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Full Length Research Paper

The identification of the first isolate of influenza B virus using a duplex RT-PCR DNA sequencing in Saudi Arabia (B/Riyadh/01/2007)

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Although Influenza virus infections threaten thousands of lives each year worldwide and bear the risk of been an epidemic, little is known about the circulating strains in Saudi Arabia. The availability of a rapid and accurate diagnostic approach is essential for effective treatment and disease control. In the current study, we optimized a duplex RT-PCR assay for the simultaneous detection of influenza A and B viruses in clinical samples. The developed assay was utilized for testing 100 nasopharyngeal aspirates collected from young children hospitalized with acute respiratory tract infection in Riyadh. Influenza B not A viruses, were identified as possible causes of the disease syndrome. Recovery of influenza B virus from clinical samples was achieved by serial passage in chicken embryos and the virus isolate was designated as Influenza B/Riyadh/01/2007. The identity of B/Riyadh/01/2007 was confirmed by sequencing the RT-PCR product. The sequenced data was submitted to the Gene Bank under the accession number GU135839. An almost complete homology was recognized with all Influenza B virus strains available on Gene Bank. Although several studies suggested the presence of influenza B virus in respiratory tract infection of Saudi children, this is the first report that describes the isolation and partial identification of the circulating virus. Further studies that fully characterize the isolated strain are ongoing.

Key words: Influenza B virus, isolation, RT-PCR, DNA sequencing, Saudi Arabia.

INTRODUCTION

Influenza viruses are members of the family Orthomyxoviridae which includes influenza A, B and C viruses in addition to Thogoto- and Isa- viruses. All members of the family contain a single-stranded, negative-sense and segmented RNA genome (Tobita, 1997; Kawaoka et al., 2005). In particular, Influenza A and B viruses are recognized as major causes of highly contagious respiratory disease in young children, the elderly and immunocompromised patients with potential fatal outcomes. They also cause local epidemics and worldwide pandemics with significant infection rates and severe economic losses (Wright et al., 2007).

Since 2003, only influenza B viruses and two subtypes of influenza A viruses (H1N1 and H3N2) are persistently circulating in the human populations (Xu et al., 2004). However, few subtypes of influenza A viruses like H5N1, H7N3, H7N7, H9N2 and an altered H1N1 (Swine flu) have recently emerged and/or have crossed the restrictive species barrier (Yu et al., 2006; CDC, 2009). This situation announces possible epidemic and severe public health problems. Since a wide spectrum of different bacterial and viral pathogens are commonly involved in the acute respiratory disease syndrome of humans, it is important to identify influenza viruses in suspected clinical samples directly and rapidly. This will facilitate application of the proper preventive strategies and initialization of antiviral therapy with significant impact (Claas et al., 1993; Poehling et al., 2002).

Classical diagnosis of influenza virus infections is based on virus isolation in cell culture or chicken embryos with subsequent haemagglutinin and neuramindase sub typing by serological methods (Dwyer et al., 2006). Despite the accuracy and sensitivity of such methods they require time, high costs, specific technical skills and strict safety measures. Faster results are obtained by partial propagation of the virus on cell culture

followed by antigen detection using imunofluorescence (Ziegler et al., 1995). Other serological and molecular techniques were adopted for rapid diagnosis of influenza viruses although they still have some limitations with regard to sensitivity, specificity and/or availability (Doller et al., 1992; Church et al., 2002; Masaki et al., 2006). At this moment RT-PCR is well established as the most sensitive and specific technique available for the detection of influenza virus infections (Claas et al., 1993). Different types and formats of the technique have been introduced to enable detection, typing, subtyping and quantification of the virus genome in short time, this enable proper intervention and better medical management (Ellis et al., 1997; Templeton et al., 2004; Minosse et al., 2007; Wu et al., 2008).

Although influenza B viruses are important human pathogens and are responsible for the majority of influenza cases in specified geographical regions, they have obtained much less attention in comparison with influenza A viruses (Barclay and Palese, 1995). The reasons for such bias may be a result of their lower mutation rates especially with genetic reassortment; their restricted host range that limits the extensive virus spread; and their relatively tolerable health problems (Lindstrom et al., 1999; McCullers et al., 1999). In Saudi Arabia, influenza viruses, and more specifically influenza B viruses did not receive much attention in literature, rather than the identification of influenza viruses as important causes of respiratory tract infections among pilgrims during the Hajj season (El-Sheikh et al., 1998; Kholeidi et al., 2001; Balkhy et al., 2004; Al-Saleh et al., 2005; Rashid et al., 2008), and few reports on the impact of different respiratory viruses including influenza viruses in the hospitalization of young children (Al-Hajjar et al., 1998; Bakir et al., 1998; Al-Shehri et al., 2005). A significant lack of information regarding the influenza viruses circulating in Saudi Arabia in terms of prevalent types and subtypes, virulence and risk factors as well as virus diversity/phylogeny still exists and necessitates comprehensive studies.

