology Laboratory, San Raffaele Hospital, Milan, Italy. Influenza A H3N2/Hong Kong/8/68 and seasonal H1N1/Puerto Rico/8/34 were included in each experiment. Amplicons were visualized by ethidium bromide staining following electrophoresis on 1.5% agarose gel. The presence of DNA bands at the specific sizes of controls was considered as positive.

Sequencing HA and NA genes of A(H1N1)pdm09 subtype

Four isolates of novel A(H1N1)pdm09 subtype were randomly chosen for sequencing the HA and NA genes. The supernatants of MDCK cell culture were collected and 140μl of each were processed for RNA extraction. Reverse transcription was conducted using primer Uni 12 (M) (5'-AGCRAAAGCAGG-3') with the ThermoScript RT-PCR System(Invitrogen).The reaction was performed at 65°C for 60 minutes and was terminated by heating at 85°C for 5 minutes[9].

PCR amplification of complete HA and NA genes was performed in the Veriti Gradient thermal cycler using the protocol described previously [9] with the GoTaq PCR core systems (Promega, Madison, WI,USA). The PCR products were purified using the GenElute PCR Clean-up Kit (Sigma, St. Louis, MO,USA), and the DNA purification was checked following electrophoresis on 1% agarose gel by visualizing the specific sizes of HA (1778bp) and NA (1410 bp) bands stained with ethidium bromide. Purified preparations of the HA and NA genes were sent for direct sequencing (BMR Genomics, Padova, Italy).

Table 1. Primer sequences used for influenza A virus typing and sequencing

Type/subtype	Target gene	Primer	Primer Structure (5' - 3')	Product length (bp)	Reference
Influenza type A	M	M30F2/08	ATGAGYCTTYTAACCGAGGTCGAAACG	244bp	7
		M264R3/08	TGGACAAANCGTCTACGCTGCAG		
Influenza A (H1N1) 2009 virus	H1-2009	H1-F1	TGCATTTGGGTAAATGTAACATTG	349bp	7
		H1-R1	AATGTAGGATTTRCTGAKCTTTGG		
Complete sequence	HA	HA-1	AGCAAAAGCAGGGGAAAATA	1778bp	9
		HA-1778	AGTAGAAACAAGGGTGTTTT		
	NA	NA-1	AGCAAAAGCAGGAGTGAAAA	1413bp	9
		NA-1413	AGTAGAAACAAGGAGTTTTTT		
H3N2	HA3	H3 P1	CCTTGATGGAGAAAACTGCACAC	338bp	8
		H3 P2	TGTTTGGCATAGTCACGTTCA		
Seasonal H1N1	H1s	H1 P1	GAATCATGGTCCTACATTGTAGAAA	814bp	8
		H1 P2	ATCATTCCAGTACATCCCCCTTCAAT		

Phylogenetic analysis

The complete HA and NA sequences of influenza A (H1N1) 2009 viruses isolated from humans in different countries of Asia (Cambodia, China, Hong Kong, India, Japan, Taiwan and Thailand), Europe (England, Germany, Italy and France), Australia, and America (USA, Mexico, Canada) from April 2009 to November 2009 were obtained from the GenBank at http://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi for comparison and construction of phylogenetic trees.

MEGA5 software (MEGA, Tempe, AZ, USA) was used for phylogenetic analysis of HA and NA sequences, and the evolutionary distance was inferred by the maximum likelihood method based on the Tamura-Nei model [10]. The phylogenetic trees were constructed using MEGA5 software with the 100 replicates bootstrap. Geneious 4.85 software (http://www.geneious.com/) was used for alignment and comparison of amino acid sequences.

Results

Detection and subtyping of influenza A viruses

Of the 53 samples collected from patients with suspected influenza A(H1N1)pdm09 virus infection, 41 were tested by both isolation and RT-PCR and 12 were tested only by RT-PCR. Viral isolation in cell culture was positive in 24 out of 41 samples (58.5%) and RT-PCR was positive in 27 (65.9%). Among these, 22 samples were positive by both isolation and RT-PCR, 2 samples were positive with RT-PCR only after isolation, and 5 samples were detected only by RT-PCR. Therefore, the total number of positive samples by both methods was 29, making the sensitivity for RT-PCR and viral isolation 93.1% and 82.7%, respectively.

In 12 samples tested only with RT-PCR, 3 were positive for influenza A(H1N1)pdm09 virus. The total number of positive samples for influenza type A virus was 32 (60.3%) of the 53 samples; after subtyping, 30 (93.7%) were influenza A(H1N1)pdm09 virus and 2 (6.3%) were seasonal H1N1 subtype, while subtype H3N2 was not identified in this study.

Characterization and phylogenetic analysis of HA and NA genes of Influenza A(H1N1)pdm09 virus in Thua Thien Hue

The complete HA gene of isolates 6, 7, 10, 15 and the complete NA gene of isolates 6, 10 and 15 were sequenced and submitted to GenBank, where they are available with the following accession numbers: Isolate 6: JN896300 for HA, JN896301 for NA; Isolate 7: JN935017 for HA; Isolate 10: JN896302 for HA, JN896303 for NA; Isolate 15: JN393307 for HA, JN656969 for NA.

Alignment of HA sequences among isolates 6, 7, 10 and 15 showed high similarities and the nucleotide identity percentages ranged from 99.48% to 99.77% for HA, with nucleotide differences of 9 to 4 nucleotides. When Hue HA sequences were aligned with 19 reference isolates, similarities ranging from 99.01% to 99.77% (16 to 4 nucleotide differences, respectively) were found. Hue isolates also showed above 99% identity with the prototypic A/California/07/2009 H1N1 strain. In the phylogenetic tree of HA sequences, Hue isolates were clustered together with A/Japan/NHRC0001/2009(H1N1) and showed closer similarity to the A/Firenze/10/2009(H1N1) and A/Cambodia/NHRCC00011/2009(H1N1) isolates (Figure 1).

Comparing NA sequences of Hue isolates 6, 10 and 15 showed similarities from 99.57% to 99.86% with differences from 6 to 2 nucleotides. After alignment with 19 reference NA sequences, identities ranged from 99.36% (9 different nucleotides) to 98.65% (19 different nucleotides). In the phylogenetic tree, Hue NA sequences are seen together in a separate cluster, and show greater similarity to A/Vienna/INS291/2009(H1N1), A/Firenze/10/2009(H1N1), A/Japan/NHRC0001/2009(H1N1), A/Taiwan/526/2009(H1N1), and A/England/313/2009(H1N1) (Figure 2).

Differences in amino acid

The comparison of amino acid sequences predicted from the HA nucleotide sequence of Hue isolates with the sequences of the prototypic A/California/07/2009 H1N1 and 18 other reference strains showed a high degree of similarity (Table 2). However, point mutations were detected with the NA sequence of three Hue isolates at S35N, I54L, A86T, S95N, D103N, R130K, R156P, V176I, A178P, A181T, D199N in comparison with the prototypic A/California/07/2009 H1N1 and the 18 other reference strains (Table 3).

Figure 1. The phylogenetic tree of the HA genes of four influenza A(H1N1)pdm09 viruses in Thua Thien Hue and 19 reference sequences by using Maximum Likelihood method, the length scale measured the number of substitutions per site