In the current report, we describe the development and establishment of a highly specific and sensitive duplex RT-PCT assay for the simultaneous detection and typing of influenza viruses in clinical samples. This assay was applied to the analyzes of 100 nasopharyngeal aspirate samples (NPAs) collected from young children hospitalized at King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia during the winter season 2007/08. The test results facilitated the identification and further isolation of the first Saudi influenza B virus isolate (B/Riyadh/10/2007).

MATERIALS AND METHODS

Clinical specimens

One-hundred nasopharyngeal aspirates (NPAs) were collected from hospitalized children, aging from one-month to three-years and suffering from acute respiratory tract illness, at King Khalid University Hospital, Riyadh, Saudi Arabia. NPAs were collected during the period starting from September 2007 to April 2008 by trained nurses through insertion of a sterile catheter into the nasal cavity of patients with application of gentle suction using an electronic suction device. Clinical samples were transported in minimal esssential medium (MEM) supplemented with 500 U penicillin and 500 µg streptomycin per ml to the Research Central laboratory, College of Science, King Saud University, Riyadh for processing, testing and storage at -80°C.

Chicken embryos` inoculation

Specific-Pathogen-Free (SPF) chicken embryos of 9 - 11 days-old were inoculated with 0.2 ml of the clinical sample preparations via the allantoic route. The eggs were incubated for 4 - 7 days at 37°C and were examined daily for embryonic death. Eggs containing dying embryos after 24 h of incubation and those that stayed alive till the end of incubation period were chilled for 2 h and the allantoic fluid was harvested. Three successive passages were performed for complete adaptation and propagation of the virus isolates. The virus titer was confirmed by plate haemagglutination assay (Hierholzer et al., 1969).

RNA isolation

Extraction of viral RNA from infected allantoic fluid samples was conducted using QIAamp Viral RNA Mini Kit (Qiagen GMBH, Hilden, Germany). Each sample aliquot was used only once to avoid the loss of viral genomic material during repetitive freezing and thawing. 140 µl sample volumes were processed according to the manufacturer's instructions to obtain 60 µl RNA elutes. A portion of the RNA was used immediately in RT-PCR, while the rest was divided into aliquots and kept frozen at -20°C for further analysis.

Reverse transcription

cDNA was synthesized from the extracted viral RNA using Sensiscript® Reverse transcription kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. A reaction mixture containing 5 µl of template RNA, 2 µl of 10x buffer RT, 2 µl of 5mM dNTPs mix, 2 µl of 100 µM of random primers, 1 µl (10U) of RNase inhibitor and 1 µl of sensiscript® Reverse Transcriptase was added to 20 µl total reaction volume by RNase free water. The mixture was incubated for 60 min at 37°C and then cooled to 4°C till use for PCR. As in-process control the synthesized cDNA products were analyzed by agarose gel electrophoresis for further confirmation.

Multiplex PCR

Identification of Influenza A and B viruses in cDNA samples was achieved by simultaneous amplification of specific sequences located within the NS-2 gene of either virus using the primer sets designed by Claas et al. (1993) (Table 1). The PCR reaction was performed using the Multiplex PCR Kit (Qiagen GmbH, Hilden, Germany). A reaction volume containing 25 µl of 2x Qiagen Multiplex PCR Master Mix, 0.5 µl of 100 µM stock of each primer (Influenza A and B primer sets) and 2 µl of the cDNA product was added to 50 µl volume using RNase free water. All sample tubes were incubated in a thermal cycler (Primus 96 plus, MWG AG Biotech, Ebersberg, Germany) for one cycle at 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 50°C for 90 s and 72°C

Table 1. Oligonucleotide primers for Multiplex RT-PCR.