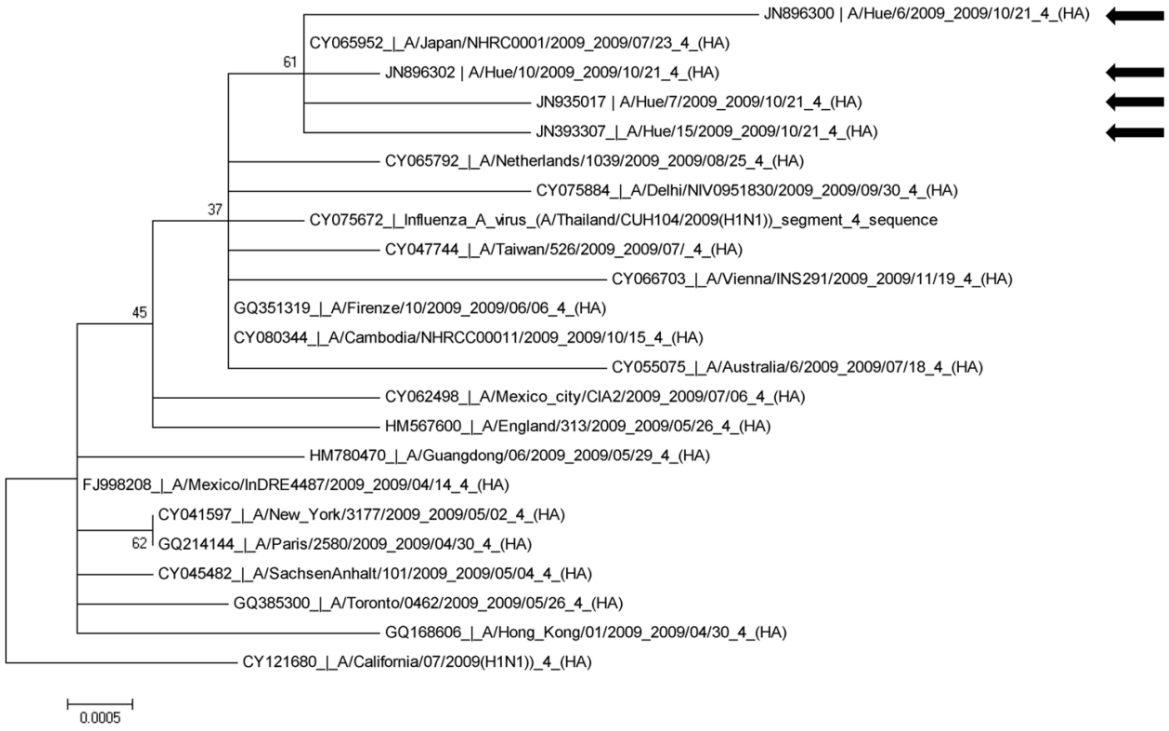
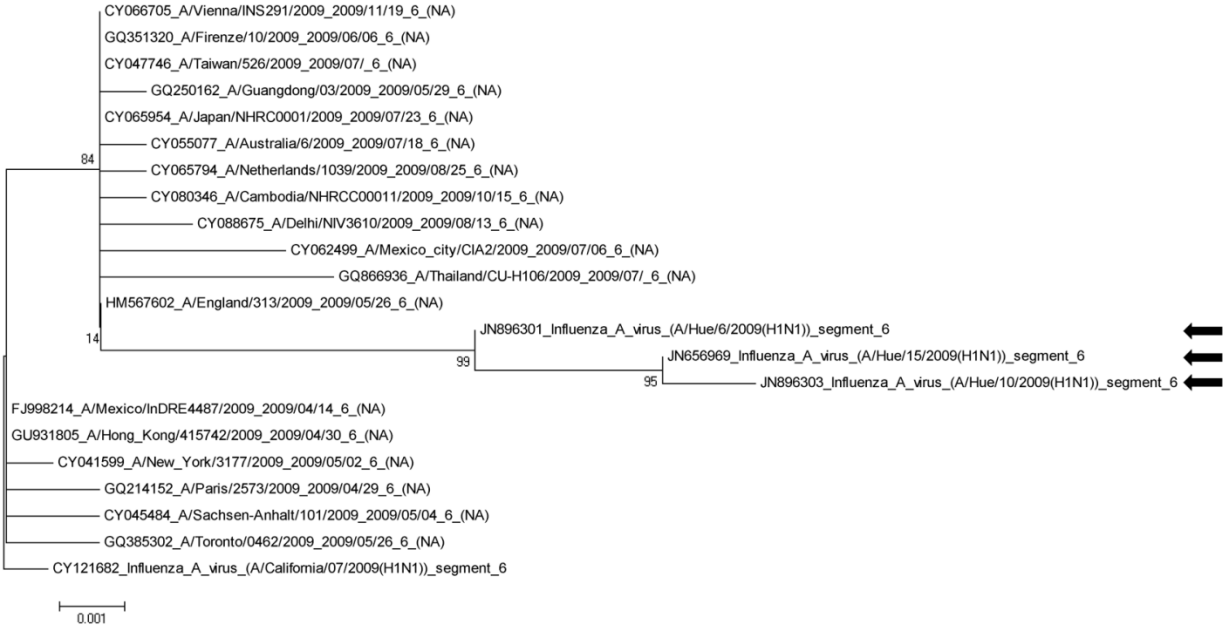


Figure 2. The phylogenetic tree of the NA genes of three influenza A(H1N1)pdm09 viruses in Thua Thien Hue and 19 reference sequences was constructed by using Maximum Likelihood method, the length scale measured the number of substitutions per site.