Target virus	Primer name	Primer sequence	PCR product
Influenza A	INF-A-cDNA	5`- AAG GGC TTT CAC CGA AGA GG -3`	190 bp
	INF-A-rev	5`- CCC ATT CTC ATT ACT GCT TC -3`	
Influenza B	INF-B-cDNA	5`- ATG GCC ATC GGA TCC TCA AC -3`	238 bp
	INF-B-rev	5`- TGT CAG CTA TTA TGG AGC TG -3	

for 90 s, and finally one cycle of 72°C for 10 min. PCR products were separated in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide; the bands were identified in comparison with 1 kbp DNA ladder (Invitrogen, San Diego, CA, USA) and documented using an image analysis system (IMAGO compact imaging system, B&L, USA).

Nucleotide sequence analysis

Specific PCR products were excised from agarose gel and purified by Montáge DNA gel extraction kit (Millipore, Concord Road Billerica, MA, USA). The nucleotide sequence of the purified fragments was determined using an automated DNA sequencer at King Faisal Specialist Hospital Research Center (KFSHRC), Riyadh. Sequence result analysis was conducted using BLAST search tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Lasergene software, version 3.18 (DNASTAR, Madison, Wis.). Multiple sequence alignment was performed to determine the relationship between the clinical virus isolates and several reference strains using Clustal W, version 1.81; and GeneDoc, version 2.7.

RESULTS

Optimization and validation of the duplex RT-PCR assay

A duplex RT-PCR assay was developed for simultaneous detection of influenza A and B viruses. The test made use of two primer sets homologous with a highly conserved sequence of the NS-2 gene of each influenza virus type. Different variables of the RT-PCR assay were tested to identify the best possible conditions for getting highly specific, sensitive and reproducible results, including type of reverse transcriptase and polymerase enzymes, buffering system, primer concentration and thermal cycling conditions. The assay specificity was confirmed by parallel testing of influenza A (H1N1) and B viruses with different human respiratory viral agents. While influenza A and B viruses elucidated strong positive signals, no cross reactivity was observed with respiratory syncytial virus types A and B, para influenza virus types 2 and 3, human coronaviruses OC43 and E229, human metapneumo viruses and Measles virus.

Analysis of clinical samples for influenza viruses

One-hundred NPA samples were collected from children (below 3 years of age) hospitalized at KKUH, Riyadh during the late autumn period of 2007 to spring of 2008 for acute respiratory tract infections. The different samples were tested before and after a preliminary passage in embryonating chicken eggs using the developed duplex RT-PCR assay. While no sample reacted specifically with the influenza A primers, nine samples showed positive reactivity with the influenza B primer set (Figure 1). A representative positive sample was chosen for further propagation in chicken embryos and the recovered virus isolate was designated (B/Riyadh/01/2007).

Nucleotide sequence analysis

To further confirm and validate the viral identity, the RT-PCR product of influenza B/Riyadh/01/2007 was purified from agarose gel and subjected to DNA sequence analysis. The obtained nucleotide sequence was submitted to the Gene Bank and was given the accession number GU135839. Multiple sequence alignment of B/Riyadh/01/2007 with the different Influenza B virus sequences available at the Gene Bank using BLAST sequence search program and Megalign program of the Lasergene package showed an almost complete homology (99.58%) with only one nucleotide uncertainty [No. 229] (Figure 2).

DISCUSSION

Saudi Arabia is one of the major countries at risk from exotic and emerging viral disease infections. In addition to the relatively high population of imported working force, the Hajj season constitutes one of the major annual gatherings in the world (Alzeer, 2009). Such conditions favor virus spread making Saudi Arabia a virgin soil for potential health problems and possible epidemic.

More specifically, respiratory viral infections including influenza viruses are seen as highly suspected challenges for the health and economy in Saudi Arabia due to their massive spread, shorter incubation periods, and higher mutation frequencies (Ison et al., 2002). During the last few months, Saudi Arabia has experienced increasing exposure rates of influenza virus H1N1; commonly recognized as swine flu. Therefore, the Saudi ministry of health has taken into consideration all possible means to counter such problem. It requires,