Discussion

The outbreak of A(H1N1)pdm09 infection in Vietnam started on 30 May 2009 with an infected student returning from the United States, and by 15 July, 309 laboratory-confirmed cases were reported from 29 provinces and cities across the country [2]. Most of the cases (87%) were imported through airline passengers arriving in Ho Chi Minh City, with infected people arriving from Australia, the United States, Thailand, Singapore, Germany, Hong Kong and New Zealand [2]. In the following months the new influenza A(H1N1) pdm09 showed a high capacity to spread rapidly in all parts of the country, quickly replacing the pre-existing epidemic strains, particularly H1N1 [11,12]. In our study, sample collection was performed from the beginning of October to the middle of November 2009; this period corresponded with the peak of the A(H1N1)pdm09 epidemic spreading throughout the community in Vietnam. In spite of the favorable epidemic conditions, influenza A virus was identified in only 60.3% of the clinically suspected patients. This observation may indicate that another virus or viruses could have been responsible for the respiratory infection. In fact, recent results have shown the circulation of a different respiratory virus in children during the peak period of the influenza epidemic [13].

Another factor influencing the successful isolation of the virus was the time of sample collection, since viral load in respiratory secretions is higher at the beginning of the symptomatic phase and patients usually seek hospital care a few days after the onset of the disease, when viral release is going down [14].

It is interesting to note that 93.7% of the influenza A isolates belong to the new A(H1N1)pdm09 virus; the remaining 6.3% belong to the previously circulating seasonal H1N1 subtype. This observation is in accordance with the high spreading capacity of the new influenza virus and its ability to compete with the previously dominant viral population, leading to an almost complete disappearance of the seasonal H1N1 virus, as shown in more recent papers [11,12].

Sequencing of the HA and NA genes of some representative isolates from Thua Thien Hue indicates that they are genetically very close and most probably originated from the same clade of A(H1N1)pdm09. This hypothesis is further confirmed by the phylogenetic tree constructed with the HA and NA sequences of the Hue isolates and the 19 reference sequences obtained by the Gene Bank. The Hue isolates are clustered together both for the HA and NA genes, and show high identities with the reference isolate sequences. Several reports have shown that influenza A(H1N1)pdm09 virus circulating in a

Table 2. Comparison of amino acid sequences of 4 Hue HA from A(H1N1)pdm09 isolates with the prototypic A/California/07/2009 H1N1 strain and the 18 other reference HA sequences

Strain name	12	17	27	58	59	110	113	146	165	183	230	250	257	262	287	320	322	348	415	453	501	530	572	582
A/California/07/2009	K	L	A	N	L	P	D	S	H	N	S	R	Y	E	N	Q	I	I	T	V	T	V	C	R
A/New York/3177/2009	–	–	–	–	–	S	–	–	–	–	–	Q	–	–	–	–	–	V	–	I	–	–	–	–
A/Toronto/0462/2009	–	–	–	–	–	S	–	–	–	–	–	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Mexico/InDRE4487/2009	–	–	–	–	–	S	–	–	–	–	–	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Mexico city/CIA2/2009	–	–	–	–	–	S	–	–	–	K	–	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/England/313/2009	–	–	–	–	–	S	–	–	–	–	–	Q	–	–	–	H	–	V	–	–	M	–	–	–
A/Paris/2580/2009	–	–	–	–	I	S	–	–	–	–	–	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Firenze/10/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Vienna/INS291/2009	–	–	–	D	–	S	–	–	N	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Netherlands/1039/2009	–	–	T	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Sachsen-Anhalt/101/2009	–	M	–	–	–	S	–	–	–	–	–	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Australia/6/2009	–	–	–	–	–	S	G	–	–	–	T	Q	H	–	–	–	–	V	–	–	–	–	–	–
A/Hong Kong/01/2009	–	–	–	–	–	S	–	–	–	–	–	Q	–	–	–	–	–	V	I	–	–	–	–	–
A/Guangdong/06/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	I	Y	–
A/Taiwan/526/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	K
A/Thailand/CU-H104/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	D	–	–	V	–	–	–	–	–	–
A/Japan/NHRC0001/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Delhi/NIV0951830/2009	E	–	–	–	–	S	–	–	–	–	T	Q	–	K	–	–	V	V	–	–	–	–	–	–
A/Cambodia/NHRCC00011/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Hue/7/2009	–	–	–	–	–	S	–	N	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Hue/6/2009	–	–	–	–	–	S	Y	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Hue/10/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–
A/Hue/15/2009	–	–	–	–	–	S	–	–	–	–	T	Q	–	–	–	–	–	V	–	–	–	–	–	–

Table 3. Comparison of amino acid sequences of 3 Hue NA from A(H1N1)pdm09 isolates with the prototypic A/California/07/2009 H1N1 strain and the 18 other reference NA sequences

Strain name	9	35	48	54	55	73	86	95	103	106	130	156	176	178	181	199	248	274	371	372	453	469
A/California/07/2009	T	S	T	I	T	N	A	S	D	V	R	R	V	A	A	D	N	Y	F	E	V	K
A/New York/3177/2009	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Toronto/0462/2009	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Mexico/InDRE4487/2009	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Mexico city/CIA2/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	H	–	–	M	–
A/England/313/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Paris/2573/2009	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Firenze/10/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Vienna/INS291/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Netherlands/1039/2009	I	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Sachsen-Anhalt/101/2009	–	–	I	–	–	–	–	G	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Australia/6/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Hong Kong/415742/2009	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A/Guangdong/03/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Taiwan/526/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Thailand/CU-H106/2009	–	–	–	–	A	D	–	–	–	I	–	–	–	–	–	–	D	–	L	G	–	N
A/Japan/NHRC0001/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Delhi/NIV3610/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Cambodia/NHRCC00011/2009	–	–	–	–	–	–	–	–	–	I	–	–	–	–	–	–	D	–	–	–	–	–
A/Hue/6/2009	–	N	–	L	–	–	T	N	N	I	K	P	–	–	–	–	D	–	–	–	–	–
A/Hue/10/2009	–	N	–	L	–	–	T	N	N	I	K	P	I	P	T	N	D	–	–	–	G	–
A/Hue/15/2009	–	N	–	L	–	–	T	N	N	I	K	P	I	P	T	N	D	–	–	–	–	–

geographic region may result from different original lineages which are more closely related in evolution [15,16]. Our analyses were conducted on only four randomized isolates, which were collected in the same place in a short period of time and after a few months from the beginning of the pandemic, limiting the possibility of greater genetic variability.

Worldwide, recent reports have shown that a small proportion of influenza A(H1N1)pdm09 viruses have an NA mutant substitution at H275Y (N1 numbering, H275Y) that has been reported to give a high degree of resistance to oseltamivir [17,18,19,20]. This mutant isolate of influenza A(H1N1)pdm09 virus was also detected in Vietnam [18,20,21]. Thua Thien Hue isolates had several mutated substitutions in the NA amino acid sequences; however, they were not harboring the mutant substitution at H275Y. Our results are in agreement with those in a report on treatment response for the outbreak of influenza A(H1N1)pdm09 from the health service in Thua Thien Hue province, in which a total of 2,051 clinical cases of A(H1N1)pdm09 infection in 2009 received oseltamivir (Tamiflu) in the local health stations and communities; all infected people responded well to the antiviral drug and 89% of the patients showed no fever after two days of treatment [4].

Conclusion

Our study showed that viral isolation on MDCK and RT-PCR are useful and sensitive for the detection of the novel influenza A(H1N1)pdm09 virus. Thua Thien Hue representative isolates were highly similar in the HA and NA gene sequences to reference isolates; several mutations were found in the NA amino acid sequence, but no oseltamivir resistant mutation could be demonstrated.