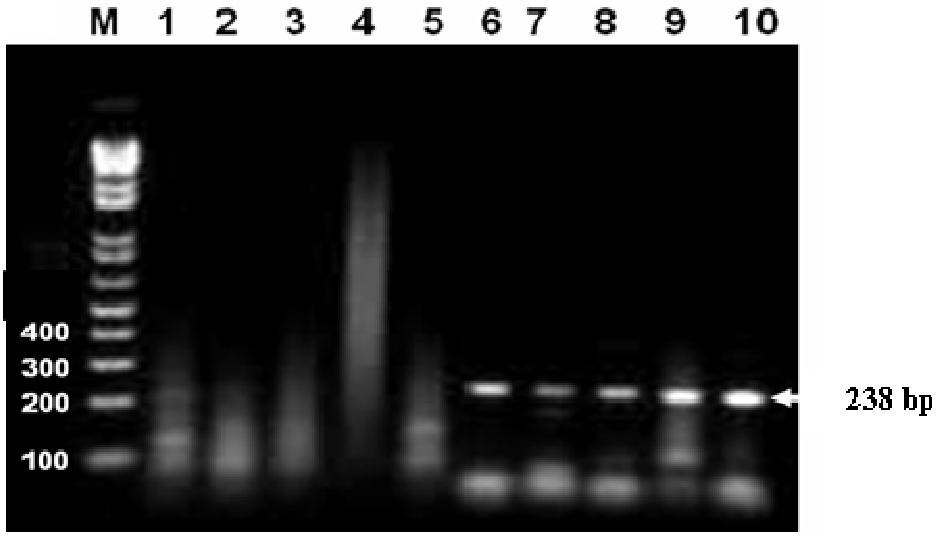

Figure 1. Identification of influenza B virus in selected clinical samples after propagation in embryonating chicken eggs using two-step duplex RT-PCR assay. M: 1 Kbp DNA molecular weight ladder plus; Lanes 1 - 5: Influenza B negative samples; Lanes 6 - 9: Influenza B positive samples as detected by amplification of a 238 bp fragment of the NS2 gene Lane 10: Positive influenza B virus control (B/Hong Kong/5/72, VR-823™, ATCC).

according to the recommendation of World health Organization (WHO), multi step process which includes improvement of the diagnostic approaches, preventive measures and hygiene level.

The availability of a diagnostic approach that combines the high degree of detection sensitivity and specificity usually achieved by the gold standard "virus isolation", along with the economic and rapid advantages of several serological and rapid antigen detection tests is necessary for physicians and health organizations to enable more accurate treatment and control decisions (Kendal et al., 1982). Several studies have evaluated RT-PCR for the diagnosis of influenza viruses using different strategies, either for virus detection [single RT-PCR] (Claas et al., 1993 and Steininger et al., 2002); typing [Duplex RT-PCR] (Herrmann et al., 2001; Carraro et al., 2007); and quantification [Real-time RT-PCR] (Daum et al., 2007; Wu et al., 2008). All of these trials proved that RT-PCR is the most suitable assay for diagnosis of influenza viruses. Therefore in the current study, we optimized a duplex RT-PCR assay for detection and differentiation of influenza A and B viruses and utilized such assay in identification of the circulating influenza viruses in Saudi Arabia.

Careful evaluation of the different factors affecting the performance of RT-PCR, utilizing standard Influenza A and B viruses as positive controls permitted the development of a highly specific, sensitive and reproducible assay. Exploitation of the developed assay in testing 100 clinical samples collected from young children suffering from acute respiratory tract infections in Riyadh identified influenza B virus in nine samples. No influenza A viruses were detected in any sample. Similar studies conducted in Saudi Arabia using a wide diverse of virological, serological and molecular techniques were able to identify ratios ranging from 6 - 23% of influenza virus infections in suspected clinical samples (El-Sheikh et al., 1998; Al-Hajjar et al., 1998; Bakir et al., 1998; Kholeidi et al., 2001; Balkhy et al., 2004; Al-Saleh et al., 2005; Al-Shehri et al., 2005; Meqdam et al., 2005; Rashid et al., 2008). We can conclude that influenza viruses are still circulating with a parallel prevalence, however, the lack of any influenza A positive sample was totally unexpected. Influenza A viruses were the prevalent type recorded in most studies and was always circulating every year except during the winter season of 1987/1988 (Hossain et al., 1988). We can expect that during the winter season of 2007/2008 in Riyadh, a restrictive range of pathogens, excluding influenza A viruses predominate possible causes of the respiratory disease syndrome. This presumption was supported by the identification of Respiratory syncytial and Parainfluenza 2 and 3 viruses in addition to influenza B viruses in the majority of test samples (data not shown).

Isolation and propagation of influenza B virus in embryonated chicken eggs is not recommended by WHO since it requires multiple serial passages and usually do not yield satisfactory virus titers. However, more recent studies identified a group of influenza B viruses that grow well and produce high virus titers in eggs (Lugovtsev et al., 2005). These strains were selected for use in the European vaccines although in certain cases the growth of chosen viruses is still unsatisfactory causing a potential reduction in the number of available vaccine doses. In the current report, adaptation and propagation of the selected influenza B virus strain in chicken embryos were quite successful from the first passage.

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B-Riyadh-01-2007            ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Shiga-T30-1998            ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Taiwan-70061-2006         ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Malaysia-2506-2004        ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Guangzhou-01-2007         ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Houston-B57-1997          ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Perth-25-2002             ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Johannesburg-06-1994      ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Nipal-1331-2005           ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-HongKong-310-2004         ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60
B-Tehran-80-2002            ATGGCCATCGGATCCTCAACTCACTCTTCGAGCGTCTTAATGAAGGACATTCAAAGCCAA  60

B-Riyadh-01-2007            TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Shiga-T30-1998            TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Taiwan-70061-2006         TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Malaysia-2506-2004        TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Guangzhou-01-2007         TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Houston-B57-1997          TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Perth-25-2002             TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Johannesburg-06-1994      TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Nipal-1331-2005           TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-HongKong-310-2004         TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120
B-Tehran-80-2002            TTCGAGCAGCTGAAACTGCGGTGGGAGTCTTATCCCAATTTGGTCAAGAGCACCGATTAT  120

B-Riyadh-01-2007            CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Shiga-T30-1998            CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Taiwan-70061-2006         CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Malaysia-2506-2004        CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Guangzhou-01-2007         CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Houston-B57-1997          CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Perth-25-2002             CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Johannesburg-06-1994      CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Nipal-1331-2005           CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-HongKong-310-2004         CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180
B-Tehran-80-2002            CACCAGAAGAGGGAGACAATTAGACTGGTCACGGAAGAACTTTATCTTTTAAGTAAAAGA  180

B-Riyadh-01-2007            ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCNTAATAGCTG    238
B-Shiga-T30-1998            ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Taiwan-70061-2006         ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Malaysia-2506-2004        ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Guangzhou-01-2007         ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Houston-B57-1997          ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Perth-25-2002             ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Johannesburg-06-1994      ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Nipal-1331-2005           ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-HongKong-310-2004         ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
B-Tehran-80-2002            ATTGATGATAACATATTGTTCCACAAAACAGTAATAGCTAACAGCTCCATAATAGCTG    238
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Figure 2. Multiple sequence alignment of the DNA sequence represents the RT-PCR product of B/Riyadh/01/2007 with the corresponding sequences of different influenza B virus strains available at the GeneBank. The shadow identifies the variable nucleotide in the original sequence.

The virus titer increased exponentially from a passage to another, as identified by the plate haemagglutination test using human RBCs (type O), till it reached 5120 HA unit/ml by the third passage. The virus isolate designed (B/Riyadh/01/2007), is therefore a promising candidate for future use in vaccine production after full characterization.

DNA sequence analysis of the RT-PCR product of Influenza virus B/Riyadh/01/2007 enabled further confirmation of the virus identity. Since the primer set used for PCR amplification specifically targeted a highly conserved sequence of the virus genome within the NS-2 gene, no distinct variation between B/Riyadh/01/2007 and any of the influenza B virus strains available on the Gene Bank was observed (Figure 2). Only a single nucleotide at the position 229 was identified as (N), which may not be a real substitution of the (A) nucleotide always identified for the other strains.

Currently circulating influenza B viruses are divided into two distinct phylogenetic lineages; B/Yamagata/16/88 (B/Yam) and B/Victoria/2/87 (B/Vic) (Kanegae et al., 1990). B/Vic-like strains were dominant worldwide in the

1980s, but became confined to Eastern Asia during the last decade (Nakagawa et al., 2000). However, B/Vic-like viruses re-emerged outside East Asia during the 2001/02 season (Shaw et al., 2002) and have since been isolated sporadically with occasional localized outbreaks world-wide. In contrast, circulation of B/Yam lineage viruses was not geographically-restricted at any time period. Reassortants of the two lineages have been observed frequently since 2002 (Motta et al., 2006; Lee et al., 2009). Antigenic and genomic identification of the isolated Influenza B/Riyadh/01/2007, particularly the phylogenetic relationship of HA, NA and MP genes with the different Influenza B virus strains will provide valuable data that will enable the recognition of the circulating lineage, identification of possible reassortants and further understanding of the epidemiology and evolution of Saudi strains.

Conclusion

The present study describes the development of an accurate and rapid diagnostic approach that detects and differentiates influenza A and B viruses in a single reaction. We present the first Saudi influenza B virus isolate circulating in Riyadh District during the winter season of 2007/08. Further studies concerning antigenic and genomic characterization of the virus isolate, as well as the basis of its diversity and evolution are in progress and are expected to provide valuable information.

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