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References

1. Hien TT, Boni MF, Bryant JE, Ngan TT, Wolbers M, Nguyen TD, Truong NT, Dung NT, Ha do Q, Hien VM, Thanh TT, Nhu le NT, Uyen le TT, Nhien PT, Chinh NT, Chau NV, Farrar J, van Doorn HR(2010) Early Pandemic Influenza (2009 H1N1) in Ho Chi Minh City, Vietnam: A clinical, virological and epidemiological analysis. PLoS Med 7: e1000277.
2. Nguyen VB, Lan PT, Tinh PT, Nguyen TMN (2010) Description of the first pandemic influenza A (H1N1) cases in Vietnam, June-July 2009. OSIR 3:10-13. Available: www.osirjournal.net/upload/files/2_H1N1_Vietnam_new.pdf. Accessed 25 August 2011.
3. Vietnamese Ministry of Health, Report no1193-21-Sept-2009.
4. Nguyen Dung, Hoang Huu Nam, Duong Quang Minh, Nguyen Mau Duyen, Nguyen Khoa Nguyen, Nguyen Dinh Son, Nguyen Thai Hoa (2010) An assessment of the treament response for the epidemic of Influenza A (H1N1) in Thua Thien Hue Province in 2009. J Scienc, Hue University 6: 93-108.
5. WHO (2009) Clinical management of human infection with pandemic (H1N1) 2009: revised guidance. www.who.int/csr/resources/publications/swineflu/clinical_-management_H1N1.pdf.
6. WHO (2011) WHO global influenza surveillance network: Manual for the laboratory diagnosis and virological surveillance of influenza. Global Influenza Programme. Geneva, Switzerland.
7. WHO(2009)WHO information for laboratory diagnosis of pandemic (H1N1)2009 virus in humans - revised. http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf. Accessed 25 August 2011.
8. Percivalle E, Rovida F, Piralla A, Rognoni V, Zavattoni M, Baldanti F, Marchi A, Gerna G (2008) Rapid typing, subtyping and RNA quantification of influenza virus type A strains in respiratory secretions. New Microbiol 31: 319-327.
9. Chan CH, Lin KL, Chan Y, Wang YL, Chi YT, Tu HL, Shieh HK, Liu WT (2006) Amplification of the entire genome of influenza A virus H1N1 and H3N2 subtypes by reverse-transcription polymerase chain reaction. J Virol Methods 136: 38-43.
10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. Available: http://kumarlab.net/pdf_new/TamuraKumar11.pdf. Accessed 25 August 2011.
11. Beauté J, Broberg E, Plata F, Bonmarin I, O'Donnell J, Delgado C, Boddington N, nacken R (2012) Overrepresentation of influenza A(H1N1) pdm09 virus among severe influenza cases in the 2011/12 season in four European countries, Euro Surveill 17: available: http://www.eurosurveillance.org/. Accessed 8 June 2012.
12. Broor S, Krishnan A, Roy DS, Dhakad S, Kaushik S, Mir MA, Singh Y, Moen A, Chadha M, Mishra AC, Lal RB(2012) Dynamic patterns of circulating seasonal and Pandemic A(H1N1)pdm09 influenza viruses from 2007–2010 in and around Delhi, India. PLoS ONE 7: e29129.
13. Mak GC, Wong AH, Ho WYY, Lim W(2012) The impact of pandemic Influenza A (H1N1) 2009 on the circulation of respiratory viruses 2009–2011. Influenza and Other Respiratory Viruses 6: e6–e10.
14. Meschi S, Selleri M, Lalle E, Bordi L, Valli MB, Ferraro F, Ippolito G, Petrosillo N, Lauria FN and Capobianchi MR (2011) Duration of viral shedding in hospitalized patients infected with pandemic H1N1. BMC Infect Dis11:140.
15. Kawano H, Haruyama T, Hayashi Y, Sinoda Y, Sonoda M, and Kobayashi N (2011) Genetic analysis and phylogenetic characterization of Pandemic (H1N1) 2009 influenza viruses that found in Nagasaki, Japan. Jpn J Infect Dis 64: 195-203.

16. Nelson MI, Tan Y, Ghedin E, Wentworth DE, St George K, Edelman L, Beck ET, Fan J, Lam TTY, Kumar S (2011) Phylogeography of the spring and fall waves of the H1N1/09 pandemic influenza virus in the United States. J Virol 85: 828-834.
17. Duan S, Boltz DA, Seiler P, Li J, Bragstad K, Nielsen LP, Webby RJ, Webster RG, Govorkova EA (2010) Oseltamivir-resistant Pandemic H1N1/2009 Influenza Virus possesses lower transmissibility and fitness in ferrets. PLoS Pathog 6(7): e1001022.
18. Kiso M, Shinya K, Shimojima M, Takano R, Takahashi K, Katsura H, Kakugawa S, Mai Thi Quynh Le, Yamashita M, Furuta Y, Ozawa M, Kawaoka Y (2010) Characterization of oseltamivir-resistant 2009 H1N1 Pandemic Influenza A viruses. PLoS Pathog 6: e1001079.
19. Pizzorno A, Bouhy X, Abed Y, Boivin G (2011) Generation and characterization of recombinant Pandemic Influenza A(H1N1) viruses resistant to neuraminidase inhibitors. J Infect Dis 203: 25-31.
20. WHO (2010) Update on oseltamivir-resistant pandemic A (H1N1) 2009 Influenza virus: Weekly Epidemiol Record 85: 37-48.
21. Le QM, Wertheim HF, Tran ND, van Doorn HR, Nguyen TH, Horby P (2010) Vietnam H1N1 Investigation Team. A community cluster of oseltamivir-resistant cases of 2009 H1N1 influenza.N Eng J Med 362: 86-87.

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